Surgical Removal of Articular Cartilage Leads to Loss of Chondrocytes from Cartilage Bordering the Wound Edge

Ernst B. Hunziker and Thomas M. Quinn


This information is current as of August 13, 2007

**Reprints and Permissions**  
Click here to [order reprints or request permission](#) to use material from this article, or locate the article citation on [jbjs.org](http://www.jbjs.org) and click on the [Reprints and Permissions] link.

**Publisher Information**  
The Journal of Bone and Joint Surgery  
20 Pickering Street, Needham, MA 02492-3157  
[www.jbjs.org](http://www.jbjs.org)
Surgical Removal of Articular Cartilage Leads to Loss of Chondrocytes from Cartilage Bordering the Wound Edge

By Ernst B. Hunziker, MD, and Thomas M. Quinn, PhD

Background: A number of arthroscopic procedures that are used in the treatment of focal cartilage lesions or osteoarthritic joints, such as shaving, débridement, and laser abrasion, involve the removal of both diseased and healthy articular cartilage. The excision of such tissue has the effect of generating lesions within the articular cartilage. The fate of the chondrocytes that border such lesions has not been evaluated. The purpose of this investigation was to ascertain whether the surgical creation of lesions in articular cartilage induces irreversible loss of chondrocytes over time from tissue bordering the wound edge and to determine whether the synthetic activity of cells in this region is compromised.

Methods: Partial-thickness defects of defined dimensions were created in the femoral condyle and/or trochlear groove of rabbits and miniature pigs. Cell volumes, cell volume densities, and numerical cell densities within tissue close to (within 100 µm) and remote from (control site) the wound edge were determined by quantitative histomorphometry at various time intervals up to six months after surgery. Rates of proteoglycan synthesis by cells in both regions were determined by quantitative autoradiography following \(^{35}\)S-sulphate labeling in vivo.

Results: The surgical creation of partial-thickness lesions in articular cartilage induced a significant and long-term loss of cells from tissue near the wound edge. However, the surviving cell population maintained a normal rate of matrix proteoglycan deposition.

Conclusions: This study illustrates that maintenance and remodeling of cartilage matrix close to wound edges in articular cartilage lesions is compromised, since fewer cells, with an unchanged metabolic activity rate, are left to sustain matrix domains.

Clinical Relevance: The long-term benefits of arthroscopic treatments such as shaving, débridement, and laser abrasion may be deleteriously affected by their adverse biological effects on the cells and matrix of articular cartilage.

In surgical practice, the removal of articular cartilage by débridement, shaving, or laser abrasion is widely used in the treatment of osteoarthritic diseases\(^1-3\). The surgical preparation of chondral or osteochondral lesions for the receipt of transplanted tissue plugs also involves the incision of healthy articular cartilage\(^4\).

It has not been shown previously whether surgical incisions in adult articular cartilage are associated with deleterious effects such as the loss of chondrocytes from tissue bordering the wound edge, nor is it known whether therapeutic excision of tissue elicits a beneficial repair response. Several previous studies, including our own, have demonstrated an apparent decrease in cellularity within tissue bordering articular cartilage defects\(^5-9\). Furthermore, Walker et al.\(^10\) demonstrated, in cultured fetal cartilage, that chondrocytes in the vicinity of surgically created lesions undergo apoptosis without subsequent replacement.

The purpose of the current investigation was to determine quantitatively in vivo in rabbits and in Goettingen miniature pigs whether the surgical removal of tissue from adult articular cartilage is associated with a long-term loss of cells from the wound edge. We also determined whether matrix proteoglycan synthesis in the remaining chondrocytes near the wound edge was impaired, sustained, or enhanced compared with that in chondrocytes of articular cartilage remote from the wound.

