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Original article

Evaluation of reparative cartilage after autologous chondrocyte implantation for osteochondritis dissecans: histology, biochemistry, and MR imaging

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Abstract

Background. The aim of this study was to investigate the biochemical properties, histological and immunohistochemical appearance, and magnetic resonance (MR) imaging findings of reparative cartilage after autologous chondrocyte implantation (ACI) for osteochondritis dissecans (OCD).

Methods. Six patients (mean age 20.2 ± 8.8 years; 13–35 years) who underwent ACI for full-thickness cartilage defects of the femoral condyle were studied. One year after the procedure, a second-look arthroscopic operation was performed with biopsy of reparative tissue. The International Cartilage Repair Society (ICRS) visual histological assessment scale was used for histological assessment. Biopsied tissue was immunohistochemically analyzed with the use of monoclonal antihuman collagen type I and monoclonal antihuman collagen type II primary antibodies. Glycosaminoglycan (GAG) concentrations in biopsied reparative cartilage samples were measured by high performance liquid chromatography (HPLC). MR imaging was performed with T_1- and T_2-weighted imaging and three-dimensional spoiled gradient-recalled (3D-SPGR) MR imaging.

Results. Four tissue samples were graded as having a mixed morphology of hyaline and fibrocartilage while the other two were graded as fibrocartilage. Average ICRS scores for each criterion were (I) 1.0 ± 1.5; (II) 1.7 ± 0.5; (III) 0.6 ± 1.0; (IV) 3.0 ± 0.0; (V) 1.8 ± 1.5; and (VI) 2.5 ± 1.2. Average total score was 10.7 ± 2.8. On immunohistochemical analysis, the matrix from deep and middle layers of reparative cartilage stained positive for type II collagen; however, the surface layer did not stain well. The average GAG concentration in reparative cartilage was 76.6 ± 4.2 μg/mg whereas that in normal cartilage was 108 ± 11.2 μg/mg. Common complications observed on 3D-SPGR MR imaging were hypertrophy of grafted periosteum, edema-like signal in bone marrow, and incomplete repair of subchondral bone at the surgical site. Clinically, patients had significant improvements in Lysholm scores.

Conclusions. In spite of a good clinical course, reparative cartilage after ACI had less GAG concentration and was inferior to healthy hyaline cartilage in histological and immunohistochemical appearance and on MRI findings.

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Introduction

Articular cartilage is composed of hyaline cartilage containing a relatively small number of chondrocytes embedded in abundant extracellular matrix materials such as type II collagen and proteoglycan.[1] Articular cartilage has limited intrinsic repair capacity, and damage to cartilage or mechanical damage to the joint surface can be a risk factor for more extensive joint damage.[2] Over the past 10 years, autologous chondrocyte implantation (ACI) has been a widely used technique for treatment of articular cartilage lesions, and good to excellent clinical results have been reported.[3,4] However, several investigators have conducted histological and/or biochemical analyses of ACI repair sites and reported that the reparative cartilage was not always identical to native hyaline cartilage found in normal articular cartilage.[5] In previous reports, reparative cartilage was assessed with qualitative methods: the presence of key components such as proteoglycan and type II collagen was observed in reparative tissues.[6,7] However, only a few reports have included quantitative biochemical analysis of such matrix components. Biochemical analysis of extracellular matrix that can characterize the nature of reparative and native articular cartilage is considered to be essential to assess reparative tissue and predict prognosis after ACI. We hypothesized that reparative tissue after ACI would contain abundant proteoglycan but might have less than native cartilage. In addition, we were interested in exploring some adverse effects of ACI that have been reported, e.g., graft failure, delamination, and tissue hypertrophy.[8]

The aim of this study was to assess the efficacy of ACI as a cartilage repair method by use of histological as-

sessment such as general histology and immunohistochemistry, biochemical quantitative analysis, magnetic resonance (MR) imaging, clinical evaluation, and macroscopic assessment on follow-up arthroscopy.

Materials and methods

This study was approved by the Ethics Review Committee of Chiba University Hospital. The patients gave consent for ACI as a two-stage process, with a follow-up arthroscopy procedure including biopsy approximately 1 year after the second stage of the procedure. Additionally, patients were informed that data derived from their procedures would be submitted for publication, and they gave their consent.

