Autologous Chondrocyte Implantation and Osteochondral Cylinder Transplantation in Cartilage Repair of the Knee Joint

A Prospective, Comparative Trial

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Background: Current methods used to restore the joint surface in patients with localized articular cartilage defects include transplantation of an autologous osteochondral cylinder and implantation of autologous chondrocytes. The purpose of this study was to evaluate the clinical and histological outcomes of these two techniques.

Methods: We performed a prospective clinical study to investigate the two-year outcomes in forty patients with an articular cartilage lesion of the femoral condyle who had been randomly treated with either transplantation of an autologous osteochondral cylinder or implantation of autologous chondrocytes. Biopsy specimens from representative patients of both groups were evaluated with histological staining, immunohistochemistry, and scanning electron microscopy.

Results: According to the postoperative Lysholm score, the recovery after autologous chondrocyte implantation was slower than that after osteochondral transplantation at six months ($p \leq 0.015$), twelve months ($p \leq 0.001$), and twenty-four months ($p \leq 0.012$). On the basis of the Meyers score and the Tegner activity score, the results were equally good with the two methods two years after treatment. Histomorphological evaluation of biopsy specimens within two years after autologous chondrocyte implantation demonstrated a complete, mechanically stable resurfacing of the defect in all patients. The tissue consisted mainly of fibrocartilage, while localized areas of hyaline-like regenerative cartilage could be detected close to the subchondral bone. Although a gap remained at the site of the transplantation in all five biopsy specimens examined as long as two years after osteochondral cylinder transplantation, histomorphological analysis and scanning electron microscopy revealed no differences between the osteochondral transplants and the surrounding original cartilage.

Conclusions: Both treatments resulted in a decrease in symptoms. However, the improvement provided by the autologous chondrocyte implantation lagged behind that provided by the osteochondral cylinder transplantation. Histologically, the defects treated with autologous chondrocyte implantation were primarily filled with fibrocartilage, whereas the osteochondral cylinder transplants retained their hyaline character, although there was a persistent interface between the transplant and the surrounding original cartilage. Limitations of our study included the small number of patients, the relatively short (two-year) follow-up, and the absence of a control group.

Level of Evidence: Therapeutic study, Level II-2 (poor-quality randomized controlled trial [e.g., <80% follow-up]). See Instructions to Authors for a complete description of levels of evidence.

Articular cartilage has a limited potential to heal[1,2]. Mechanical damage to the joint surface can lead to premature arthritis[3]. Furthermore, joint replacement procedures are more prone to fail in young patients and those in early middle age[4]. Therefore, it is desirable to regenerate cartilaginous articular surfaces.

Methods such as abrasion chondroplasty[5], subchondral drilling[6], microfracture, and spongialization[7] have been used to stimulate cartilage-healing. These techniques are based on the recruitment of pluripotent mesenchymal cells from the bone marrow[8] and lead to a fibrous substitute tissue covering the cartilage defect.

Brittberg et al.[9] and Grande et al.[10] reported in vivo formation of hyaline cartilage following implantation of autologous chondrocytes. At the same time as they were carrying out their work, surgical techniques and devices for the transplanta-

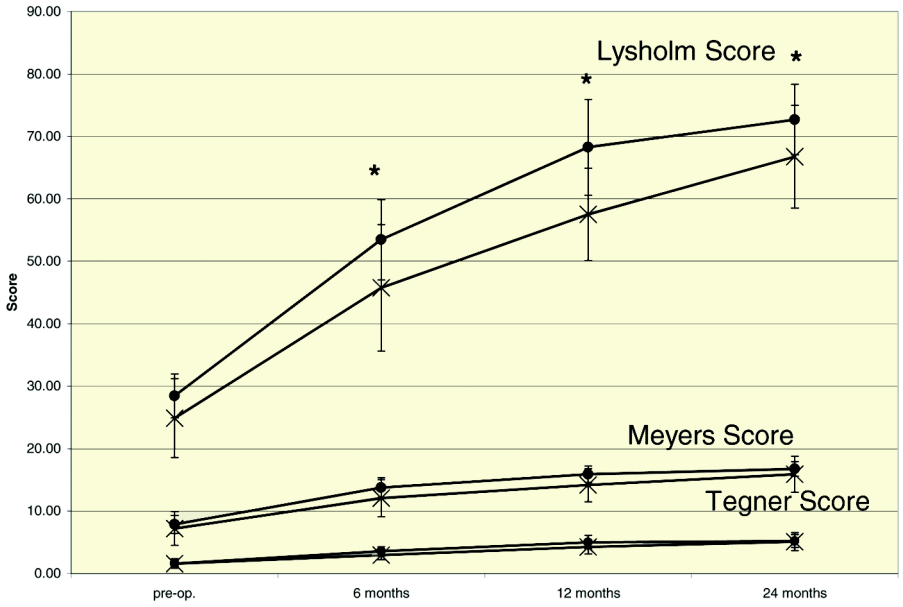

Fig. 1

Graph showing the Lysholm, Tegner, and Meyers scores, preoperatively and at three, six, twelve, and twenty-four months after the surgical procedure, for the group treated with autologous chondrocyte implantation (×) and that treated with osteochondral cylinder transplantation (•). An asterisk indicates a significant difference between groups ($p < 0.05$).