Materials and Methods

Animals, Surgery, and Tissue Processing

Twenty-four adult rabbits (Red Burgundy) and six adult Goettingen miniature pigs were used. In the rabbits, general anesthesia was induced and was maintained with ketamine. Following surgical exposure of both knee joints, a 0.15-mm-deep partial-thickness defect (1 mm in width × 8 to
10 mm in length) was created (Fig. 1) in each facet of the patellar groove and in the medial femoral condyle with use of a custom-built planing instrument (Rolf Haenggi Engineering, Grenchen, Switzerland). The joints were closed layer by layer, and the animals were permitted free cage movement until they were killed. Rabbits were killed with an overdose of anesthetic at two weeks (n = 4), four weeks (n = 4), six weeks (n = 4), three months (n = 6), and six months (n = 6). Each rabbit received an intra-articular injection in both knee joints of 35S-sulphate (350 µCi in 0.5 mL of physiological saline solution, Amersham [Rahn], Zurich, Switzerland), for \textit{in vivo} radiolabeling of newly synthesized proteoglycans, two and four days before it was killed. The dual injection served as a precautionary measure against inadequate delivery of 35S-sulphate to chondrocytes in the vicinity of the lesion.

In the adult Goettingen miniature pigs (two to four years of age; body weight, 36.3 to 54.4 kg), general anesthesia was induced with ketamine and was maintained by the delivery of a halothane/oxygen mixture. Only one knee joint per animal was exposed, and a 0.5-mm-deep partial-thickness defect (0.5 mm in width \times 8 to 10 mm in length) was then created in each facet of the femoral groove. The exposed joint was closed layer by layer, and animals were permitted free cage movement until they were killed. All six miniature pigs were killed with an overdose of potassium chloride to induce cardiac arrest under general anesthesia, eight weeks after surgery.

Following the death of the animal, the soft tissues were removed and the osseous specimens were trimmed into 10 \times 15-mm osteochondral blocks containing the surgically created lesions. Tissue blocks derived from rabbit knee joints were fixed in a 2.5% glutaraldehyde solution (buffered with 0.1 M Tris-phosphate, pH 7.4) containing 2.5% cetylpyridinium chloride for three to four hours at ambient temperature. They were then rinsed in an isotonic Tris-phosphate buffer, dehydrated in ethanol, and embedded in Epon 812 (Fluka Chemie GmbH, Buchs, Switzerland). Tissue blocks derived from miniature-pig knee joints were fixed in 2.5% formaldehyde solution (buffered with 0.1 M sodium cacodylate, pH 7.4) for three to four hours at ambient temperature. They were then rinsed in an isotonic sodium cacodylate buffer, dehydrated in ethanol, and embedded in methacrylate.

\textbf{Tissue Sampling and Stereology}

For morphometric analyses, 1-mm-thick serial saw cuts were prepared from each tissue block according to a systematic random-sampling protocol\textsuperscript{13}, with a random start at the proximal end of the lesion. Four or five slices were thereby produced along the length of each lesion. From each slice, one semi-thin section was prepared, stained with 1% toluidine blue O, and photographed in the light microscope. Articular cartilage tissue that was within 100 µm of the lesion wall was defined as being near the wound edge (Fig. 1). The cell volume density (Vv) within this zone and within tissue further removed (100 to 200 µm away [control site]) from the wound edge was estimated by point counting\textsuperscript{14,15} on light micrographs. Mean chondrocyte volumes (v[c]) were estimated according to the nucleator method\textsuperscript{16,17}. Light-microscopic images of tissue near the
and remote from the wound edge were recorded (Fig. 1), were digitized, and were sampled systematically. The number of cells per unit volume of tissue (Nv) was derived indirectly from Nv = Vv/v(c).

To investigate the possibility that changes in numerical cell density were attributable to swelling of tissue near the wound edges, all sections were systematically inspected for bulging of the defect borders into the lesion void and for a reduction in staining intensity (indicative of increased water content and decreased proteoglycan density) relative to that in control-site sections.