Patients

ACI was performed in six male patients (mean age, 20.2 ± 8.8 years; age range, 13–35 years) for osteochondritis dissecans (OCD) at a femoral condyle (five medial, one lateral). All were full-thickness cartilage defects graded as ICRS (International Cartilage Repair Society) OCD: IV and ICRS Grade 4: severely abnormal.[9] The size of the lesions ranged from 400 to 1280 mm^2 (mean, 596 ± 345 mm^2).

ACI procedure

A small biopsy of articular cartilage was collected from a non-weight-bearing area (e.g., trochlear cartilage). Biopsy specimens were sent to Genzyme (Carticel Service, Genzyme, Cambridge, MA, USA) for processing and culturing. After enzymatic digestion of the tissue and 3-week cultivation of the chondrocytes in culture medium including fetal bovine serum and gentamicin, patients were readmitted to our hospital for the second stage of the procedure. To prepare for chondrocyte implantation, a medial parapatellar arthrotomy was performed. The osteochondral lesion was debrided with minimum bleeding, and thrombin was given to stop bleeding from subchondral bone tissue. A periosteal flap was harvested from the proximal medial tibia and was fitted and sutured to the surrounding rim of cartilage with 5-0 Vicryl. The periosteal flap was sealed to the rim with fibrin glue except for one upper corner, within which the cultured chondrocytes were injected into the defect. After chondrocyte injection beneath the periosteal flap, the remaining defect between the periosteal flap and rim was sutured with 5-0 Vicryl and sealed with fibrin glue. Each defect received approximately 1.6 million cells/cm^2 of cultured chondrocytes.

Rehabilitation

The rehabilitation schedule for each patient included active and passive movement, muscle training, and weight-bearing exercise. Patients began physiotherapy with 0°–30° angle of continuous passive motion beginning 6 h after surgery. The range of motion was gradually increased until 12 weeks, culminating in full flexion. Each patient remained non-weight-bearing for the 1st to 4th postoperative week, with partial weight-bearing exercise beginning after the 4th week. By the 12th postoperative week, patients had progressed to walking with full weight-bearing. Sports activity was gradually increased after 6 months; however, hard sporting activity was allowed only after 12 months.

Tissue biopsies

At the 1-year (12.4 ± 0.7 months) follow-up, arthroscopic assessments were performed for all patients, and tissue biopsies were taken from the center of the ACI repair site and also from a normal area at the lateral side of the femoral condyle (the latter as controls). Full-depth cores of cartilage and subchondral bone were obtained from all six patients. Biopsies were taken from the center of the graft region using an 11-gauge biopsy needle (Trapsystem MDTECH, Gainesville, FL, USA). The cores were taken as closely as possible to a 90° angle to the articular surface. Biopsy samples were used for histological, immunohistochemical, and biochemical analyses.

Histological assessment

For general histology, 7-μm-thick frozen sections were stained with hematoxylin and eosin (H&E), safranin-O (0.5% in 0.1 M sodium acetate, pH 4.6, for 30 s), and Masson-trichrome stain.

The ICRS Visual Histological Assessment Scale[10] (Table 1) was used to grade the reparative tissue samples. The biopsy specimens were evaluated by a skilled cartilage research pathologist, who used both polarized and plain light microscopy to assess collagen organization and morphology of each sample.

For immunohistochemical analysis, the following primary antibodies were used: mouse monoclonal antihuman collagen type I antibody and mouse monoclonal antibody to collagen type II (Immunodiagnostika und Biotechnologie, Berlin, Germany).

Biochemical assessment

Concentrations of GAG in reparative and native cartilage samples from four patients were measured with high performance liquid chromatography (HPLC). The

Table 1. International Cartilage Repair Society (ICRS) Visual Histological Assessment scale

Feature	Score
I. Surface	
Smooth/continuous	3
Discontinuities/irregularities	0
II. Matrix	
Hyaline	3
Mixture: hyaline/fibrocartilage	2
Fibrocartilage	1
Fibrous tissue	0
III. Cell distribution	
Columnar	3
Mixed/columnar clusters	2
Clusters	1
Individual cells/disorganized	0
IV. Cell population viability	
Predominantly viable	3
Partially viable	1
<10% viable	0
V. Subchondral bone	
Normal	3
Increased remodeling	2
Bone necrosis/granulation tissue	1
Detached/fracture/callus at base	0
VI. Cartilage mineralization (calcified cartilage)	
Normal	3
Abnormal/inappropriate location	0