tion of autologous cartilage-bone cylinders were being developed. These techniques can be performed either with a series of small osteochondral plugs or with a single, larger osteochondral transplant. Both of these approaches to cartilage resurfacing have had encouraging clinical results[9,11]. However, middle and long-term prospective studies of the clinical results and the histomorphological appearance of the repair have not been adequately documented in the literature, to our knowledge.

The purpose of this prospective, randomized study was to evaluate the clinical and histomorphological outcomes of these two techniques of transplantation.

Materials and Methods

Study Design

The study was approved by the ethics commission of the Justus-Liebig University, Giessen, Germany. The patients were randomly assigned to either group, with an alternating consecutive selection, after they had provided informed consent.

The inclusion criteria were a history of a single traumatic event, a single cartilage lesion extending to or through the articular cartilage tidemark without an osseous lesion, location of the lesion in the weight-bearing area of the femoral condyle, an age between eighteen and forty-five years, and clinical symptoms such as locking of the joint, pain with weight-bearing or squatting, and swelling.

Exclusion criteria were knee joint instability, a matching lesion on the opposing tibial articular surface, axial malalignment (>10° of varus or valgus as assessed by clinical observation), an osteochondral tumor, skeletal immaturity, degenerative or rheumatoid joint disease, or the patient's refusal to be randomly assigned to a treatment group.

All study patients were followed clinically at predetermined time-points. No participant was withdrawn because of a surgical complication (see Appendix).

The clinical findings were evaluated with use of a modification of the score described by Lysholm and Gillquist[12], with the score described by Meyers et al.[13], and with the activity scale described by Tegner and Lysholm[14]; the clinical evaluations were performed preoperatively and at three, six, twelve, and twenty-four months postoperatively. The preoperative Lysholm, Tegner, and Meyers scores were similar between the two treatment groups ($p < 0.12$). In order to decrease the bias introduced by symptoms of instability, we eliminated the category for instability, as described by Raunest and Lohnert[12,15,16].

A total of forty patients were included in the study. Twenty patients (twelve women and eight men) with a mean age of 31.4 years (range, eighteen to forty-two years) were treated with implantation of autologous chondrocytes. Twenty patients (five women and fifteen men) with a mean age of 35.4 years (range, twenty-one to forty-four years) were treated with transplantation of an autologous osteochondral cylinder.

The sizes of the cartilage lesions ranged from 3.2 to 5.6 cm^2 (mean, 3.75 cm^2) in the series as a whole, 3.86 cm^2 in the group treated with autologous chondrocyte implantation, and 3.63 cm^2 in the group treated with osteochondral cylinder transplantation. The difference between these sizes was not significant.

At the time of treatment, all lesions extended beyond the tidemark layer of the articular cartilage, but none involved the subchondral bone. Of the patients treated with autologous chondrocyte implantation, seventeen had the lesion on the medial femoral condyle, with the patellofemoral articulation also affected in one of them, and three patients had the lesion on the lateral femoral condyle. In the group of patients treated with osteochondral cylinder transplantation, sixteen had the lesion on the medial femoral condyle and four, on the lateral femoral condyle.

Seven patients treated with autologous chondrocyte implantation had had a previous surgical abrasion arthroplasty or spongiolization. Two patients treated with osteochondral cylin-

der transplantation had previously been managed with abrasion arthroplasty and two, with drilling of the cartilage defect.

Surgical Techniques

Autologous Chondrocyte Implantation

The depth and extent of the osteochondral lesion was evaluated, and a slice of healthy articular cartilage (140 to 360 mg) was obtained arthroscopically from the proximal part of the medial femoral condyle of the affected knee joint during the first operation.

