

## In Vivo Changes After Mechanical Injury

*Clifford W. Colwell, Jr., MD; Darryl D. D'Lima, MD;  
Heinz R. Hoenecke, MD; Jan Fronck, MD; Pamela Pulido, RN;  
Beverly A. Morris, RN; Christine Chung, MD;  
Donald Resnick, MD; and Martin Lotz, MD*

Chondrocytes undergo apoptosis in response to mechanical injury in vitro. The current clinical study correlates arthroscopic and magnetic resonance imaging results with biopsy specimens of cartilage from patients with knee injury. Twenty patients were evaluated at a mean 2.7 months after acute knee injury. The mean age of the patients was 32 years and the mean weight was 83 kg. Cartilage lesions were graded separately on magnetic resonance images and arthroscopy in a blinded manner. During arthroscopy, a 1.8 mm diameter biopsy specimen was obtained from the edge of cartilage lesion. The biopsy specimen underwent histologic examination by safranin O staining and detection of chondrocyte apoptosis by the presence of deoxyribonucleic acid fragmentation. There was a positive correlation in 50% (10 of 20) when the presence or absence of cartilage lesions by magnetic resonance imaging was correlated with arthroscopy. All cases of partial thickness or full-thickness cartilage loss that were seen by arthroscopy also were detected by magnetic resonance images. Apoptotic cells were significantly more numerous in biopsy specimens from lesions compared with control biopsy specimens. The findings of reduced cell

viability attributable to apoptosis may have profound implications for cartilage repair. This opens potential therapeutic avenues for the treatment of posttraumatic cartilage lesions through apoptosis prevention.

### List of Abbreviations Used

DNA deoxyribonucleic acid

Cartilage injury is one of the most significant factors leading to secondary osteoarthritis. The consequences in vivo and the long-term effects have yet to be documented adequately. The current study was designed to explore the arthroscopic, histologic, and magnetic resonance imaging (MRI) findings after acute joint trauma and determine correlations between the above.

Several studies have shown cartilage matrix damage and chondrocyte death in response to mechanical injury.<sup>3,11</sup> Recently, it has been shown that chondrocytes undergo apoptosis when subjected to mechanical trauma.<sup>4,10,21</sup> Cartilage from various sources displayed a similar response to mechanical injury. Three different modes of injury were modeled in the above studies: injurious compression, blunt impact, injury leading to loss of cartilage, and chronic injury attributable to instability. Three species were tested: rabbit, bovine, and human. In addi-

From the Division of Orthopaedic Surgery, Scripps Clinic, La Jolla, CA.

Funded by the ALSAM Foundation: Skaggs Institute for Research.

Reprint requests to Clifford W. Colwell, Jr, MD, Scripps Clinic, MS126, 11025 North Torrey Pines Road, Suite 140, La Jolla, CA 92037.

tion, the apoptotic response was seen in experiments ranging from full-thickness cartilage to intact joints. These studies have been done in vitro under artificial conditions or in vivo in animals. The theory that cartilage injury results in apoptosis requires validation with clinical data. The current study determined whether cartilage lesions associated with acute joint injury showed evidence of apoptosis.

Data exist to support the theory that apoptosis can be inhibited in vitro and in vivo.<sup>20</sup> This opens possibilities of alternative therapeutic approaches to chondroprotection. Chondrocyte and matrix responses to mechanical injury are significant factors in determining the development, history, and treatment of cartilage lesions. A recent approach to the treatment of cartilage lesions is chondroprotection. Chondroprotection involves the use of therapeutic measures, usually pharmacologic agents, that maintain matrix integrity and retard the development and progression of cartilage lesions. Several chondroprotective agents (such as glucosamine and its derivatives, chondroitin sulfate, and hyaluronic acid) currently are being investigated and theoretically have the potential of reducing cartilage degeneration or enhancing repair after significant injury. Accurate noninvasive markers of cartilage damage and repair therefore would be valuable in documenting and validating the effect of various therapeutic approaches.