For quantitative autoradiography, semi-thin sections were exposed to a Kodak NTB-2 emulsion for three weeks, after which they were developed and then stained with 0.01% toluidine blue. In radiolabeled specimens, silver grains around chondrocytes near and far removed from the wound edge were counted by means of an automated image analysis. In specimens derived from the six-week rabbit group, silver-grain densities within concentric, 1-µm-broad annuli, radiating outward from the remaining chondrocytes, were additionally calculated in zones near and remote from the wound edge. As the concentration of 35S-sulphate reaching cells during in vivo labeling was expected to vary, the grain density distributions were normalized to the tissue-average values. These tissue-average values represent the local rates of proteoglycan synthesis, whereas the normalized grain distributions reflect the pericellular pattern of proteoglycan deposition as a function of distance from the plasma membrane.

For each measured parameter, the significance of differences between values derived from tissue zones near and remote from the wound edge was then estimated with use of the Student t test. Values of $p < 0.05$ were considered to be significant.

**Results**

Figure 2, A illustrates a typical partial-thickness defect created within the articular cartilage layer of the rabbit patellar groove. Chondrocytes within a 100-µm radius of the wound edge were morphologically indistinguishable from those that were further removed from it. However, cellularity in the former zone (Fig. 2, B) was clearly decreased compared with that in the latter (Fig. 2, C). These qualitative impressions were confirmed by quantification of the mean cell volume, which did not differ between the two areas (Fig. 3), and of cell volume density (Fig. 4) and numerical cell density (Fig. 5), both of which were lower in the zone closest to the wound edge. Cell volume density in each zone was lowest at two weeks after surgery, but significant differences between the two regions were maintained throughout the six-month follow-up period ($p < 0.01$ for all time points).

Although very similar trends were apparent for numerical cell density in the rabbit groups, the differences between the two tissue areas were not significant at four weeks after surgery (Fig. 5) or at later time points. This latter circumstance is readily explained. Numerical cell density was estimated indirectly from the quotient $Vv/v(c)$. The standard errors of the means for numerical cell density were thus compounded from those for cell volume density and cell volume; if these latter were large—which they were in both the rabbit and the miniature pig (see below)—then those for numerical cell density were even larger. Hence, unless differences be-

---

**Fig. 2**

A: Light micrograph of a superficial defect created in the femoral groove of an adult rabbit. The defect void is confined to the layer of hyaline articular cartilage; it does not penetrate the subchondral bone plate. AC = articular cartilage, CC = calcified cartilage, and arrowheads = defect surface.

B: High-magnification view of chondrocytes near the wound edge (arrowheads) six weeks after surgery. C: High-magnification view of an internal control site. The numerical density of chondrocytes is lower in the vicinity of the lesion wall (B) than in more remote regions (C). The 35S-sulphate autoradiographic grain densities, which represent the proteoglycan synthesis activity states of chondrocytes, are the same around individual cells at both sites (B and C). Scale bars: A = 100 µm; B and C = 50 µm.
Surgical Removal of Articular Cartilage Leads to Loss of Chondrocytes from Cartilage Bordering the Wound Edge

**Fig. 3**
Mean volumes of chondrocytes near (within 100 µm) and remote from (control site) superficial defects in rabbit articular cartilage as a function of time after surgery. Mean ± SEM; n = 19 (two weeks), 20 (four weeks), 35 (six weeks), 17 (three months), and 18 (six months).

**Fig. 4**
Volume fractions of chondrocytes (volume of cells per unit volume of tissue) near (within 100 µm) and remote from (control site) superficial defects in rabbit articular cartilage as a function of time after surgery. Mean ± SEM; n = 48 (two weeks), 24 (four weeks), 42 (six weeks), 18 (three months), and 18 (six months). Significant differences existed (p < 0.01) between all groups.

**Fig. 5**
Numerical density of chondrocytes (number of cells per unit volume of tissue) near (within 100 µm) and remote from (control site) superficial defects in rabbit articular cartilage as a function of time after surgery. Mean ± SEM; n = 42 (two weeks), 20 (four weeks), 35 (six weeks), 17 (three months), and 18 (six months). The values differed significantly (p < 0.01) between the groups at two weeks but not at the remaining points of time.
between means were very marked, they would not attain significance until a very large sample size was acquired.