Chondrocytes were isolated according to the protocol of Brittberg et al.[9]. The process of cell reproduction took two to three weeks, resulting in a total cell number of 3.2 to 6.5 × 10^6 chondrocytes in a total volume of 100 to 160 mL. The chondrocyte suspension was transplanted in a second operation, through a medial or lateral parapatellar arthrotomy in a tourniquet-controlled, bloodless field. The injured cartilage was prepared by sharp excision into completely healthy hyaline cartilage down the subchondral bone plate, but bleeding of the transplantation site was avoided. A precisely fitting periosteal flap, always taken from the medial aspect of the proximal part of the tibia, was applied to the defect with the cambium layer facing the subchondral bone and was fixed securely by interrupted sutures to the hyaline cartilage surrounding the defect. In order to exclude the possibility of viral infection, fibrin glue was not used. A watertight seal of the periosteal flap was confirmed by injecting saline solution under the flap. The suspension of cultivated autologous chondrocytes was then injected under the periosteal flap, and the injection site was closed with a final suture. This was followed by layered closure of the knee joint.

Osteochondral Cylinder Transplantation

To harvest the osteochondral transplants, a diamond bone-cutting system (DBCS; Merck, Darmstadt, Hessen, Germany) with a twin pair of carving cylinders differing in diameter by 0.1 mm was used. The donor transplant was harvested with the larger cylinder, and the lesion was carved out with the smaller cylinder, so that a press-fit transplantation of the osteochondral cylinder could be achieved. Additional fixation was not necessary. We could resurface a cartilaginous area of 0.78 cm^2 with the smallest cylinder and an area of 2.26 cm^2 with the largest. For defects that required multiple cylinders in order to maintain joint congruency or for the coverage of large defects, we used press-fit implantation of several single osteochondral transplants. Depending on the location, a medial or lateral arthrotomy was used.

Rehabilitation

The same rehabilitation program was used for both groups. It was divided into four phases: the protection phase (four weeks), the transition phase (fifth and sixth weeks), the maturation phase (seventh to twelfth weeks), and the functional activities phase. A brace was not used in either treatment group.

The patient remained non-weight-bearing for the first to fourteenth days postoperatively, began bearing weight of approximately 20 to 30 lb (9.1 to 13.6 kg) from the third to the fourth week, bore 25% of body weight from the fifth week, and gradually progressed to full weight-bearing at twelve weeks. The range of motion was limited to as little as 0° to as much as 90° for the first to tenth days, increased by 5° to 10° per day for the eleventh to twenty-first days, and was limited to as little as 0° to as much as 130° from the fourth to twelfth weeks. After twelve weeks, a free range of movement was permitted.

Active and passive physiotherapy was begun immediately in the protection phase. The physiotherapy included patellar mobilization; stretching of the hamstring, calf, and quadriceps muscles; straight-leg raises; and continuous passive motion. Beginning four weeks postoperatively, the program continued with isometric leg-press exercises, proprioceptive neuromuscu-

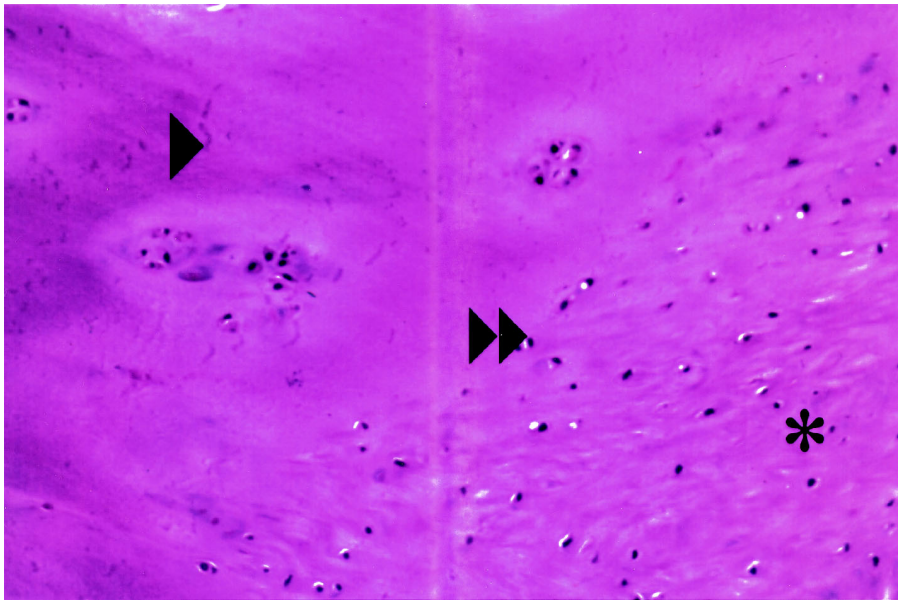

Fig. 2

Histological appearance of a biopsy specimen taken twenty-four months after autologous chondrocyte transplantation. Fibrous regenerated tissue (*) can be observed in the transplant region. The surrounding cartilage (▸) and the transplant (*) are tightly connected (▸▸) (hematoxylin and eosin; original magnification, ×200).