## MATERIALS AND METHODS

Twenty patients were recruited after appropriate Institutional Board Review approval. Inclusion criteria were age between 18 and 45 years, acute knee injury within the previous 6 months, and clinical or MRI evidence of acute joint injury necessitating an arthroscopy. Exclusion criteria were intraarticular bone fracture, preexisting joint lesions, chronic symptoms, history of previous trauma or surgery, or local anatomic deformity. There were 17 men and three women. The mean age of the patients was 32 years (range, 19–43 years) and the mean weight was 83 kg (range, 66–125 kg). Sixteen patients sustained the knee injury during sporting activities, three reported a fall, and one sustained the injury during a

routine activity of daily living. The mean time after injury was 2.7 months (range, 1–6 months).

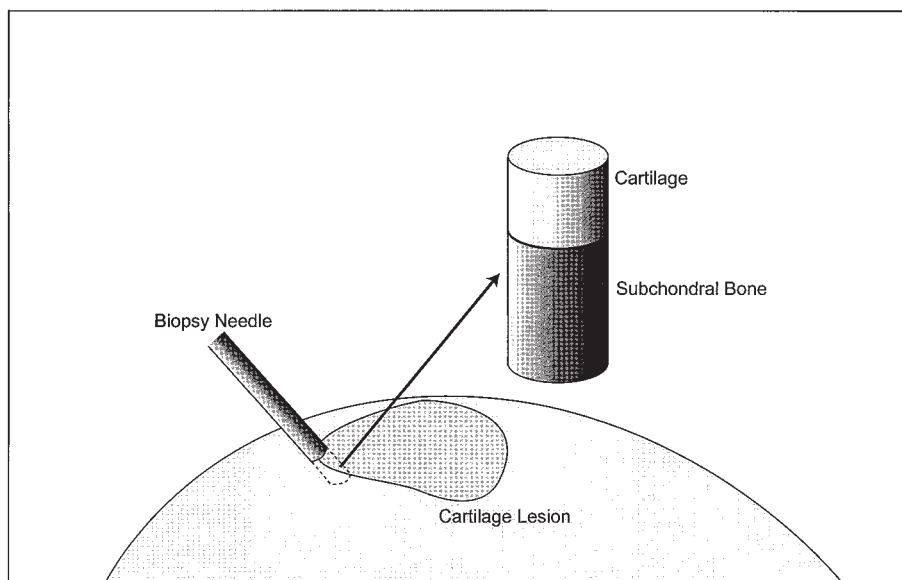
Patients underwent routine diagnostic arthroscopy as indicated by their clinical examination and initial clinical MRI findings. Before this diagnostic arthroscopy, patients underwent an additional MRI examination that included proton density fat saturated fast spin echo and three-dimensional spoiled gradient echo of the affected knee. The presence of any cartilage lesion including the size and the grade (ranging from signal heterogeneity to full-thickness loss of cartilage) was observed. The presence and grade of bone marrow edema also was documented.

During arthroscopy, the surgeon did a routine exploration of the joint cavity and recorded abnormal findings and was unblinded to the results of the research MRI. At this time, the surgeon obtained a 1.8-mm diameter biopsy specimen at the edge of any cartilage lesion if present (Figure 1). An 11-gauge Jamshidi bone marrow biopsy needle (Allergiance Healthcare Corp, McGaw Park, IL) was used. The surgeon then did any relevant surgical therapeutic procedures necessary such as debridement and lavage, meniscal excision or repair, and anterior cruciate ligament reconstruction. Cartilage lesions were graded on MRI and arthroscopic findings as shown in Table 1.

The biopsy specimen was fixed in 10% buffered formalin, decalcified and examined histologically after safranin O staining and detection of DNA fragmentation. The safranin O-stained sections were graded using the histologic scoring system of Mankin et al,<sup>12</sup> which semiquantitatively grades structural changes, cellular density, safranin O stain intensity, and tidemark integrity. Apoptotic cells were detected using the Mebstain kit (MBL, Nagoya, Japan) that uses a fluorescent labeled antibody to DNA fragments. Nonapoptotic cells were counterstained with propidium iodide. The number of apoptotic cells divided by the total number of cells present yielded the percent apoptosis in that section. Control biopsy specimens were obtained from the femoral condyles of four fresh cadaver donors and from the intercondylar notch of four patients (before notchplasty) who required anterior cruciate ligament reconstruction.

