


Lubricin: Its Presence in Repair Cartilage following Treatment with Autologous Chondrocyte Implantation

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Abstract

Objective: To determine if lubricin was present in the surface layer of repair cartilage formed after autologous chondrocyte implantation (ACI). **Design:** Forty-three biopsies of repair tissue were taken from patients who had been treated with ACI 8 to 68 months previously (mean of 18.0 ± 14.4 months); 30 had flaps of periosteum and 13 of Chondro-Gide[®]. Cryopreserved sections were stained with hematoxylin and eosin, toluidine blue, and immunostained for lubricin and type II collagen. The quality of repair tissue was scored via OsScore, and clinical improvement in patients was assessed via change in Lysholm score. Normal/control cartilage was studied for comparison ($n = 5$). **Results:** Patients' Lysholm scores improved from 48.1 ± 17 preoperatively to 69.5 ± 21.5 posttreatment. The thickness of repair tissue was 2.9 ± 1.7 mm compared with 2.3 ± 0.6 mm for control cartilage, with an OsScore of 6.7 ± 1.6 (8.9 ± 1.2 for controls). Ninety-eight percent of biopsies had staining for lubricin, with 84% having some in the surface layer (60% of periosteal treated and 100% of Chondro-Gide treated). The improvement in Lysholm score was not significantly different in patients with lubricin present at the surface compared with those without. **Conclusion:** Lubricin was present in almost all samples of repair tissue formed post ACI, often in the surface layer, resembling the distribution that is seen in normal cartilage. The presence of lubricin in the upper layer is likely to have implications for the functioning of the tissue because, via its mucin-like repeats, it appears capable of reducing the friction that could arise in articulating joints.

Keywords

superficial zone protein, proteoglycan 4, cell therapy, articular cartilage, surface zone

Introduction

Autologous chondrocyte implantation (ACI) has now been used worldwide for more than 2 decades as a means of treating chondral defects, particularly in the knee.^{1,2} There has been extensive interest as to the quality and morphology of the repair tissue that forms, whether it is hyaline cartilage, fibrocartilage, or how much type II collagen it contains.³⁻⁵ The premise is that the more it resembles hyaline cartilage and the more type II collagen within it, the better the quality of the repair. Other properties of the repair tissue, however, may be as important and indicative of the long-term successful functioning of the replacement tissue that forms. For example, normal articular cartilage contains a proteoglycan that is alternatively called superficial zone protein or lubricin⁶ (also synonymous with PRG4, megakaryocyte-stimulating factor precursor, or CAP [or CACP] protein). These all appear to have a similar primary structure, all being encoded by the gene PRG4, but they may have different posttranslational

modifications.⁷ Lubricin, which was first isolated from synovial fluid,⁸ is attributed with many functions relevant to its location in the superficial zone of articular cartilage, including antiproliferative effects and inhibiting protein or cell adhesion.⁹ Probably one of the most important properties, however, is that it functions as an efficient boundary lubricant at low striding speeds typically seen in joints.¹⁰⁻¹²

Defects in the structure or production of lubricin are suspected to be associated with abnormal joint functioning.

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For example, the genetic disorder camptodactyl-arthropathy pericarditis syndrome appears to be mapped to the same locus as that encoding for lubricin.⁷ Patients with this disorder have a large joint arthropathy, flexion contracture of interphalangeal joints, and synoviocyte hyperplasia, suggested to result from ineffective joint lubrication. Prg-4 knockout mice are born with apparently normal joints, but with aging, they develop abnormalities in the surface of the cartilage (abnormal protein deposition) and subsequent cartilage deterioration.⁹ Animal models of osteoarthritis (OA) have reduced levels of lubricin,¹³ particularly in the early stage of the disorder.¹⁴ *In vitro* studies of lubricin production show it is down-regulated by several cytokines believed to be involved in OA, for example, IL1- β and TNF- α ¹⁵; in addition, lubricin appears to be chondroprotective and to reduce structural damage when applied in an animal model of OA.¹⁶ To date, little is known of lubricin's involvement in cartilage repair in humans.

We have examined the presence of lubricin in biopsies of repair tissue from patients who had chondral or osteochondral defects treated with ACI. In some patients, this was carried out in the traditional/original manner as described by Brittberg *et al.*,² whereby autologous chondrocytes had been extracted from healthy tissue harvested from a less weight-bearing region of the knee, proliferated *in vitro*, and injected beneath a flap of periosteum stitched over the damaged but debrided area. In other patients, a collagen membrane, Chondro-Gide[®] (Geistlich Pharma AG, Switzerland), was used in place of the periosteum. This immunohistochemical study demonstrated that lubricin is present in the repair tissue after approximately 18 months of treatment in most patients using autologous periosteum and in all using a sheet of porcine collagen as the flap.