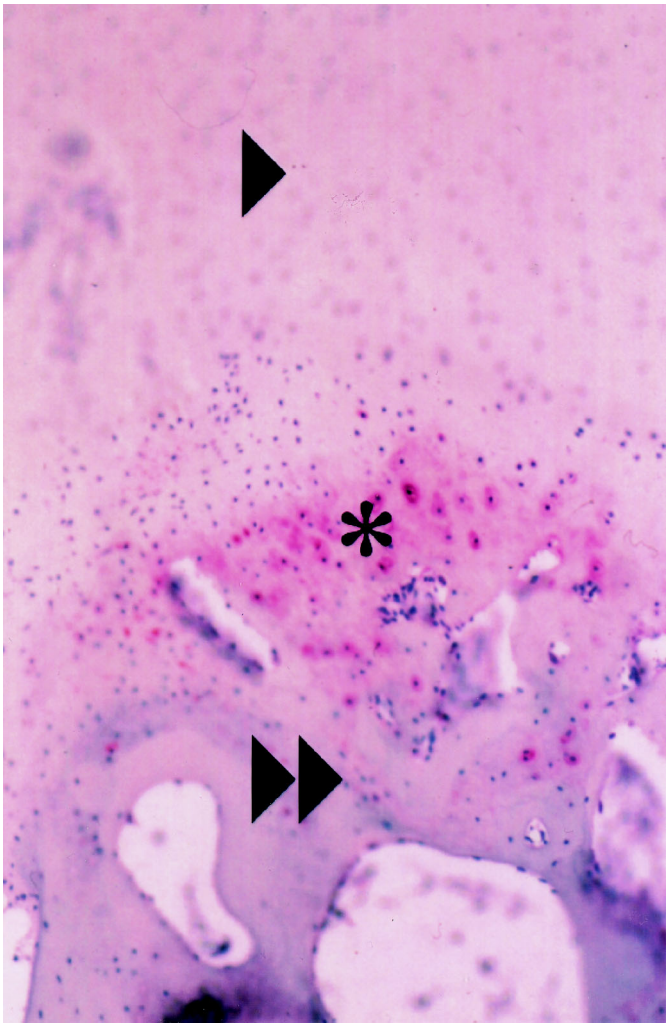

Fig. 3

In a biopsy specimen taken twenty-four months after autologous chondrocyte implantation, only the deep layer (*) is slightly positive for protein S-100. The superficial layer (▸) and the subchondral bone (▸▸) are negative (original magnification, ×100).

lar facilitation, and aqua-jogging. During the transition phase, mini-squats (with 0° to 45° of knee flexion) and closed and open-chain kinetic exercises were initiated, and the patient progressed with weight-bearing from eight to nine weeks postoperatively. The maturation phase included bilateral squats (0° to 60°), leg-press exercises (0° to 90°), a walking program, swimming, and the use of a Stairmaster. After completion of the maturation phase, patients were generally allowed full activity. However, we recommended that they permanently refrain from participation in competitive contact sports such as soccer, basketball, or hockey.

If the patient consented, an arthroscopic examination of the joint, with a biopsy with use of a cannulated 2-mm metal biopsy needle to obtain specimens for histological examination, was performed approximately three, twelve, or twenty-four months after the surgery. Histological slides were evaluated by a histologist who was blinded with regard to patient allocation.

The surface of the unprocessed biopsy specimens was evaluated under a polarized light microscope. Additionally, immunohistochemical evidence of collagen types I, II, III, VI, and X and aggrecan and protein S-100 was assessed. Chondrocytes of normal articular cartilage stain for protein S-100. Cells of all layers are usually positive, although the superficial and deepest layers stain most intensely. The presence of protein S-100 identifies chondroid cells in the repair process that have not begun to synthesize stainable extracellular matrix[17].

Next, a scanning electron microscopic examination was carried out to analyze the structure of the regenerated tissue in comparison with that of the surrounding healthy cartilage. The histological sections were evaluated by pathologists blinded to patient allocation.