## RESULTS

All patients successfully underwent the anesthesia, arthroscopic procedures, and cartilage



**Fig 1.** The biopsy technique is shown. An 1.8-mm diameter core of cartilage and subchondral bone was harvested from the edge of the cartilage lesions using an 11-gauge Jamshidi bone marrow biopsy needle.

biopsy without any adverse events. Twelve patients required debridement and lavage of the joint, eight required meniscal surgery, and 11 required anterior cruciate ligament reconstruction. The MRI findings are summarized in Table 2. Representative images of various grades of cartilage lesions are shown in Figure 2. There were 13 patients with evidence of bone marrow edema, 11 in whom the edema was adjacent to overlying cartilage lesions, and two in whom there were no detectable cartilage lesions. Figure 2F shows a patient with bone marrow edema without any overlying

cartilage lesion. The arthroscopic findings are summarized in Table 3. Ten patients had visible cartilage lesions on the lateral femoral condyle, two in the medial femoral condyle, two in the lateral tibial plateau, one in the medial tibial plateau, and one in the patella. In four of the 20 patients, no significant lesion was found. Figure 3 shows arthroscopic photographs representative of the different grades of cartilage lesions.

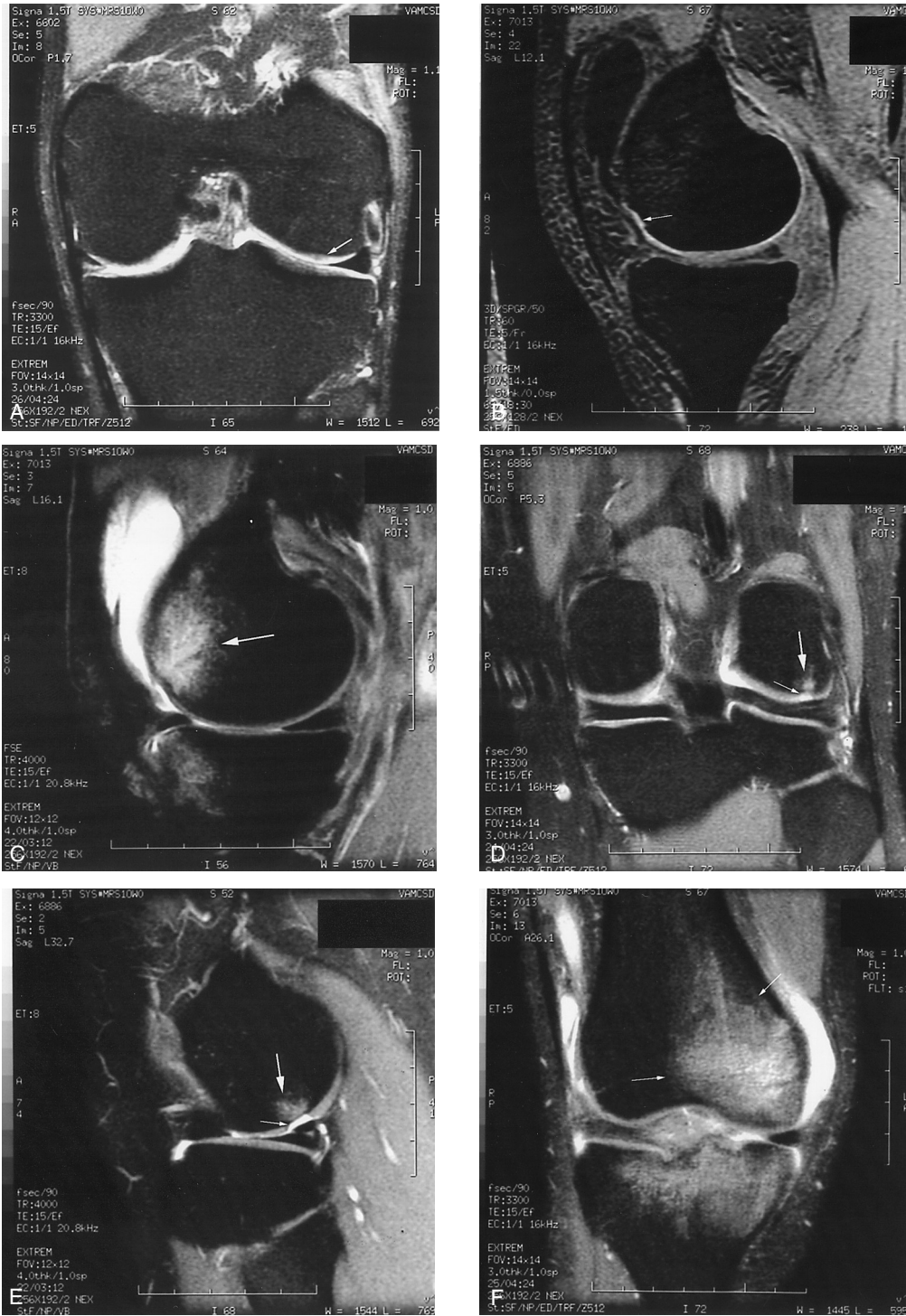
When the presence or absence of cartilage lesions by MRI was correlated with arthroscopy, there was a positive correlation in 50% (10 of