Materials and Methods

Patients and Tissue Biopsies

Forty-three full-depth biopsies were obtained from 41 patients aged 19 to 60 years at treatment (mean age, 34.8 \pm 9.7 years) who had been treated with ACI for cartilage defects; 29 were treated using a periosteal flap, and 12 with a Chondro-Gide flap. As part of their follow-up assessment, patients in our institute routinely undergo an arthroscopic examination and biopsy of the graft at approximately 1 year (ranging between 8 and 68 months, with a mean of 18.0 \pm 14.4 months), as approved by the local Ethical Committee. Biopsies of 1.8 mm in diameter were taken (using a juvenile bone marrow biopsy needle) from the center of the treated region and included both the repair cartilage tissue and, where possible, the subchondral bone, as detailed previously.¹⁷ Twenty-seven biopsies were taken from the right

knee and 16 from the left. The most common site of treatment was the medial femoral condyle ($n = 26$), with 11 biopsies taken from the lateral femoral condyle, 5 from the trochlea, and 1 from the patella. The average size of the defect area treated was 5.1 \pm 3.9 cm² (7.2 \pm 5.4 cm² for Chondro-Gide or 4.2 \pm 2.9 cm² for periosteum-treated regions). Biopsies were snap frozen and stored in liquid nitrogen until examined.

Patients were also assessed at regular intervals for clinical outcome using the Lysholm score, ranging from 0 (poor outcome) to 100 (best).¹⁸ This was recorded preoperatively, just prior to the arthroscopic investigation, and annually thereafter. Within this score is a pain component (0 = most severe pain, 25 = no pain), which was also recorded.

For comparison to the repair tissue, "normal" control articular cartilage was examined from 5 individuals: 4 from medial femoral condyles obtained at autopsy of individuals aged 22, 30, 40, and 50 years (obtained from the UK Human Tissue Bank) and 1 lateral femoral condyle of an individual aged 10 years undergoing corrective surgery.

Histologic Assessment

Cryosections were stained with hematoxylin and eosin and toluidine blue, and cartilage morphology was determined microscopically using normal and polarized light. Quality of repair tissue was assessed using a semiquantitative score (OsScore), in which the parameters smoothness of surface, morphology, metachromasia, vascularization, mineralization, and basal integration were assessed and scored as described previously.¹⁷ This uses a scale of 0 (worst) to 10 (best).

Immunostaining

For lubricin immunohistochemistry, 7- μ m-thick cryostat sections were collected onto poly-L-lysine-coated slides and fixed with 4% paraformaldehyde. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol and nonspecific staining blocked with normal goat serum. Sections were incubated overnight at 4°C with 10 μ g/mL G35 polyclonal antibody raised in rabbits against the human lubricin peptide sequence GEGYSRDAT, from the protein region encoded by exon 2.¹⁰ This antibody detects full-length lubricin on Western blotting (data not shown). Labeling was visualized using biotinylated anti-rabbit antibody and an avidin-biotin complex (Vectastain Elite ABC kit; Vector Laboratories, Peterborough, UK) and diaminobenzadine as chromogen. Sections were counterstained with Mayer's hematoxylin, dehydrated, cleared, and mounted with Pertex. Adjacent sections were used as controls by omitting the primary antibody and using a rabbit IgG in its place (Vector Laboratories).

Table 1. Details of Patients Entered into the Study^a

	Chondro-Gide	Periosteum	All Samples
Average age of patients at autologous chondrocyte implantation (years)	36 ± 9.8 (12)	34.3 ± 9.8 (29)	34.8 ± 9.7 (41)
Time post-autologous chondrocyte implantation (months)	14.9 ± 7.2 (13)	19.3 ± 16.6 (30)	18.0 ± 14.4 (43)
% improvement in Lysholm score at 1 year over preoperative score	117.6 ± 167 (9)	63.0 ± 93 (28)	76.3 ± 115 (37)
Cartilage thickness (mm)	3.0 ± 2.0 (13)	2.9 ± 1.7 (30)	2.9 ± 1.7 (43)
OsScore	6.9 ± 1.2 (13)	6.6 ± 2.1 (30)	6.7 ± 1.8 (43)

^aValues are means ± standard deviations, with number of samples in parentheses.

Immunostaining for type II collagen was also carried out on adjacent cryosections fixed in 10% formalin and enzymatically digested with ovine testicular hyaluronidase (4800 U/mL) for 2 hours at room temperature. They were then incubated with a monoclonal antibody to type II collagen (CHCI; Developmental Studies Hybridoma Bank, University of Iowa) for 30 minutes at room temperature. Visualization was carried out as for lubricin but using an antimouse secondary antibody, with antimouse IgG used as a normal control.

Statistics

Nonparametric statistical analyses were performed, for example, the Mann-Whitney *U* test, to compare outcomes in patient cohorts with or without lubricin in the surface layer analyzing parameters such as the pain score, Lysholm score, OsScore, and thickness of repair cartilage. Similarly, comparisons were made between the 2 patient groups treated with either periosteal or Chondro-Gide patch. Statistics were carried out with a software program, Analyse-it[®] (Analyse-it Software Ltd, Leeds, UK). Means are quoted ± standard deviation, and a *P* value of <0.05 was considered to indicate significance.