Histological Analysis

The samples were fixed in 4% formaldehyde, decalcified in 20% EDTA, and embedded in paraffin for light microscopy. Serial 4-μm cuts were made in the frontal plane and were stained with hematoxylin and eosin, Masson-Goldner stain, and toluidine blue. The samples, stored in 4% formalin, were treated with chondroitinase and hyaluronidase for several days, then rinsed in flowing water, and subsequently rinsed in increasing concentrations of acetone solution. This was followed by meticulous punctiform drying (in acetone medium)

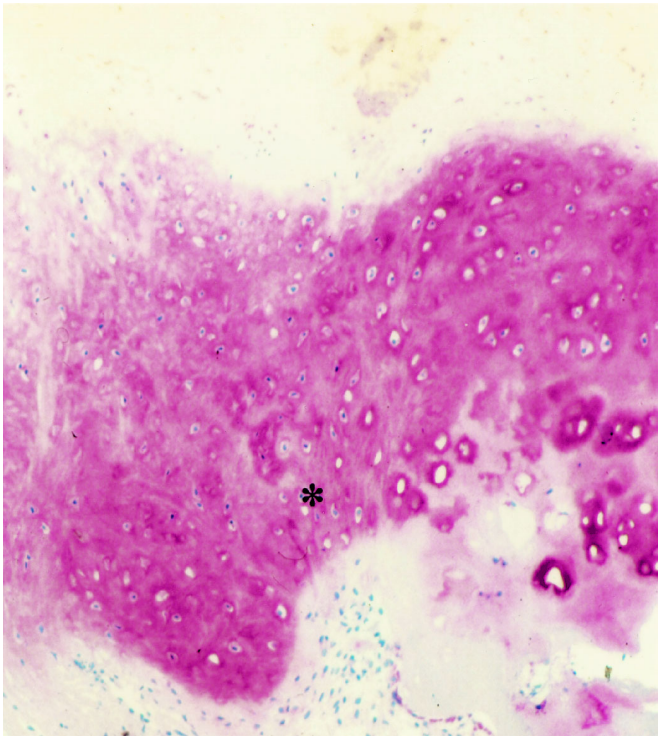

Fig. 4

In a biopsy specimen taken twenty-four months after autologous chondrocyte implantation, only the deep layers (*) stained positive for type-II collagen (original magnification, ×100).

and demasking of the collagen fibrils with use of tape. Finally, the samples were sputtered with palladium and gold.

Statistical Analysis

We applied the nonparametric Mann-Whitney U test for the statistical analysis. Because of the repeated measurements in the course of time, the level of significance was corrected according to the Bonferroni method. The level of significance was $p < 0.05$.

Results

Clinical Results

The postoperative scores (Fig. 1) showed a continuous increase in postoperative performance over the follow-up time-period. However, particularly according to the postoperative Lysholm score, the recovery of the patients treated with autologous chondrocyte implantation was significantly slower than that of the patients treated with osteochondral cylinder transplantation; this was found at six months ($p \leq 0.015$), twelve months ($p \leq 0.001$), and at twenty-four months ($p \leq 0.012$).

Of the twenty patients treated with autologous chondrocyte implantation, one (Case 2; see Appendix) complained of an increase in pain and deterioration of function of the treated knee and stated that the operation had not helped him. In this patient, the cartilage defect involved the patellofemoral joint and, measuring 5.6 cm^2, was the largest defect treated. The findings at an arthrotomy performed twenty-four months after the autologous chondrocyte implantation revealed a fibrocartilage-appearing regenerated tissue in the defect that was distinctly thinned and thus considered a sign of partial transplant failure. We then performed a transplant of two 9-mm osteochondral cylinders. The two cylinders of tissue removed from the defect were examined histologically.

Two patients had only slight improvement twenty-four months after autologous chondrocyte implantation, and a biopsy was performed in one of them. The remaining seventeen patients all reported substantial postoperative improvement. A biopsy was performed in four of those patients.

Three patients reported only slight improvement twenty-four months after osteochondral cylinder transplantation and were not certain whether the transplantation had been beneficial. A biopsy was performed in one of those patients, at twenty-two months. Seventeen patients noted substantial improvement after osteochondral cylinder transplantation. A biopsy was performed in four of those patients.

Five of the seven patients in whom an osteochondral cylinder had been harvested from the posterior aspect of the femoral condyle reported mild pain while squatting.

