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Atelocollagen-associated autologous chondrocyte implantation for the repair of large cartilage defects of the knee: results at three to seven years

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EK was involved in the design of the study, performed the clinical assessment, analysis and interpretation of data, and drafted and revised the manuscript. TK and KI were involved in the design of the study, assisted with data interpretation, and revised the manuscript for important intellectual content. KS, YO, ZT were involved in evaluations in their areas of expertise. MM, TO, DM, ST and NI were involved in the design of the study and the data acquisition and revised the manuscript critically for important intellectual content. All authors have read and approved the final manuscript.

Competing interests

The authors declare no conflicts of interest associated with this manuscript.

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Abstract

Background: Recently, various types of engineered autologous chondrocyte implantation (ACI) have been developed. Atelocollagen-associated ACI (A-ACI) is the only ACI procedure covered by Japanese Health Insurance since 2013. The indications of the A-ACI are traumatic cartilage defects and osteochondral dissecans (OCD) for knee joints.

Purpose: To evaluate midterm clinical results after implantation of A-ACI for the treatment for full-thickness cartilage defects of the knee.

Methods: Thirteen consecutive patients who underwent A-ACI between 2014 and 2018 had been prospectively enrolled in this study. There were 11 men and 2 women with a mean age of 34 years at the time of surgery. The causes of the cartilage defect were trauma in 10 knees and OCD in 3 knees. The total number of lesions was 15, which were comprised of the medial femoral condyle in 5 knees, the lateral femoral condyle in 5 knees, and the femoral trochlea in 5 knees. The mean size of the lesion was 5.3 cm². Each knee was clinically and radiologically evaluated preoperatively and postoperatively.

Results: The mean Lysholm score improved significantly from 74.0 points to 94.0 points ($p=0.008$) and each subscale in Knee injury and Osteoarthritis Outcome Score improved significantly ($p<0.001$) at the mean final follow-up period of 51 months (range, 36 to 84 months). The magnetic resonance observation of cartilage repair tissue 2.0 score at the mean follow-up of 38 months was significantly higher than that at 2 months postoperatively ($p=0.014$). According to the International Cartilage Repair Society (ICRS) grading scale, 3 knees were graded as normal, 3 knees as nearly normal, and 1 knee as severely abnormal in second-look arthroscopic evaluation at a mean of 22 months (range, 8-41 months) after A-ACI.

25 **Conclusion:** The present study showed a significantly subjective and objective clinical
26 improvement in the A-ACI for large cartilage defects of the knee at a mean follow-up of
27 51 months (range, 36 to 84 months).

28 **Keywords:** atelocollagen, autologous chondrocyte implantation, large cartilage defect,
29 knee

30

Introduction

Various surgical techniques for the cartilage lesions in the knee joint have been established. Autologous chondrocyte implantation (ACI) is one of the best candidates for large cartilage lesions. ACI was initially described by Brittberg et al., and good results were reported (1, 2). However, this technique has the risk of leakage and dedifferentiation of the implanted chondrocytes (3). To address these problems, various types of engineered ACI have been developed (4). Ochi et al. developed an ACI that utilized atelocollagen as a matrix to culture the harvested chondrocytes in three dimensions (3, 5). Recently, a few investigators have reported their clinical outcomes using this A-ACI (6-8). In Japan, this A-ACI is the only ACI procedure (JACC, Japan Tissue Engineering Co., Ltd., Gamagori, Japan) approved by the Ministry of Health, Labour and Welfare in 2012 and covered by Japanese Health Insurance since 2013. The indications of the A-ACI are categorized as traumatic cartilage defects and osteochondral dissecans (OCD) for knee joints. The use of this product is limited to patients with a defect area of over 4 cm² with no alternative therapy. Exclusion criteria for A-ACI were knee osteoarthritis and autoimmune disease such as rheumatoid arthritis. However, the previous studies included patients with a cartilage defect area of under 4 cm² or osteoarthritis (5-8). To our best knowledge, this is the first study reporting the results of the A-ACI in patients with cartilage lesions larger than 4 cm² caused by trauma or OCD after health insurance coverage in Japan.

The purpose of this study was to evaluate midterm clinical results after A-ACI for the treatment for large-sized and full-thickness cartilage defects of the knee.