**TABLE 1. Grading of Cartilage Lesions**

Grade	MRI	Arthroscopy
I	Signal heterogeneity	Softening
II	Fraying, fissuring	Fraying, fissuring
III	Partial loss of cartilage thickness	Partial loss of cartilage thickness
IV	Complete loss of cartilage thickness	Complete loss of cartilage thickness

**TABLE 2. Magnetic Resonance Imaging Lesions**

Grade	Number of Patients
I	8
II	4
III	1
IV	1
Bone marrow edema without cartilage lesion	2
No detectable lesion	4



**Fig 2A–F.** Magnetic resonance images showing different locations and grades of cartilage lesions. (A) Proton density fat saturated fast spin echo image showing signal heterogeneity (arrow) suggestive of a Grade I lesion in the lateral femoral condylar cartilage; (B) Three-dimensional spoiled gradient echo image showing partial-thickness (Grade III) cartilage loss (arrow) in the anterior femoral condyle; (C) Proton density fat saturated fast spin echo image of the same section as shown in Figure 2B, highlighting the area of bone marrow edema (arrow); (D) Proton density fat saturated fast spin echo image showing full-thickness (Grade IV) cartilage loss (small arrow) associated with a small area of bone marrow edema (large arrow) in the coronal plane; (E) The same lesion as shown in Figure 2D, in sagittal section (small arrow points to cartilage lesion, large arrow points to bone marrow edema); and (F) Large area of bone marrow edema (arrows) not associated with any obvious overlying cartilage lesion.