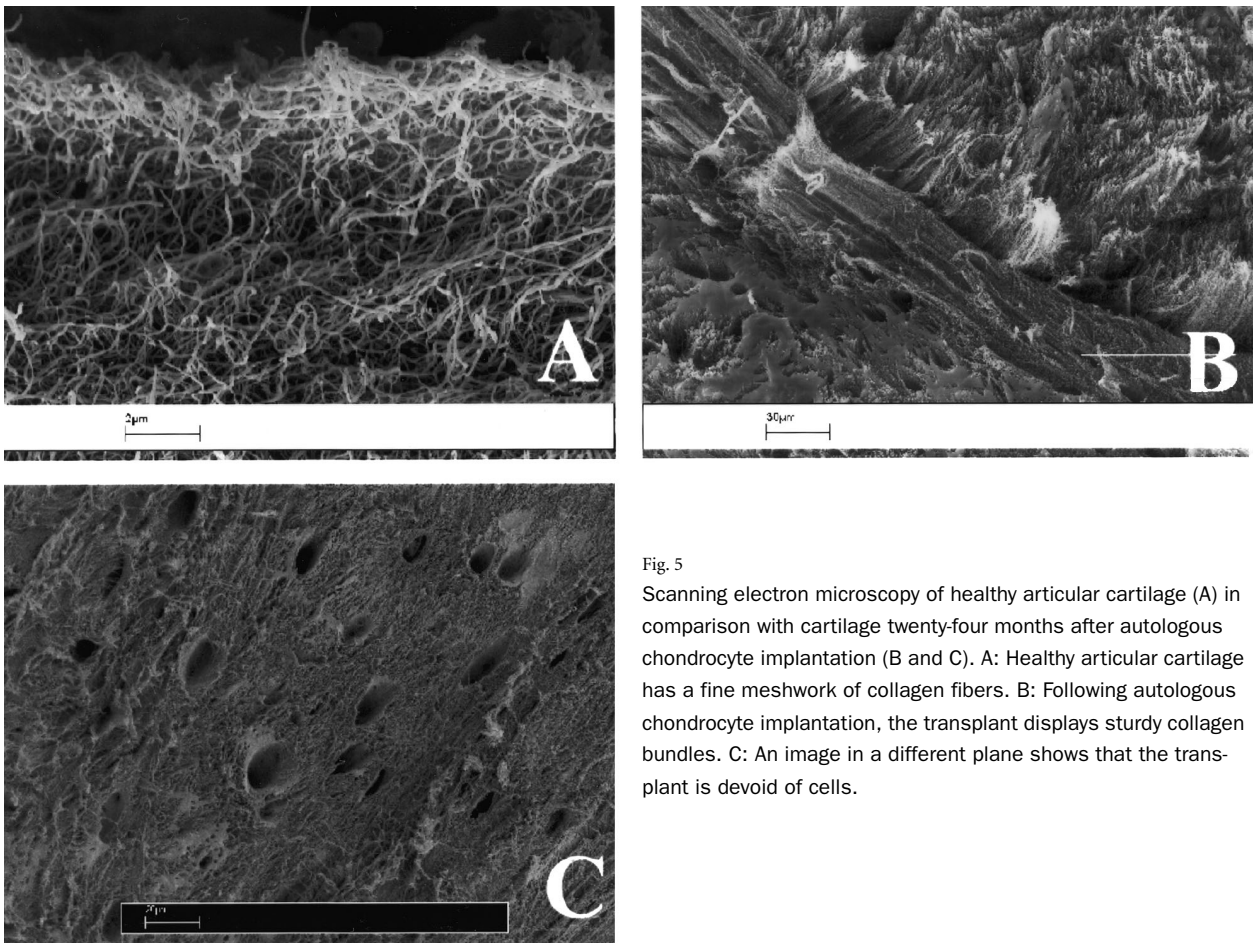

Fig. 5

Scanning electron microscopy of healthy articular cartilage (A) in comparison with cartilage twenty-four months after autologous chondrocyte implantation (B and C). A: Healthy articular cartilage has a fine meshwork of collagen fibers. B: Following autologous chondrocyte implantation, the transplant displays sturdy collagen bundles. C: An image in a different plane shows that the transplant is devoid of cells.