Results

The average age of both treatment groups was similar, and the time post-ACI was 19.3 months for the periosteum-treated group and 14.9 months for the Chondro-Gide-treated group (Table 1). Patients treated with periosteum had an average preoperative Lysholm score of 48.8 ± 17 (*n* = 29), which had improved to 70.3 ± 21 at 1 year. The average preoperative Lysholm score for the patients treated with Chondro-Gide was 39.1 ± 17.9 (10), which increased to 60.3 ± 21.1 at 1 year after treatment; there was no significant difference in clinical improvement between those treated with periosteum or Chondro-Gide (*P* = 0.17). After the 1st year posttreatment, there was little increase in Lysholm score with time for all patients (Fig. 1a). The pain score followed a similar trend, with little difference between patients treated with Chondro-Gide or periosteal flaps (Fig. 1b).

The morphology of the repair tissue in the periosteum group was predominantly hyaline cartilage in 5 samples; 2 were a mixture of hyaline and fibrocartilage, 20 were

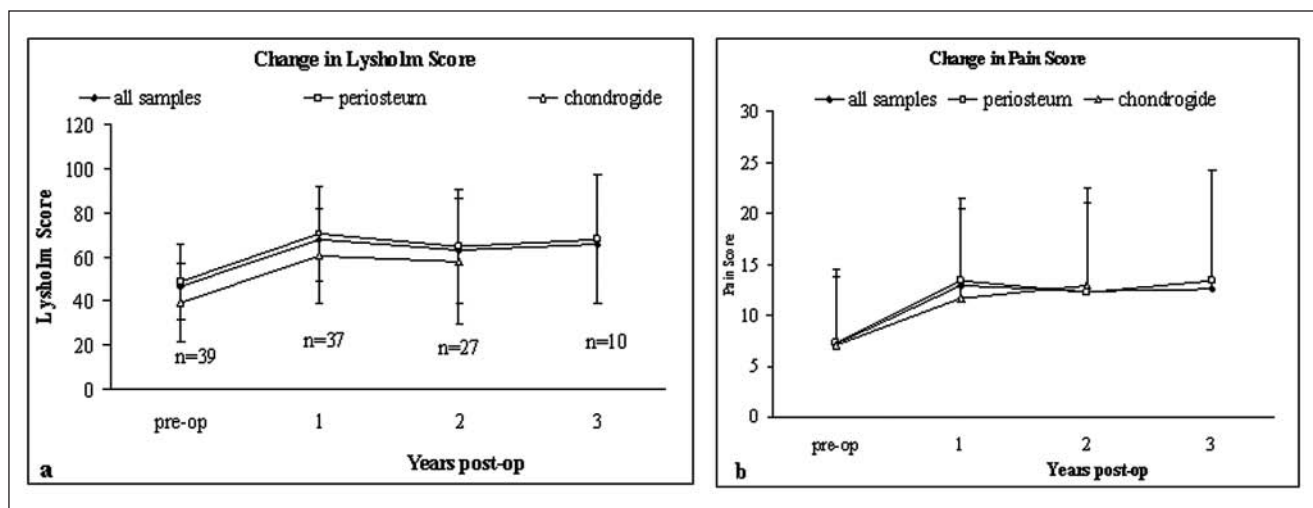


Figure 1. Change in (a) Lysholm score and (b) pain score with time posttreatment in autologous chondrocyte implantation (ACI) patients. Results are mean values; bars represent standard deviations. *n* = the number of biopsies at each time point.

mainly fibrocartilage, and 3 were fibrous tissue. In the Chondro-Gide–treated group, only 1 biopsy was predominantly hyaline cartilage, 1 was a mixture of hyaline and fibrocartilage, and 11 were mostly fibrocartilage. There was little difference between the groups in the thickness of repair tissue formed (2.9 mm average) or the OsScore (6.7; **Table 1**). Control samples were all classed as hyaline cartilage with an average thickness of 2.7 ± 1.1 mm and an OsScore of 8.5 ± 2.7 .

Immunostaining for lubricin was present in all of the control samples of normal cartilage in the superficial zone; in the 22-year-old, this occurred only in the cells, but for the 10-, 30-, 40-, and 50-year-olds, it was in both cells and some matrix (**Fig. 2e-g**). There were also a few cells within the rest of the tissue that were positive, with the strongest staining seen in those at the base of the tissue toward the bone. In the few samples with cell clusters (3 or more cells) present, some of these cells were positively stained.