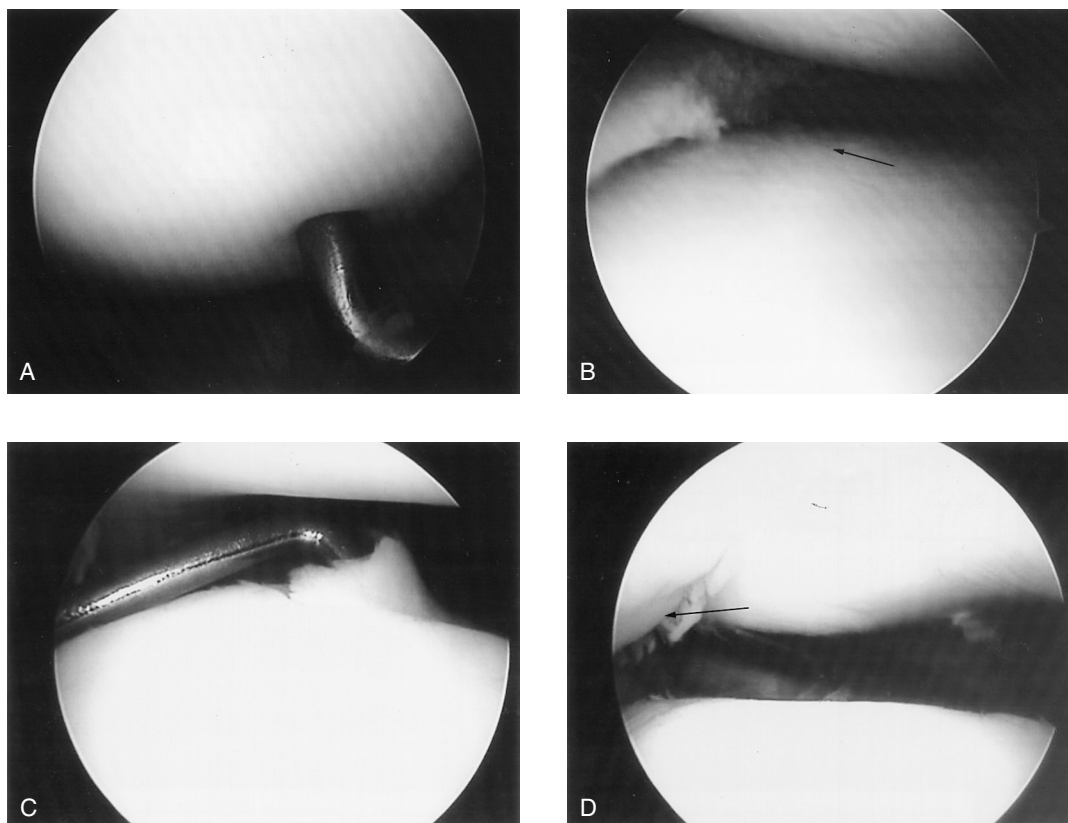
**TABLE 3. Arthroscopic Lesions**

Grade	Number of Patients
I	5
II	7
III	3
IV	1
No detectable lesion	4

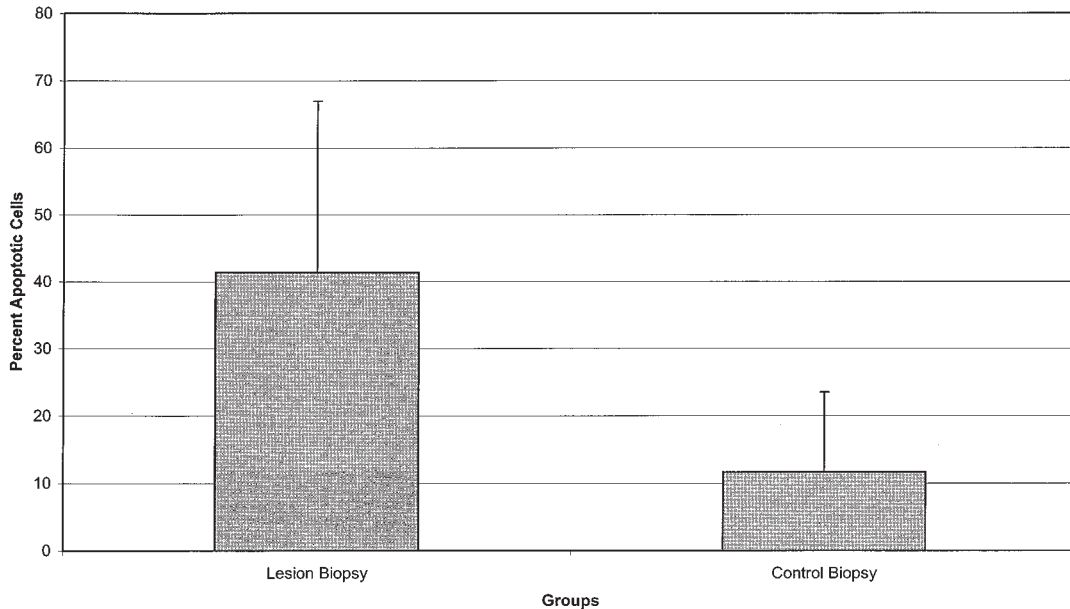
20). When the presence of bone marrow edema without overlying cartilage lesions was included, the number of MRI lesions that matched arthroscopic findings increased to 12 (60%). All four cases of partial-thickness or full-thickness

cartilage loss that were seen by arthroscopy also were detected by MRI (although the grades did not always concur).

Control biopsy specimens (from cadavers and femoral intercondylar notch) showed uniform safranin O stain and a normal Mankin score. Biopsy specimens from cartilage lesions showed mild to moderate reduction in safranin O stain intensity especially in the superficial third. The Mankin score ranged from 3 to 7 with a median of 4. Cells that were positive for DNA fragmentation were significantly more numerous in biopsy specimens from lesions compared with control biopsy specimens (Fig 4,  $p = 0.002$ ).