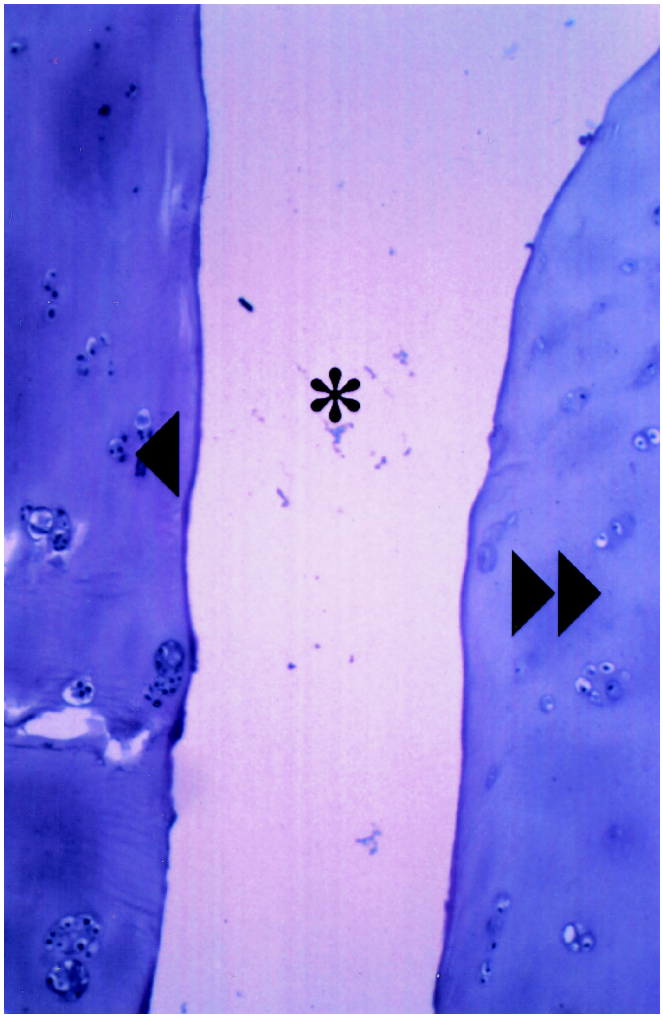

Fig. 6

Histological appearance of a biopsy specimen taken twenty-two months after osteochondral transplantation. A cleft (*) remains between the residential cartilage (◀◀) and the osteochondral transplant (◀) in the cartilage layer (toluidine blue; original magnification, ×200).

The patients in whom the cylinder had been obtained from the anterior-superior part of the medial condyle had only mild pain in the early postoperative phase during straight-leg raises and tenderness in the patellofemoral joint with the knee extended. Except for the mild pain, all of the other symptoms disappeared by the maturation phase (twelve weeks postoperatively) of the rehabilitation program.

Histological Results

Autologous Chondrocyte Implantation

Six patients who had been treated with the autologous chondrocyte implantation were reexamined arthroscopically, and eight biopsies were performed, within twenty-four months postoperatively. The arthroscopic examination, performed with a probe, revealed regenerated tissue with a rigid, elastic consistency and a rippled surface; in five of the eight cases, there was a distinct, rough surface. In two patients, the regenerated tissue had overgrown the level of the surrounding cartilage.

The biopsy specimens showed thickening of the subchondral bone and adjoining fibrous tissue covering the defect on staining with hematoxylin and eosin at twenty-four months. Single hyaline-like cartilaginous areas with chondrocyte clusters containing up to three chondrocytes were found only near the base of the regenerated tissue; however, a rather fibrous regenerated tissue with a large number of cells was detected in the central and superficial layers. A tidemark was not seen in the regenerated tissue. The basal adhesion was intact (Fig. 2). The surface of the regenerated tissue was irregularly polypoid and showed fibrous lamellae, with chondroid metaplasia. In the toluidine-blue reaction, a blotchy staining typical of fibrocartilage was observed in the regenerated tissue, while the resident surrounding hyaline cartilage stained homogeneously.

All eight samples obtained within twenty-four months after autologous chondrocyte implantation were only slightly positive for protein S-100 in the deeper layer of the regenerated tissue but positive in the surrounding original hyaline cartilage (Fig. 3). Staining for type-I collagen was multifocally positive in the regenerated tissue and negative in the adjoining original cartilage. Conversely, staining for type-II collagen was distinctly positive in the original cartilage and only focally verifiable in the regenerated tissue, where it was essentially limited to the deep layers (Fig. 4).

Scanning electron microscopy twenty-four months after autologous chondrocyte implantation showed regenerated tissue that was tightly united with the original cartilage (Fig. 5). Sturdy monodirectional collagen bundles either originated from the subchondral bone or ran across the interface in a few thick layers (Fig. 5, *B*). These collagen layers contained a large number of holes that corresponded in size to chondrocytes without actually hosting any cells (Fig. 5, *C*). In contrast, the surrounding normal cartilage demonstrated a three-dimensional network of thinner collagen fibrils that was condensed and flattened out toward the surface and could be seen to lie above a continuous tidemark (Fig. 5, *A*).

Osteochondral Cylinder Transplantation

Following osteochondral cylinder transplantation, three patients were reexamined arthroscopically at three months and two patients, at twenty-one or twenty-two months. Each had a biopsy specimen taken from the interface between the resident cartilage and the transplant for histological examination. The arthroscopic examination revealed similar findings in all patients, which consisted of macroscopically vital cartilage with a persistent, almost circular, gap at the level of the cartilage but seamless integration in the osseous layer. There was no obvious difference between the transplanted and surrounding resident cartilage macroscopically, and the consistency was the same when tested by palpation with a probe. There were no clinical signs of degeneration of the articular cartilage (Fig. 6). The surface was smooth and appeared adapted to the natural convexity of the knee joint. The donor areas were filled with fibrous-appearing tissue.