HPLC procedures were performed in accordance with the method described by Shinmei et al.[11]

MR imaging and assessment

MR imaging was performed with a 1.5-Tesla magnet (Signa Horizon General Electronic, Milwaukee, WI, USA) using a knee coil preoperatively and at 1 year postoperatively. Imaging was performed in the sagittal plane, and a series of T_1-weighted and T_2-weighted images were obtained as routine sequences. In addition, fat-suppressed three-dimensional spoiled gradient-recalled (3D-SPGR) sequences[12] were performed to obtain more morphological details, with 1.5-mm slice thickness, repetition time of 52 ms, echo time of 10 ms, flip angle of 60°, field of view 130 × 130 mm, and matrix of 512 × 512 pixels. Imaging was performed using the same MR scanner, coil, and sequence each time.

Evaluation of MR images was performed based on the scoring systems used by Sally Roberts (Roberts et al.[5]) (Table 2) and by Henderson et al.[13] (Table 3), by a skilled musculoskeletal radiologist who was unaware of the histological evaluation.

Clinical evaluation

The clinical status of patients was evaluated before ACI and 1 year after the operation using the Lysholm score[14] and the overall Brittberg clinical grading score.[1]

Table 2. Magnetic resonance (MR) imaging score applied by Roberts et al.[5]

Feature	Score
I. Surface integrity and contour	1 = normal or near normal, 0 = abnormal
II. Cartilage signal in graft region	1 = normal or near normal, 0 = abnormal
III. Cartilage thickness	1 = normal or near normal, 0 = abnormal
IV. Changes in underlying bone	1 = normal or near normal, 0 = abnormal
Maximum total possible	4 (minimum: 0 is the worst)

Table 3. MR imaging score applied by Henderson et al.[13]

Feature	Score
I. Fill of the repair site	Complete = 1, >50% of the defect = 2 <50% of the defect = 3 Full-thickness defect = 4
II. Signal at the repair site	Normal = 1, Nearly normal = 2, Abnormal = 3, Absent = 4
III. Bone marrow edema	Absent = 1, Mild = 2. Moderate = 3, Severe = 4
IV. Joint effusion	Absent = 1, Mild = 2. Moderate = 3, Severe = 4
Minimum total possible	4 (maximum: 16 is the worst)

Macroscopic assessment

Arthroscopic assessment of reparative tissue was performed based on the ICRS cartilage repair assessment (Protocol A)[9] by a skilled orthopedic surgeon. The scoring system assigns a maximum of 12 points and is based on the degree of defect repair, integration into border zones, and macroscopic appearance.

Statistical analysis

A paired *t* test was used for statistical evaluation of both the GAG concentrations and the assessment of the Lysholm score. A significant difference was defined as $P < 0.05$.

Results

Histology

Four samples displayed mixed morphology of hyaline cartilage and fibrocartilage, in which several rounded chondrocytes were observed in typical lacunae. In contrast, the two remaining samples showed only fibrocartilage (Table 4). In the samples graded as fibrocartilage, however, there were several small islands that resembled hyaline-like cartilage. In the samples with mixed morphology (Fig. 1a,b), half or more of the matrix stained positive for proteoglycans with safranin-O (Fig. 1c). Positive safranin-O staining was observed throughout the matrix except for the fibrous tissue on the sur-

Table 4. Details of individuals, their histology scores, and GAG concentrations

Patient number	Age	Sex	Location of defect	Size of defect (mm^2)	ICRS Visual Histological Assessment Scale							GAG concentration	
					I	II	III	IV	V	VI	Total	ACI (μg/mg)	Control (μg/mg)
1	16	M	MFC	437	0	1	0	3	0	3	7	N/A	N/A
2	35	M	MFC	500	0	2	2	3	3	0	10	77	97
3	27	M	MFC	1280	0	2	0	3	0	3	8	N/A	N/A
4	15	M	MFC	400	3	1	0	3	2	3	12	81	115
5	15	M	LFC	361	3	2	0	3	3	3	14	78	120
6	15	M	MFC	600	0	2	2	3	3	3	13	71	100

ICRS, International Cartilage Repair Society; GAG, glycosaminoglycans; ACI, autologous chondrocyte implantation; MFC, medial femoral condyle; LFC, lateral femoral condyle; N/A. not available