In biopsies of repair tissue, there was immunostaining for lubricin in a very thin strip of the superficial zone in 84% of biopsy samples where the articulating surface was completely intact (33/38; **Fig. 3**); unfortunately, in 5 samples the surface was damaged or absent (probably during the process of obtaining the biopsy), so it was not possible to assess the location in these. Staining occurred either in the uppermost cells, the matrix, or both (**Fig. 2**). In the group treated with ACI and periosteum, the superficial zone matrix was positively stained in 60% (15/25) of the samples, and 64% (16/25) had positive staining in some cells. In contrast, all the biopsies from patients treated with Chondro-Gide (13/13) showed positive staining of the cells in the superficial zone, and 83% of these samples had positive staining within the matrix here.

In the tissue, further from the surface, cells that stained positively for lubricin were seen in 40 samples (93%) in both the mid and the deep zones, with the strongest staining occurring in the deep zone (**Fig. 4**). Clusters of cells were seen in 11 samples (26%), and some of the cells within the clusters were immunopositive. There was only slight ($n = 8$) or no ($n = 35$) immunopositivity seen in the extracellular matrix of the mid and deep zone of the samples. Only 1 sample from a patient (periosteal patch) was completely negative for lubricin; this biopsy was fibrocartilage throughout, with the upper surface containing very thin fibroblast-like and distorted cells.

Bone was present in 40 of the biopsies (but partially detached from the cartilage in 1 sample). Positive immunostaining for lubricin was seen in either bone, osteoid, or both in all of the intact biopsies.

There was immunostaining for type II collagen in all of the samples investigated but to very varying degrees, ranging from throughout the whole depth of repair tissue to only a small region being positive. Where staining was

partial, it was always the region closest to the bone that was positive, with the region toward the articulating surface being weaker or negatively stained. The distribution of staining for lubricin and type II collagen in the upper layer appeared to be completely unrelated, whereas the cellular staining for lubricin that was present in the deeper zones did occur with stronger matrix staining for type II collagen (**Fig. 4f**). There were, however, no obvious trends with the presence of or staining pattern for lubricin and the OsScore, tissue morphology, or clinical parameters investigated and no significant difference for any parameters between patients who had lubricin present in the surface layer and those who did not.

Discussion

The repair tissue that formed post-ACI in this study was of a similar thickness to that of the normal control articular cartilage, even though it was mostly fibrocartilaginous rather than of hyaline cartilage morphology. In most samples, a thin layer of lubricin was demonstrated to occur in the superficial region at the articulating surface. The pattern of immunostaining was a little more widespread throughout the matrix at the surface than in the older control samples examined here and in those illustrated in some other studies (although this was in bovine cartilage).¹⁹ There was an average of a 20-point increase in clinical benefit in both groups of patients (regardless of the type of flap used), which generally occurred within the 1st year and was sustained thereafter, as has been reported in other studies.²⁰

The location of lubricin at the articulating surface could arise either via lubricin within the synovial fluid binding to the upper layers (as has been shown to occur *in vitro*¹⁰) or via production by the surface cells, particularly in response to shear stresses on the tissue and cells. Grad *et al.*²¹ found that increasing surface motion via reciprocating sliding on tissue-engineered cartilage constructs increased the expression for and production of lubricin. The loads and strains that arose in this model were not characterized, but it is thought that fluid flow, cell deformation, or shear would occur. Lubricin production increased when shear was applied to chondrocytes cultured in alginate.²² Lubricin has been reported on the surface of other connective tissues and at possible sites of high shear stresses, for example, in tendons,^{23,25} meniscus,²⁶ and between annular lamellae within the intervertebral disc.²⁷ The most intense staining for lubricin was at the surface of tendons subjected to the highest levels of compressive, shear, tensile, and frictional forces.²³

One of the most commonly suggested functions for lubricin occurring at the articular surface is that it is likely to reduce friction, possibly by virtue of its large mucin-like domain characterized by the repeat motif KEPAPTTT/P.⁹