**Fig 3A–D.** Arthroscopic images representative of the different grades of cartilage lesions seen are shown. (A) Grade I lesion with intact cartilage surface showing softening on probing; (B) Superficial fibrillation seen in a Grade II lesion (arrow) in association with an acute meniscal tear that was excised; (C) Partial-thickness Grade III lesion with a flap being raised by the arthroscopic probe; and (D) Full-thickness Grade IV lesion in the femoral condyle (arrow).



**Fig 4.** The mean chondrocyte apoptosis in cartilage biopsy specimens is shown. Cells positive for DNA fragmentation were counted and apoptosis was quantitated as a percentage of all cells. Mean ( $\pm$  standard deviation) percent apoptosis in biopsy specimens from cartilage lesions was significantly higher than control biopsy specimens ( $p = 0.002$ ).

## DISCUSSION

Mature articular cartilage has poor intrinsic repair capability. If repair tissue forms spontaneously, it is predominantly fibrous in nature and is lacking in biomechanical properties. Various surgical procedures currently are being used for replacement or regeneration of cartilage.<sup>1,6-8,13,14</sup> None has been consistently successful in restoring the topographic organization of normal hyaline articular cartilage, the biomechanical properties, or in integrating the edge of the repair tissue with adjacent normal cartilage. Perhaps this may explain the lack of durable long-term results.

Magnetic resonance imaging has potential as a safe and noninvasive means of detecting cartilage lesions that may progress to degeneration and significant disability. Magnetic resonance imaging sequences have been developed to optimize cartilage imaging. Fast spin echo<sup>2,15,22</sup> and fat-suppressed three-dimensional spoiled

gradient echo<sup>5,18</sup> are established MRI techniques that allow excellent assessment of cartilage lesions with a high sensitivity and specificity. Between 60% and 80% of patients with MRI or arthroscopically documented cartilage injury had cartilage degeneration develop by the 5-year followup.<sup>9</sup> The correlation of arthroscopic evaluation, histologic features, and sequential MRI examination should provide valuable information in defining the natural history of posttraumatic cartilage degeneration. The current authors will continue to monitor these patients with sequential MRI scans as many as 2 years after the initial arthroscopy. In the current study, the overall correlation between MRI and arthroscopic findings was positive in 50% of the patients. However, all four cases of full-thickness or partial-thickness cartilage loss as seen on arthroscopy also were detected by MRI. This suggests that MRI is relatively more accurate in detecting Grade III and IV lesions. Two pa-

tients with only bone marrow edema on MRI had Grade I or Grade II lesions on arthroscopy. When these patients were included in the correlation, the overall MRI accuracy increased to 60%.

A high incidence of bone marrow edema on MRI has been seen in association with anterior cruciate ligament injuries.<sup>19</sup> Bone marrow edema seems to be an important marker of acute injury because there was disappearance of this signal on sequential MRI scans (at 6 months and 1 year after arthroscopy, data not shown). This may serve to differentiate acute from chronic cartilage injury. In addition, the two patients who had bone marrow edema alone on MRI were found to have Grade I or Grade II cartilage lesions on arthroscopy, suggesting that bone marrow edema is supportive evidence of significant joint trauma.

The histologic findings of decreased safranin O stain and increased Mankin score provide evidence of cartilage matrix damage. In addition, the high percentage of cells positive for DNA fragmentation suggests that chondrocytes undergo apoptosis after joint injury. Recent studies have reported that chondrocytes undergo apoptosis in response to wounding and injurious compression.<sup>4,10,21</sup> The current study provides additional support by showing apoptosis with acute joint trauma.

Cartilage cellularity varies among species and changes during skeletal development and aging. Only 19 cells/mm<sup>3</sup> are present in the cartilage of young adults.<sup>17</sup> This cell number decreases by approximately 75% during the aging process. A study on human cartilage aging indicated that individuals older than 90 years with intact knee articular cartilage surfaces are distinguished by a significantly increased number of chondrocytes per tissue volume.<sup>16</sup> The level of cartilage cellularity determines the tissue volume that is being maintained by one chondrocyte and may have profound implications for cartilage repair. It therefore may be possible to limit cartilage degeneration and promote repair if cell viability is maintained. This opens potential therapeutic

avenues for the treatment of posttraumatic cartilage lesions through chondroprotection.