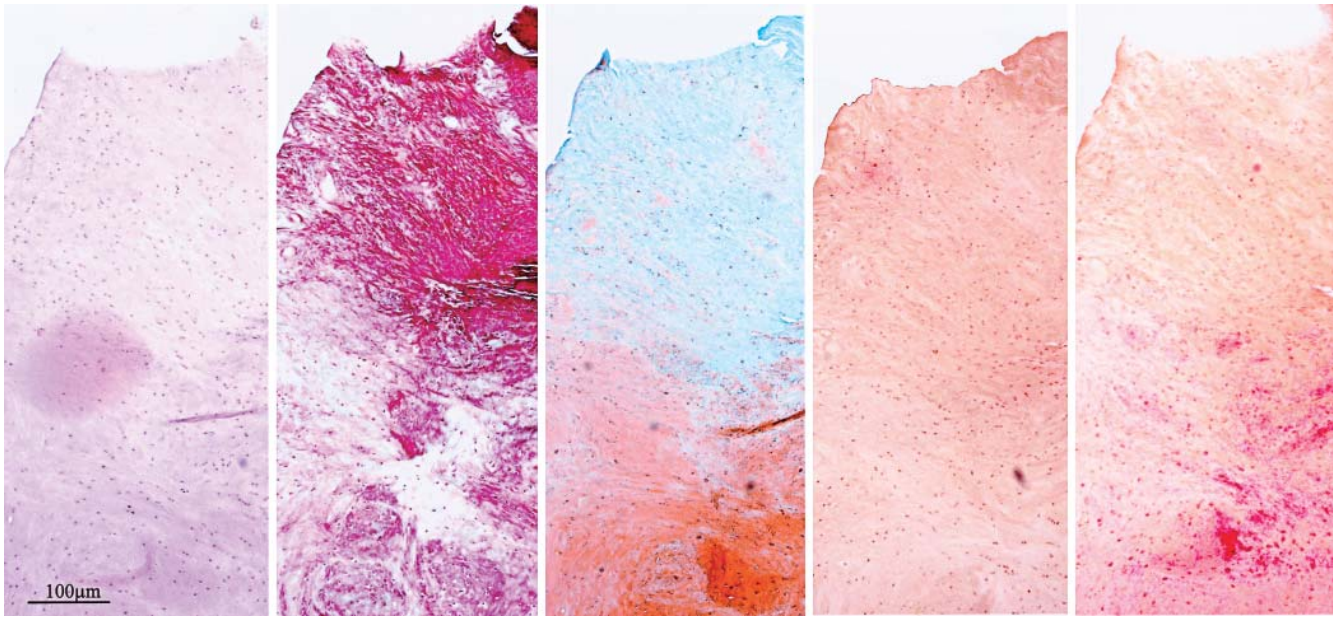

Fig. 1. Histology and immunohistochemistry of sample 5. **a** With hematoxylin and eosin staining, good cell viability was observed. **b** In the upper layer, roughly distributed collagen fibers were observed with Masson-trichrome staining. **c** With safranin-O staining (Saf-O), the deeper layer took up more stain because of the presence of proteoglycans. **d** The stain for type I collagen was observed in the upper part of the lesion; however, the stain for type II collagen was observed from the middle to deep layers (**e**)

face, and in three cases it was stronger in deeper zones than in superficial zones (Fig. 1c). Four samples showed surface irregularity, and in one of them the surface irregularity appeared to be caused by a remnant of periosteum, which was clearly demarcated from reparative cartilage originating from implanted chondrocytes. With respect to cell distribution, two samples showed a tendency to form columnar organization with some clusters of cells and were thus graded as mixed/columnar clusters. The other samples showed no organized distribution of cells. In contrast, all samples showed good viability of cell populations. Regarding the subchondral bone, one sample showed increased remodeling, two samples had a detached appearance, and three samples appeared normal. No sample showed a tidemark reorganization.

Immunohistochemistry

Type I collagen was slightly positive in all samples, but its distribution was more dispersed than that of type II collagen. In the samples graded as fibrocartilage, sparse immunoreactive type I collagen was observed throughout the matrix, whereas in samples with mixed morphology, the distribution of type I collagen was discrete and usually restricted to the uppermost regions (Fig. 1d). The tissue that appeared to be a periosteal remnant stained positive for type I collagen. All samples stained positive for type II collagen, which appeared throughout the reparative cartilage matrix, except in fibrous tissue derived from periosteum. Deep zones stained more strongly positive for type II collagen than upper zones (Fig. 1e). Additionally, deeper zones were more positive for type II than for type I collagen.