Systematic inspection of sections revealed neither bulging of the defect borders into the lesion void nor a reduction in the intensity of staining with toluidine blue O in the region near the wound. Hence, no evidence of a tissue-swelling phenomenon was observed.

In the larger-animal model (Goettingen miniature pig), estimation of the same parameters (Figs. 6, 7, and 8) revealed a picture similar to that manifested in the rabbit, although differences in cell volume density and numerical cell density between the two zones were more pronounced. In particular, differences in numerical cell density were significant in the miniature pig, indicating very strong trends toward cell loss in this large-animal model (Fig. 8).

In the rabbit group, quantitative autoradiographic analysis of silver-grain densities around chondrocytes within 100 µm of the wound edge and in the zone remote from the wound edge revealed no differences around individual cells between the two regions (Fig. 9). This finding indicates that the synthetic activity of the remaining, viable cells near the wound edge was not impaired, at least with respect to the pro-
Surgical Removal of Articular Cartilage Leads to Loss of Chondrocytes from Cartilage Bordering the Wound Edge

Production and deposition of proteoglycans. Specimens derived from the six-week rabbit group were subjected to a more detailed analysis, involving estimation of silver-grain density as a function of distance (up to 15 µm) from the chondrocyte plasma membrane (Fig. 10). The analysis revealed an expected decrease in density with increasing distance from the cell surface. The autoradiographic grain distributions recorded for the remaining viable chondrocytes located near the wound edge did not differ from those of cells remote from the wound edge.

Discussion

Histologic examination of articular cartilage tissue within 100 µm of surgically created lesions and of articular cartilage remote from this zone (control site) revealed no differences in either the morphology or the size of individual cells, this latter impression being confirmed by morphometric estimation of the chondrocyte volume. Visual inspection did, however, disclose a difference in cellularity between the two regions, the cellularity being obviously decreased within tissue near the wound edge. Morphometric data confirmed that a significant number of cells had been lost from the zone near the wound edge, the effect being more pronounced in the miniature pig than in the rabbit. As neither bulging of the defect borders into the lesion void nor a measurable loss of proteoglycans occurred, tissue-swelling phenomena were not evident. The implication of this finding is that the reduction in chondrocyte number near the wound edges represents a true loss of cells over long periods.

Whether this cell loss is the result of simple necrosis or of apoptosis remains unclear, since a clear distinction between these two mechanisms cannot be made by mere morphologic inspection. The considerable length of time that ensued after surgery before tissue was analyzed (several weeks) precluded further investigation of the mechanisms underlying chondrocyte loss, since by this juncture all traces of erstwhile cells had disappeared. However, there are indications that apoptosis accounts for the cell loss that occurs after the creation of lesions in cultured fetal sternal cartilage.

Quantitative autoradiographic data revealed no significant difference in the matrix synthesis activity levels between chondrocytes near the wound edge and those located farther away. The patterns of deposited proteoglycans associated with individual chondrocytes were likewise similar in each of the two regions. The implication of these findings is that the surviving chondrocytes in the vicinity of the wound edge function normally. The fluctuations in absolute activity levels observed with time probably reflect differences in the doses of 35S-sulphate reaching the lesion sites. That the absolute numerical densities of silver grains counted close to and farther away from the wound edge changed in parallel at each sampling point supports this interpretation (not shown). Indeed, the relatively high viscosity of the synovial fluid does not favor the rapid and homogeneous distribution of any substance injected intra-articularly.

As a significant number of chondrocytes were lost from tissue near the wound edge and the synthetic activity level of the surviving ones remained apparently unchanged, it follows that matrix deposition rates in this region were possibly lower than normal. In any case, it is certain that the surviving cells near the defect become responsible for the maintenance of an abnormally large volume of matrix. Hence, the surgical removal of articular cartilage tissue not only fails to have a beneficial biological effect—that is, it does not induce spontaneous repair—but also has a detrimental one. The surgical removal of articular cartilage tissue from large osteochondral
Surgical removal of articular cartilage leads to loss of chondrocytes from cartilage bordering the wound edge.