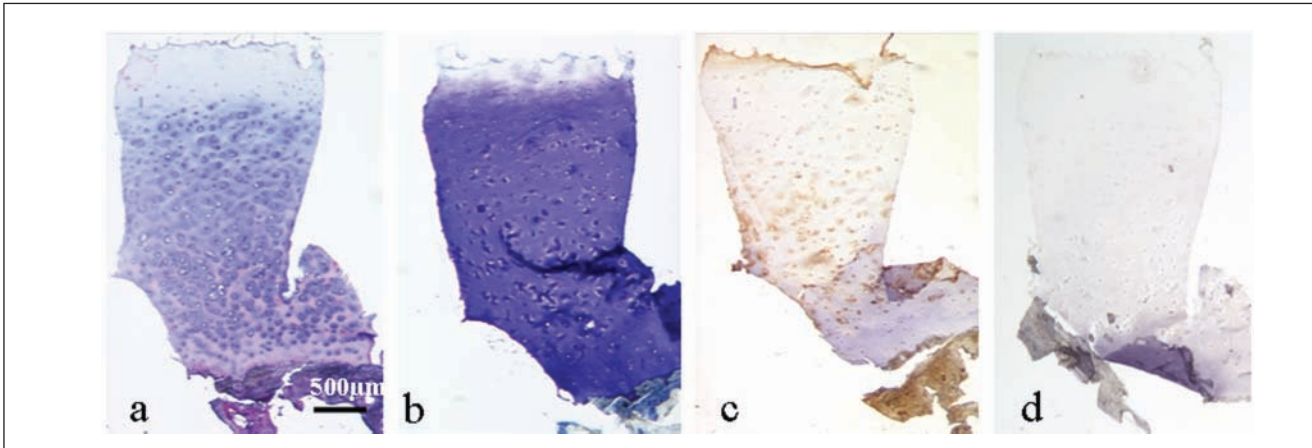


Figure 2. Full-depth sections of repair tissue from a 44-year-old man treated with autologous chondrocyte implantation and Chondro-Gide 15 months previously. Sections stained with (a) hematoxylin and eosin, (b) toluidine blue, and (c) immunostained for lubricin demonstrating the typical distribution with a thin layer at the surface and some cells toward the base of the tissue. (d) Adjacent control section labeled with rabbit IgG.

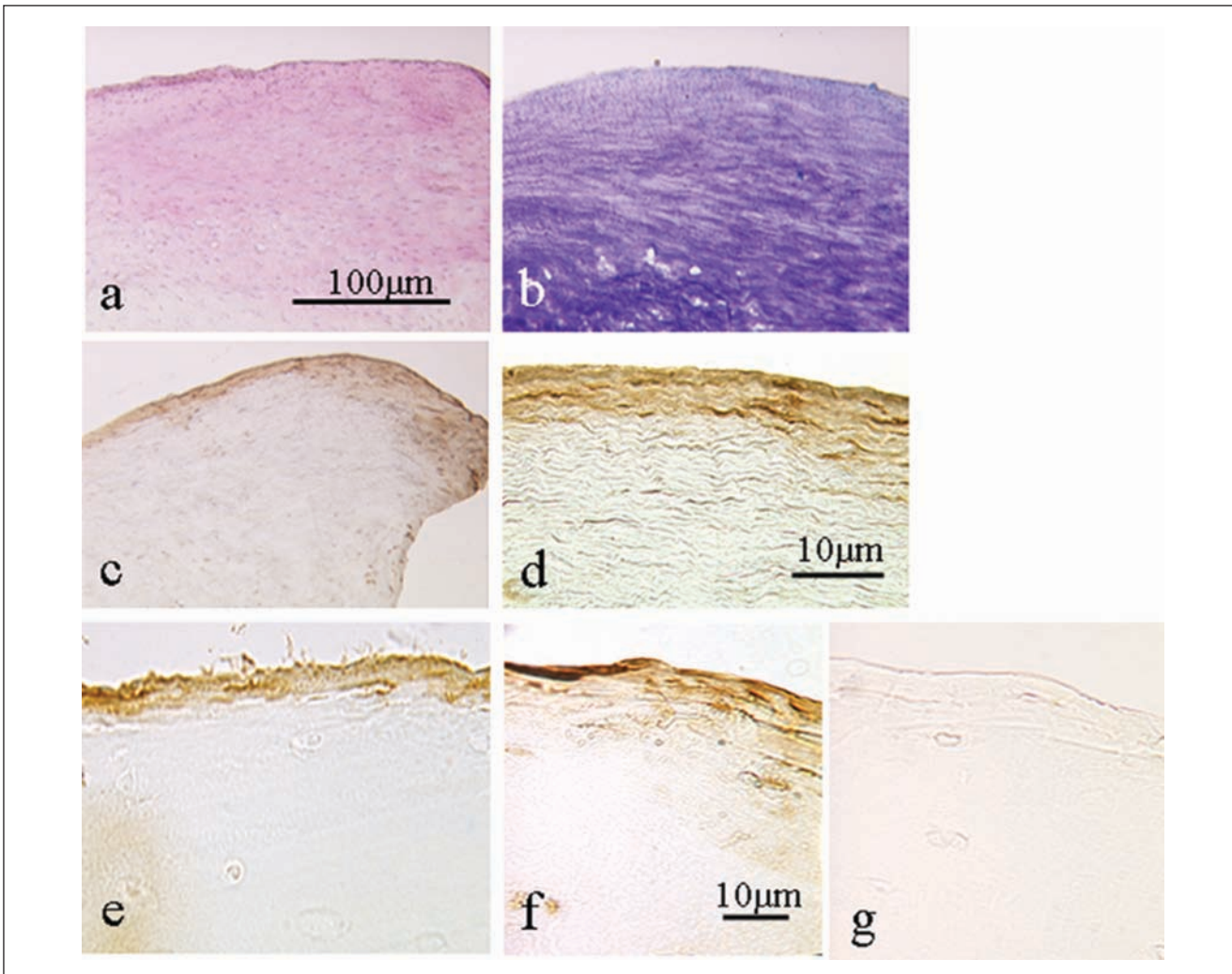


Figure 3. Repair tissue formed 13 months following autologous chondrocyte implantation in (a-d) of a 37-year-old man treated with a periosteal flap; sections stained with (a) hematoxylin and eosin, (b) toluidine blue, and (c, d) immunostained for lubricin. (a-c) Lower magnification and (d) higher magnification as shown by bar. Normal cartilage immunostained for lubricin from a 30-year-old (e) and a 40-year-old (f). (g) Adjacent control section labeled with rabbit IgG.