GAG concentration

The average GAG concentration in reparative tissue was 76.6 ± 4.2 μg/mg (range, 71–81 μg/mg), 71.5% ± 5.9% of the GAG concentration present in native cartilage, for which the average GAG concentration was 108 ± 11.2 μg/mg (range, 97–120 μg/mg). This difference in concentration was statistically significant ($P < 0.05$).

MR imaging

In all cases, the defect was completely filled with reparative tissue, which was iso-intense with the surrounding cartilage in four patients and of lower intensity than the surrounding cartilage in the remaining two patients (Table 5). In the sample with a deep osteochondral defect, signal intensity of reparative tissue was also almost homogeneously higher than that of underlying bone (Fig. 2a) on 3D-SPGR MR imaging. Hence, the reparative sample was considered cartilage-like tissue, and there was no finding of reparative subchondral bone in reparative tissue.

The surface of the reparative tissue was level with surrounding tissue in four patients; the remaining two patients showed overgrowth at the surface on 3D-SPGR MR imaging (Fig. 2b). The surface of subchondral bone was smooth and regular in three patients, but irregular in the other three cases. Three samples displayed bone marrow edema in subchondral bone at the repair site on T_1- and T_2-weighted images.

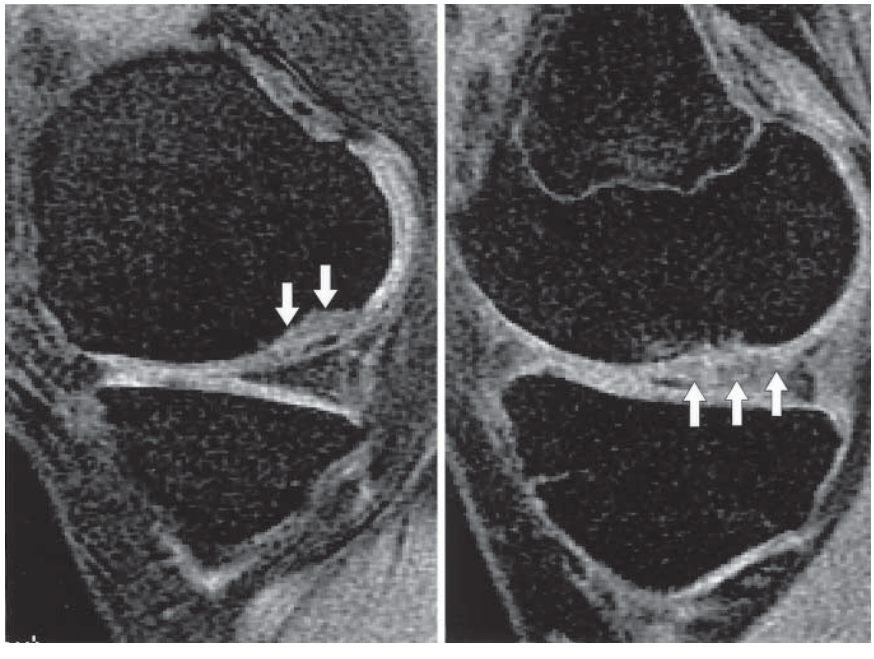

Fig. 2. Magnetic resonance (MR) images obtained 1 year after autologous chondrocyte implantation (ACI). **a** Cartilage filling in the original subchondral defect was identified on three-dimensional spoiled gradient-recalled (3D-SPGR) MR imaging (*arrows*). **b** The surface of the reparative cartilage showed some irregularity and overgrowth (*arrows*)

Table 5. Details of MRI score, Lysholm score, and arthroscopic assessment

Patient number	MRI score of Robert					MRI score of Henderson					Lysholm score		ICRS Cartilage Repair Assessment				
	I	II	III	IV	Total	I	II	III	IV	Total	Before ACI	1y after ACI	I	II	III	Total	Grade
1	0	0	0	1	1	1	2	1	1	5	74	95	4	4	2	10	II: Nearly Normal
2	0	1	0	0	1	1	1	2	1	5	64	96	4	4	3	11	II: Nearly Normal
3	0	1	0	1	2	1	1	1	1	4	66	99	4	4	3	11	II: Nearly Normal
4	1	1	0	0	2	1	1	2	1	5	56	91	4	4	4	12	I: Normal
5	1	1	0	1	3	1	1	1	1	4	76	100	4	4	3	11	II: Nearly Normal
6	0	0	1	0	1	1	2	2	1	6	71	100	4	4	3	11	II: Nearly Normal