## References

1. Brittberg M, Lindahl A, Nilsson A, et al: Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 331:889–895, 1994.
2. Broderick LS, Turner DA, Renfrew DL, et al: Severity of articular cartilage abnormality in patients with osteoarthritis: Evaluation with fast spin-echo MR vs arthroscopy. *AJR Am J Roentgenol* 162:99–103, 1994.
3. Calandruccio RA, Gilmer WS: Proliferation and repair of articular cartilage of immature animals. *J Bone Joint Surg* 44A:431–455, 1962.
4. D’Lima DD, Hashimoto S, Chen PC, et al: Impact of mechanical trauma on matrix and cells. *Clin Orthop* 391 (Suppl):S90–S99, 2001.
5. Disler DG: Fat-suppressed three-dimensional spoiled gradient-recalled MR imaging: assessment of articular and physal hyaline cartilage. *AJR Am J Roentgenol* 169:1117–1123, 1997.
6. Ghazavi MT, Prizker KP, Davis AM, et al: Fresh osteochondral allografts for post-traumatic osteochondral defects of the knee. *J Bone Joint Surg* 79B:1008–1013, 1997.
7. Hangody L, Kish G, Karpati Z, et al: Mosaicplasty for the treatment of articular cartilage defects: Application in clinical practice. *Orthopedics* 21:751–756, 1998.
8. Homminga GN, Bulstra SK, Bouwmeester PS, et al: Perichondral grafting for cartilage lesions of the knee. *J Bone Joint Surg* 72B:1003–1007, 1990.
9. Johnson DL, Urban WPJ, Caborn DN, et al: Articular cartilage changes seen with magnetic resonance imaging-detected bone bruises associated with acute anterior cruciate ligament rupture. *Am J Sports Med* 26:409–414, 1998.
10. Loening AM, James IE, Levenston ME, et al: Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. *Arch Biochem Biophys* 381:205–212, 2000.
11. Mankin HJ: Localisation of tritiated thymidine in cartilage: I. Growth in immature cartilage. *J Bone Joint Surg* 44 A:682–688, 1962.
12. Mankin HJ, Dorfman H, Lippiello L, et al: Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips: II. Correlation of morphology with biochemical and metabolic data. *J Bone Joint Surg* 53A:523–537, 1971.
13. Meyers MH, Akesson W, Convery FR: Resurfacing of the knee with fresh osteochondral allograft. *J Bone Joint Surg* 71A:704–713, 1989.
14. Peterson L, Minas T, Brittberg M, et al: Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop* 374:212–234, 2000.
15. Potter HG, Linklater JM, Allen AA, et al: Magnetic resonance imaging of articular cartilage in the knee: An evaluation with use of fast-spin-echo imaging. *J Bone Joint Surg* 80A:1276–1284, 1998.
16. Quintero M, Mitrovic DR, Stankovic A, et al: Cellular

- aspects of the aging of articular cartilage: I. Condylar cartilage with a normal surface sampled from normal knees. *Rev Rhum Mal Osteoartic* 51:375–379, 1984.
17. Quintero M, Mitrovic DR, Stankovic MA, et al: Cellular aspects of the aging of the articular cartilage: II. Condylar cartilage with fissured surface taken from normal and arthritic knees. *Rev Rhum Mal Osteoartic* 51:445–449, 1984.
  18. Recht MP, Resnick D: MR imaging of articular cartilage: Current status and future directions. *AJR Am J Roentgenol* 163:283–290, 1994.
  19. Rosen MA, Jackson DW, Berger PE: Occult osseous lesions documented by magnetic resonance imaging associated with anterior cruciate ligament ruptures. *Arthroscopy* 7:45–51, 1991.
  20. Rudel T: Caspase inhibitors in prevention of apoptosis. *Herz* 24:236–241, 1999.
  21. Tew SR, Kwan AP, Hann A, et al: The reactions of articular cartilage to experimental wounding: Role of apoptosis. *Arthritis Rheum* 43:215–225, 2000.
  22. Yao L, Gentili A, Thomas A: Incidental magnetization transfer contrast in fast spin-echo imaging of cartilage. *J Magn Reson Imaging* 6:180–184, 1996.