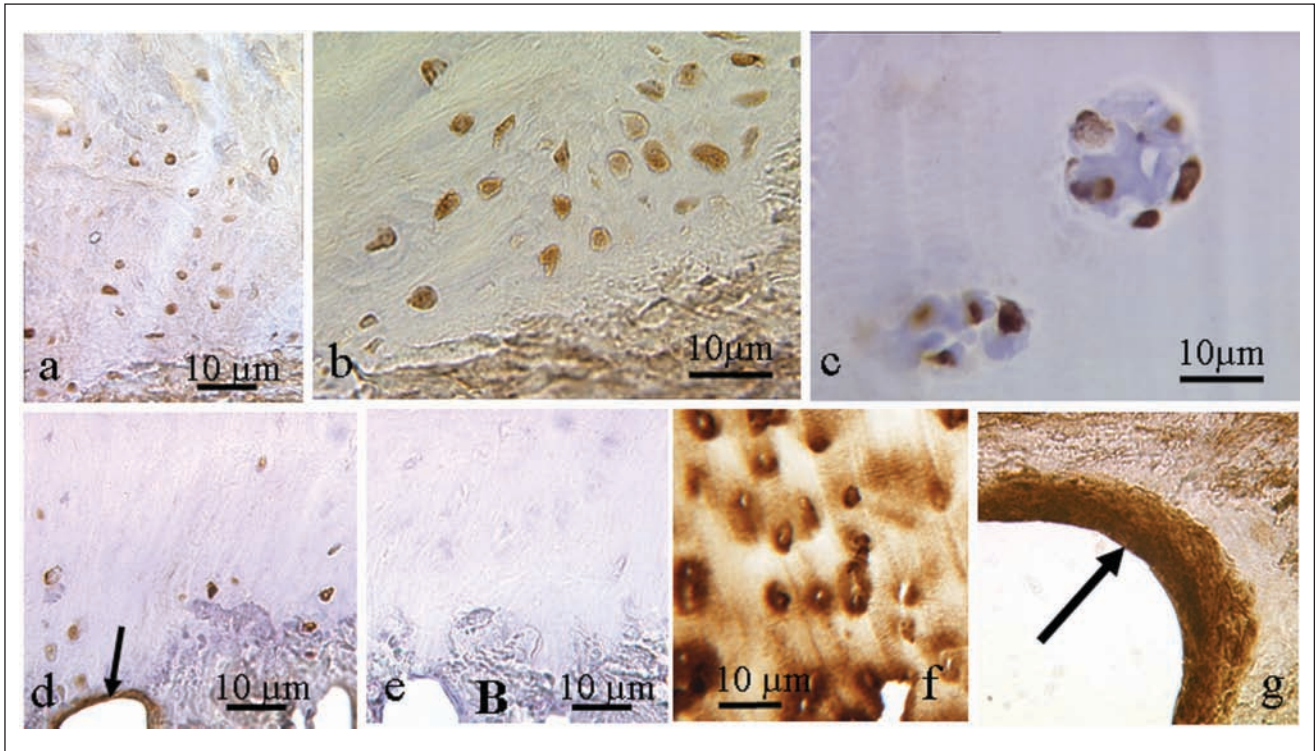


Figure 4. Repair tissue showing immunostaining for lubricin in cells in the deeper zones. This occurred in single cells ((a) 35-year-old woman treated with autologous chondrocyte implantation [ACI] and periosteum 13 months before, (b) 30-year-old man treated with ACI and periosteum 12 months before) but was particularly common in cell clusters (c) in tissue from a 44-year-old man treated with ACI and Chondro-Gide 15 months previously. Lubricin staining was also seen in the bone, in the osteoid (arrows) in tissue (g) from a 35-year-old man 13 months after ACI or (d) 41-year-old man treated with ACI and periosteum 35 months before; (e) adjacent section to (d) labeled with rabbit IgG. (f) Adjacent section to (b) immunostained for type II collagen showing positive staining around cells that were positive for lubricin B: bone. Sections were counterstained with hematoxylin.