ICRS cartilage repair assessment: (I) degree of defect repair, (II) integration to border zones, (III) macroscopic appearance. Grade: I, normal: 12P; II, nearly normal: 11-9P; III, abnormal: 7-4P; IV, severely abnormal: 3-1P

Clinical evaluation

One year after ACI, all patients had reduced knee pain and swelling, and locking sensation had disappeared. Before their operations, all patients had scored poorly on the overall Brittberg clinical grading score. One year after the operation, two patients scored as excellent and the other four scored as good. There was a significant improvement in the patients' Lysholm score, from a mean of 67.8 ± 7.4 points before surgery to a mean of 96.8 ± 3.5 points 1 year after ACI (see Table 5). Two patients showed full recovery of Lysholm scores. The other four patients complained of catching sensations and some difficulties with squatting or climbing stairs. However, after trimming of hypertrophied reparative tissue at the follow-up operation, catching sensations were diminished and the patients no longer complained of any pain or joint swelling. All patients returned to normal daily living and sports activities by 1 year after ACI.

Arthroscopy

One case was grade I on the ICRS cartilage repair assessment; the other five cases had grade II reparative cartilage with "a fibrillated surface" or "small, scattered fissures or cracks" (see Table 5). All patients had refilling of the defect; however, in three patients there was overgrowth of the graft. The patients who complained of an obvious catching sensation underwent trimming of the overgrown tissue. The tissue was similar in color and texture to the surrounding cartilage observed underneath the overgrown tissue.

Discussion

In many reports, ACI has been described as a safe and accepted technique for repair of articular cartilage lesions that can provide durable reparative tissue up to about 10 years after the procedure.[4] However, Roberts et al. reported that graft morphology of reparative cartilage after ACI varied from predominantly hyaline, through mixed, to predominantly fibrocartilage[5]; implantation effectiveness might be considered controversial. In addition, the adverse effects of graft failure, delamination, and hypertrophic tissue have been reported.[8] On the other hand, some reports have suggested that various other procedures, such as osteochondral cylinder transplantation or microfracture, may be able to yield equivalent or better results than ACI for cartilage repair in some patients in terms of both histological appearance and clinical outcome.[15,16] The present study was the first to include multilateral results and analyses of reparative tissue after ACI such as his-

tology, quantitative biochemistry, MR imaging, clinical evaluation and arthroscopic assessment; the efficacy of ACI was discussed based upon our results in addition to previous reports by other researchers.

In this study, on histological evaluation at 1 year after ACI, the matrices of reparative tissue stained well with safranin-O and type II collagen antibody. This finding indicated that the reparative tissue qualitatively contained GAG and type II collagen, which are present in native hyaline cartilage. However, on biochemical analysis, the GAG concentration in the reparative tissue matrix was significantly lower than the concentration in native cartilage. These findings, that reparative tissue was inferior to native cartilage, are consistent with those of a previous report.[17] On histological assessment, Richardson et al. reported that in post-ACI reparative tissue the superficial zone of reparative tissue was more fibrocartilaginous with more type I collagen, whereas the deeper zone was predominantly composed of matrix components similar to native cartilage.[6] Additionally, with respect to the distribution of collagen fibers in reparative tissue, Richardson reported that type II collagen was spread throughout the matrix.[6] Thus, our results are consistent with these reports with respect to the qualitative state of reparative tissue at 1 year after ACI: post-ACI reparative tissue contained the same components as normal cartilage. However, the amount and distribution of such components differed from those found in normal cartilage. In particular, reparative tissue contained significantly less GAG than normal cartilage obtained from the same patient.

In contrast, we found that a predominantly viable cell population was observed in reparative tissue. Briggs et al. have reported that transplanted chondrocytes were immature in the phenotype at 1 year after ACI but would probably continue to proliferate and mature.[17] Hence, further long-term follow-up is required to evaluate maturation of the transplanted chondrocytes and biochemical and structural maturation of reparative tissue.