**Fig. 9**
Mean density of $^{35}$S-sulphate autoradiographic grains in association with chondrocytes (a measure of proteoglycan synthesis and extracellular deposition rates at the tissue level) near (within 100 µm) and remote from (control group) superficial defects in rabbit articular cartilage as a function of time after surgery. Mean ± SEM; n = 19 (two weeks), 20 (four weeks), 35 (six weeks), 17 (three months), and 18 (six months).

**Fig. 10**
Density of $^{35}$S-sulphate autoradiographic grains as a function of distance from the plasma membrane of chondrocytes (a measure of proteoglycan synthesis and pericellular deposition patterns at the cell level) near (within 100 µm) and remote from (control site) superficial defects in rabbit articular cartilage six weeks after surgery. Mean ± SEM; n = 16.
defects, which is undertaken to improve contiguity between
the wall of a pathological lesion and the transplanted material,
is understandable and reasonable, despite the biological disad-
vantages. However, surgical therapies whose sole purpose is to
improve the biological condition of the remaining tissue by
excision, such as débridement, shaving, and laser abrasion²,³,
are clearly detrimental to the biological microenvironment of
chondrocytes.

Corresponding author:
Ernst B. Hunziker, MD

References

1. Bert J.M. Role of abrasion arthroplasty and débridement in the management
2. Bert J.M., Maschka K. The arthroscopic treatment of unicompartmen-
tal gonarthrosis: a five-year follow-up study of abrasion arthroplasty plus arthroscopic
debriement and arthroscopic débridement alone. Arthroscopy. 1989;
   5:25-32.
3. Glossop ND, Jackson RW, Koort HJ, Reed SC, Randle JA. The excimer laser
4. Goldman RT, Scuderi GR, Kelly MA. Arthroscopic treatment of the degenera-
5. McLaren AC, Blokker CP, Fowler PJ, Roth JN, Rock MG. Arthroscopic debr-
6. Hunziker EB. Articular cartilage repair: are the intrinsic biological constraints
    undermining this process insuperable? Osteoarthritis Cartilage. 1999;
    7:15-28.
7. Calandruccio RA, Gilmer WS Jr. Proliferation, regeneration, and repair of
    articular cartilage of immature animals. J Bone Joint Surg Am. 1962;44:
    431-55.
8. Shapiro F, Koide S, Glimcher MJ. Cell origin and differentiation in the repair
    75:532-53.
    Press; 1979.
    Immunolocalization of matrix metalloproteinase in partial-thickness defects
    83:B20-38.
11. Walker EA, Verner A, Flannery CR, Archer CW. Cellular responses of embry-
    18:25-34.
12. Hunziker EB, Rosenberg LC. Repair of partial-thickness defects in articular
cartilage: cell recruitment from the synovial membrane. J Bone Joint Surg
13. Gundersen HJ, Jensen EB. The efficiency of systematic sampling in stereol-
15. Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Ny-
    engaard JR, Pakkenberg B, Sorensen FB, Vesterby A, et al. Some new, sim-
ple and efficient stereological methods and their use in pathological research
16. Gundersen HJ, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N,
    Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, et al. The new stereologi-
cal tools: disector, fractionator, nucleator and point sampled intercepts and
    their use in pathological research and diagnosis. APMIS. 1989;96:857-81.
17. Moller A, Strange P, Gundersen HJ. Efficient estimation of cell volume and
18. Quinn TM, Grodzinsky AJ, Buschmann MD, Kim YJ, Hunziker EB. Mechani-
    cal compression alters proteoglycan deposition and matrix deformation
19. Buschmann MD, Maurer AM, Berger E, Hunziker EB. A method of quantita-
tive autoradiography for the spatial localization of proteoglycan synthesis
20. [Apoptosis: biological and clinical significance. Italian Society of Experi-
21. Tew SR, Kwan AP, Hann A, Thomson B, Archer CW. The reactions of articu-
22. Hunziker EB, Kapfinger E. Removal of proteoglycans from the surface of de-
    fects in articular cartilage transiently enhances coverage by repair cells. J