Certainly, the coefficient of friction for articular cartilage increased from 0.06 N to 0.09 N following trypsin digestion, with the increase suggested as being due to the digestion and loss of the lubricin.²⁸ It is also thought that lubricin might protect the cartilage surface from cell adhesion¹⁹ and possible synovial overgrowth.⁹ Down-regulation of lubricin in the superficial zone of cartilage occurs as an early event in a sheep model of OA,¹⁴ which could reduce the chondroprotection lubricin normally offers. In the correct location, lubricin thus produces the desirable effect of preventing fusion of apposing surfaces in the articular joint. It might, however, also inhibit the lateral integration of fully formed cartilage, either *in vitro*^{29,30} or perhaps *in vivo* in the surgical treatment of mosaicplasty.

The significance of lubricin staining in the cells within the lower regions of the cartilage is unknown, but because the lubricin molecule has several other multifunctional motifs (e.g., somatomedin B and hemopexin-like domains), it can be involved in many distinctive biological processes

other than just reducing friction. Although there is undoubtedly some lubricin in the deeper regions of the tissue, the degree of immunostaining within the matrix is less than in the surface layer; this is similar to the *in vitro* production of lubricin reported to be much less by mid and deep cells compared with those from the surface.³¹ Interestingly, lubricin has recently been reported in the human intervertebral disc, with the greatest extent of immunostaining being seen in the central nucleus pulposus.³² We are also unaware of the significance of results from this study reporting lubricin immunostaining within the osteoid of bone, although lubricin previously has been reported to occur in the subchondral bone of human joints,³³ particularly within cartilaginous deposits.

This study is the 1st report to our knowledge of the presence of lubricin in repair tissue formed after ACI treatment. Although it occurred in patients treated with either periosteal or Chondro-Gide flaps, it was more consistently present in the surface zone of Chondro-Gide-treated

patients. This is despite these patients having larger defect areas than those treated with periosteum (and biopsies being taken on average slightly earlier posttreatment). We have not studied the degree and type of posttranslational modification of the lubricin in terms of glycosylation in the repair tissue. Variations in these could have important implications for function because they are known to influence and organize the location of water to support loading and shear stress.³⁴ Nonetheless, this study does demonstrate that repair tissue that forms after ACI treatment resembles normal articular cartilage in at least some of its properties, such as the occurrence of lubricin in the surface layer.

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Declaration of Conflicting Interests

The authors have no known conflict of interest, although C.R.F. is employed by Pfizer Inc.

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References

1. Brittberg M. Autologous chondrocyte implantation: technique and long-term follow-up. *Injury*. 2008;39:S40-S49.
2. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med*. 1994;331:889-95.
3. Dickinson SC, Sims TJ, Pittarello L, Soranzo C, Pavesio A, Hollander AP. Quantitative outcome measures of cartilage repair in patients treated by tissue engineering. *Tissue Eng*. 2005;11:277-87.
4. Mainil-Varlet P, Aigner T, Brittberg M, Bullough P, Hollander AP, Hunziker E, et al. Histological assessment of cartilage repair: a report by the histology endpoint committee of the International Cartilage Repair Society (ICRS). *J Bone Jt Surg*. 2003;85-A:45-57.
5. Roberts S, Menage J, Sandell LJ, Evans EH, Richardson JB. Immunohistochemical study of collagen types I and II and procollagen IIA in human cartilage repair tissue following autologous chondrocyte implantation. *Knee*. 2009;16(5):398-404.
6. Jones ARC, Gleghorn JP, Hughes CE, Fitz LJ, Zollner R, Wainwright SD, et al. Binding and localization of recombinant lubricin to articular cartilage surfaces. *J Orthop Res*. 2007;25:283-92.
7. Jay GD, Tantravahi U, Britt DE, Barrach HJ, Cha C-J. Homology of lubricin and superficial zone protein (SZP): products of megakaryocyte stimulating (MSF) gene expression by human synovial fibroblasts and articular chondrocytes localized to chromosome Iq25. *J Orthop*. 2001;19:677-87.
8. Swann DA, Silver FH, Slayter HS, Stafford W, Shore E. The molecular structure and lubricating activity of lubricin isolated from bovine and human synovial fluids. *Biochem J*. 1985;225:195-201.
9. Rhee DK, Marcelino J, Baker M, Gong Y, Smits P, Lefebvre V, et al. The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. *J Clin Invest*. 2005;115:622-31.
10. Jay GD, Torres JR, Rhee DK, Helminen HJ, Hytinen MM, Cha CJ, et al. Association between friction and wear in diarthrodial joints lacking lubricin. *Arthritis Rheum*. 2007;56:3662-9.
11. Wright V, Dowson D. Lubrication and cartilage. *J Anat*. 1976;121:107-18.
12. Radin EL, Paul IL. Joint function. *Arthritis Rheum*. 1970;13:276-79.
13. Elsaid KA, Jay GD, Chichester CO. Reduced expression and proteolytic susceptibility of lubricin/superficial zone protein may explain early elevation in the coefficient of friction in the joints of rats with antigen-induced arthritis. *Arthritis Rheum*. 2007;56:108-16.
14. Young AA, McLennan S, Smith MM, Smith SM, Cake MA, Read RA, et al. Proteoglycan 4 downregulation in a sheep meniscectomy model of early osteoarthritis. *Arth Res Ther*. 2006;8:R41.
15. Jones ARC, Flannery CR. Bioregulation of lubricin expression by growth factors and cytokines. *Eur Cells Mater*. 2007;13:40-5.
16. Flannery CR, Zollner R, Corcoran C, Jones AR, Root A, Rivera-Bermúdez MA, et al. Prevention of cartilage degeneration in a rat model of osteoarthritis by intraarticular treatment with recombinant lubricin. *Arthritis Rheum*. 2009;60:840-7.
17. Roberts S, McCall IW, Darby AJ, Menage J, Evans EH, Harrison PE, et al. Autologous chondrocyte implantation for cartilage repair: monitoring its success by magnetic resonance imaging and histology. *Arth Res Ther*. 2003;5:R60-R73.
18. Lysholm J, Gillquist J. Evaluation of knee ligament surgery results with special emphasis on use of a scoring scale. *Am J Sports Med*. 1982;10:150-4.
19. Schumacher BL, Hughes CE, Kuettner KE, Catterson B, Aydelotte MB. Immunodetection and partial cDNA sequence of the proteoglycan, superficial zone protein, synthesized by cells lining synovial joints. *J Orthop Res*. 1999;17:110-20.
20. Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Rel Res*. 2000;374:212-34.