In the current study, the periosteal patch was often likely to be the cause of low scores on histological assessment because of resultant surface irregularity and hypertrophy of periosteum. Brittberg et al. mentioned that the function of the periosteal flap was to close off the defect, to stimulate reproduction of transplanted cultured chondrocytes, and to stimulate the chondrocytes in surrounding native tissue or periosteal cells themselves to enter the defect and repair it.[3] However, recently, the effect of transplanted periosteum has not been consistent among several reports. One group of investigators reported that cells from the cambium layer of the periosteum may possibly be the cell source for repair of cartilage in rabbits.[18] Stimulation of chondrogenesis in periosteum with transforming growth factor[19] and stimulation of the remodeling process in subchondral bone from periosteum have been presented in other reports.[20] In contrast, Henderson et al. reported that any enhancing effect of periosteum on cell proliferation and cell numbers in chondrocyte cultures had not been demonstrated.[21] Several reports even have presented overgrowth of the graft, either in the periosteum or underlying neocartilage, as a major complaint,[3,7,21] along with the need to trim hypertrophic tissue. Nehrer et al. mentioned that early complications after ACI were the result of hypertrophy of the periosteal graft or its inadequate fixation.[8] To prevent these early problems, the use of collagen membrane as a seal instead of periosteum has been suggested.[20] Haddo et al. showed a lower rate of graft hypertrophy in ACI with a collagen membrane than with a periosteal flap.[22] Thus, the use of a periosteal patch might not be the best way to seal the defect during ACI. A collagen membrane may be better to seal the defect to avoid significant postoperative hypertrophy of the graft.

The regeneration of subchondral bone is also an important aspect in regenerating adequate joint components. In this study, more than half of the patients showed normal subchondral bone with the ICRS Visual Histological Assessment Scale. Although in ACI only laboratory-cultivated chondrocytes in suspension are applied to the defect, the effect of such injected cells is not comprehensible. Recent experiments have shown that articular cartilage cells have the potential to form bone tissue.[23] MR imaging after ACI has produced a few reports indicative of reparative subchondral bone in reparative tissue.[5] Normally, osteochondral defects were reported to have refilled with cartilage-like tissue.[12] Qiu et al. suggested that the quality of biological repair of osteochondral defects would be improved if subchondral bone responses were better regulated with proper plate reconstitution, which would more closely approach the level of native tissue.[24] When deep osteochondral defects are treated by conventional ACI, reparative tissues are slow to mature and there are some difficulties in restoring the congruity of articular cartilage.[7] The ACI sandwich technique, which includes cancellous bone grafting to fill the bone defect and periosteal grafting at the level of the subchondral bone plate to prevent bleeding into the cartilage defect, was developed for treatment of such deep osteochondral defects by Peterson.[25] Moreover, tissue engineering of osteochondral composite and special collagen scaffold composites for cartilage and subchondral bone[26] for treatment of osteochondral defects is one possible way to facilitate subchondral bone regeneration and make the operative procedure easier and simpler. In the future, tissue engineering techniques for cartilage[27] along with subchondral bone repair may become the basis for a significant therapeutic option in this field.

With respect to evaluation of reparative cartilage, Roberts et al. reported that there was a significant correlation between MR imaging score and the OsScore (so called because it originated in the Oswestry Laboratory) of samples obtained after ACI.[5] Watanabe et al. reported that the SI index (signal intensity of reparative cartilage divided by the signal intensity of normal cartilage) with 3D-SPGR MR imaging may be a useful parameter for noninvasive evaluation.[28] Furthermore, several sequences have recently been developed, such as delayed gadolinium-enhanced MR imaging for cartilage (dGEMRIC)[29] and T_2 mapping,[30] which can be used to assess cartilage GAG content and collagen arrangement. Because the availability of human biopsy samples after ACI is limited because of the need for invasive techniques to obtain the biopsy, MR imaging seems to be the best tool for morphological assessment of cartilage for long-term follow-up because it can provide detailed information on components noninvasively.

In summary, ACI provided good to excellent clinical outcomes at 1 year with our patients that were consistent with those of several previous reports, even though reparative cartilage varied from hyaline-like cartilage to fibrous cartilage, which is inferior to native cartilage. The biochemical nature of the matrix in reparative tissue was also inferior to that of native cartilage: there was significantly less GAG content in reparative tissue than in normal cartilage at 1 year after ACI. However, the long-term durability of reparative tissue has not yet been reported. Additional long-term, large-scale studies are required to determine the indications, advantages, and limitations of ACI.

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