The specimens of the cartilage-bone cylinders taken from the interface at three months in three patients and at twenty-one or twenty-two months in two patients showed an unreactive hyaline cartilage transplant adjacent to the resident hyaline cartilage with hematoxylin and eosin staining and a gap reaching down to the bone (Fig. 6).

Immunohistochemical staining for collagen types II and VI as well as that for protein S-100 was characteristic of hyaline cartilage in the five samples obtained after osteochondral cylinder transplantation. Both the surrounding original cartilage and the transplanted cartilage showed these characteristics.

Scanning electron microscopy revealed that the transplant had maintained its original tidemark and did not appear different from the surrounding cartilage in either the deep or the superficial layer.

Discussion

We studied isolated full-thickness defects of the articular cartilage. The defects were of a predetermined size, since healing is highly dependent on the size of the lesion[2,18-20], and all were in the weight-bearing areas of the femoral condyles[21,22]. In addition, the ages of the patients and the maturation stage of the tissue, which seem to influence the structural effectiveness of articular cartilage repair[23], were controlled in this study. Despite the lack of a nonoperative control group, we believe that our findings are important because we performed an extensive histopathological analysis.

During the first six months after autologous chondrocyte implantation, arthroscopic examination showed a transformation of the regenerated tissue from a primarily smooth surface and soft consistency to a rigid, somewhat elastic tissue with partially distinct roughening. At six months, the histomorphological examinations revealed only fibrocartilage in the central and superficial layers of the regenerated tissue. Staining for type-II collagen and aggrecan-proteoglycan, which is characteristic of articular hyaline cartilage, appeared only in isolated deep-layer areas, where hyaline-like staining could also be detected.

Following autologous chondrocyte implantation, the main part of the regenerated tissue shared histomorphological characteristics with early forms of osteophytes arising from the periosteum of osteoarthritic joints[24]. This points to the pluripotent cells[8,25] and cytokines originating from the periosteal flap[26] as important stimuli for the regenerative process. However, further investigation is needed to determine if it is the transplanted autologous chondrocytes or the mesenchymal cells of the periosteal flap[3,8,25,27-30] that develop into the cellular component of the regenerated tissue.

Scanning electron microscopy of the regenerated tissue following autologous chondrocyte implantation also showed characteristics of fibrocartilage and demonstrated plenty of empty chondrocyte-sized holes in the central and deep layers between the monodirectional collagen bundles. In contrast, in the adjoining hyaline cartilage of the same samples, spaces were often found to enclose two or three chondrocytes. The disappearance of the cells from the regenerated tissue could have been caused iatrogenically. On the other hand, this phenomenon could also have been due to the premature death and resorption of the implanted cells (apoptosis). Because the histological appearance of the regenerated cartilage differed from that of the original articular cartilage in our middle-term analysis of autologous chondrocyte implantation, we believe that the long-term results of autologous chondrocyte implantation must be determined before this method is used on a vast number of patients.

Despite the gap remaining in the cartilage at the interface area twenty-two months following osteochondral cylinder transplantation, the transplanted cartilage remained viable and, on examination with a probe, resembled its donor region. Observations in other studies have emphasized the importance of the shape of the transplanted cartilage surface with regard to the surrounding cartilage[31], as multiple or large persistent gaps might affect joint congruency and create a starting point for cartilage degeneration over the long term. Donor site morbidity does not seem to be a disadvantage of osteochondral cylinder transplantation compared with autologous chondrocyte implantation, since the level of activity and other scores were similar to, or even higher than, those in the group treated with autologous chondrocyte implantation. When adequate transplants could not be harvested from the anterior-superior part of the condyle, the posterior part of the condyle served as a source of additional osteochondral transplants. Since transplantation of autologous osteochondral cylinders is limited by the size of the available donor areas, heterogenous transplants[2,32-34], which have no donor-site limits or morbidity, should be considered as a graft source.

Although osteochondral cylinder transplantation is limited by donor-site availability, we consider it to be an appropriate treatment for articular cartilage lesions. Additional experimental research on the use of scaffolds for autologous chondrocytes[35,36], the preparation of the subchondral bone[37,38], the use of cytokines[39,40], or even gene therapy[41-43] to stimulate cartilage regeneration and formation of hyaline cartilage will influence the future utilization of the procedures described in this report.

Appendix

eA Tables providing details on the two treatment groups and a figure showing the locations of the lesions are available with the electronic versions of this article, on our web site at www.jbjs.org (go to the article citation and click on "Supplementary Material") and on our quarterly CD-ROM (call our subscription department, at 781-449-9780, to order the CD-ROM). ■

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