21. Grad S, Lee CR, Gorna K, Gogolewski S, Wimmer MA, Alini M. Surface motion upregulates superficial zone protein and hyaluronan production in chondrocyte-seeded three-dimensional scaffolds. *Tissue Eng.* 2005;11:249-56.
22. Wong M, Siegrist M, Goodwin K. Cyclic tensile strain and cyclic hydrostatic pressure differentially regulate expression of hypertrophic markers in primary chondrocytes. *Bone.* 2003;33:685-93.
23. Rees SG, Davies JR, Tudor D, Flannery CR, Hughes CE, Dent CM, et al. Immunolocalisation and expression of proteoglycan 4 (cartilage superficial zone proteoglycan) in tendon. *Matrix Biol.* 2002;21:593-602.
24. Taguchi M, Zhao C, Sun YL, Jay YL, An KN, Amadio PC. The effect of surface treatment using hyaluronic and lubricin on the gliding resistance of human extrasynovial tendons *in vivo*. *J Hand Surg.* 2009;34:1276-81.
25. Funakoshi T, Martin SD, Schmid TM, Spector M. Distribution of lubricin in the ruptured human rotator cuff and biceps and tendon: a pilot study. *Clin Orthop Relat Res.* Epub ahead of print, October 2, 2009.
26. Schumacher BL, Schmidt TA, Voegtline MS, Chen AC, Sah RL. Proteoglycan 4 (PRG4) synthesis and immunolocalization in bovine meniscus. *J Orthop Res.* 2005;23:562-8.
27. Shine KM, Spector M. The presence and distribution of lubricin in the caprine intervertebral disc. *J Orthop Res.* 2008;26:1398-406.
28. Sun Y, Chen M-Y, Zhao C, An K-N, Amadio PC. The effect of hyaluronidase, phospholipase, lipid solvent and trypsin on the lubrication of canine flexor digitorum profundus tendon. *J Orthop Res.* 2008;26:1225-9.
29. Schaefer DB, Wendt D, Moretti M, Jakob M, Jay GD, Heberer M, et al. Lubricin reduces cartilage—cartilage integration. *Biorheology.* 2004;41:503-8.
30. Englert C, McGowan KB, Klein TJ, Giurea A, Schumacher B, Sah RL. Inhibition of integrative cartilage repair by proteoglycan 4 in synovial fluid. *Arthritis Rheum.* 2005;52:1091-9.
31. Niikura T, Reddi AH. Differential regulation of lubricin/superficial zone protein by transforming growth factor beta/bone morphogenetic protein superfamily members in articular chondrocytes and synoviocytes. *Arthritis Rheum.* 2007;56:2312-21.
32. Shine KM, Simpson JA, Spector M. Lubricin distribution in the human intervertebral disc. *J Bone Joint Surg Am.* 2009;91:2205-12.
33. Zhang D, Johnson LJ, Hsu H-P, Spector M. Cartilaginous deposits in subchondral bone in regions of exposed bone in osteoarthritis of the human knee: histomorphometric study of PRG4 distribution in osteoarthritic cartilage. *J Orthop Res.* 2007;25:873-87.
34. Jay GD, Harris DA, Cha C-J. Boundary lubrication by lubricin is mediated by O-linked β (1-3) Gal-GalNAc oligosaccharides. *Glycoconj J.* 2001;18:807-15.