Study Design

Consecutive patients who underwent A-ACI between 2014 and 2018 had been

prospectively enrolled in this study. The indications of A-ACI were as follows: (a) full-thickness cartilage lesion was caused by traumatic injury or OCD; (b) the cartilage lesion had either not been improved or was not anticipated to be improved by conventional treatments such as debridement, bone marrow stimulation (BMS), or autologous osteochondral transplantation (AOT); (c) the size of the cartilage defect was greater than 4.0 cm². Patients were not found eligible if the cartilage lesion was caused by osteoarthritis, rheumatoid arthritis, or other systemic joint diseases. All patients were followed up for at least three years, with a mean follow-up of 51 months (range, 36-84 months). Each knee was clinically and radiologically evaluated over time. Clinical evaluations were performed preoperatively, at 6, 12, and 24 months postoperatively, and at the final follow-up period. Radiological evaluation was carried out preoperatively, at 2, 6, and 12 months postoperatively, and at the time of the last magnetic resonance imaging (MRI) scan. Ethical approval for the study was obtained from the institutional review board and informed consents were obtained from all patients to participate in the present study.

Patient Demographics

A total of thirteen patients were operated on at a single institution by a single surgeon. The characteristics of patients are listed in Table 1. There were 11 men and 2 women with a mean age of 34 years at the time of A-ACI procedure. The causes of the cartilage defect were trauma in 10 knees and OCD in 3 knees. The total number of lesions was 15. The mean size of the lesion was 5.3 cm² (range, 4.0-10.5 cm²). In the case of anterior cruciate ligament (ACL) tear, meniscal tear, fracture, small cartilage defect, and malalignment of the lower limb, we addressed these comorbidities simultaneously (Table 1).

79

80 **Surgical Procedure**

81 The surgical technique involved two steps as previously described (9). Briefly, the first
82 step was to harvest articular cartilage (>0.4 g) from a non-weight-bearing area of the
83 femur. The harvested cartilage tissue was delivered to the facility (Japan Tissue
84 Engineering Co., Ltd.), where chondrocytes were isolated and suspended into
85 atelocollagen solution (3% type I collagen; Koken, Tokyo, Japan). The tissue-engineered
86 cartilage was then cultured in a three-dimensional environment for four weeks. Thus, the
87 atelocollagen gel containing three-dimensional cultured cartilage was delivered in the
88 form of gel discs. In the second step, a minimum arthrotomy was performed over the
89 affected lesion of the knee. The cartilage or osteochondral lesion was debrided until the
90 normal surrounding healthy cartilage and subchondral plate appeared (Fig. 1A, B). The
91 periosteal flap was harvested from the antero-medial side of the tibia and designed to
92 match the outline of the cartilage defect. The half side of the flap was sutured to the
93 surrounding rim of the normal cartilage using pull-out technique and 1.4 mm diameter
94 suture anchors (Jugger knot soft anchor system, Zimmer Biomet, Warsaw, IN, USA).
95 Afterward, the chondrocyte–atelocollagen gel was trimmed and positioned in the defect.
96 Finally, the remaining side of the periosteal flap was sutured (Fig. 1C, D).

97

98 **Postoperative Rehabilitation Protocol**

99 A postoperative immobilizer was applied for 2 weeks after surgery. Partial weight bearing
100 was introduced 4 weeks after surgery and was gradually increased to full weight bearing
101 over the first 8 weeks after surgery. Return to sporting activities was permitted from 12
102 months postoperatively. The rehabilitation protocol was modified individually depending

on each patient's comorbidities.

Clinical Evaluation

Each patient underwent clinical examinations at preoperative, 6, 12, 24 months postoperatively, and at the mean final follow-up of 51 months (range, 36-84 months). Clinical outcomes were assessed using the Lysholm score as the functional outcome and Knee injury and Osteoarthritis Outcome Score (KOOS) as the patient-reported subjective outcome. The KOOS score includes five subscales: symptoms, pain, function in daily living (ADL), function in sport and recreation (Sport/Rec), and knee-related quality of life (QOL). The activity levels before injury and at the final follow-up period were evaluated using the Tegner activity scale. Patient demographic data (age, BMI, Tegner activity scale, defect size), radiological, arthroscopic, and histological evaluations were analyzed for correlation with clinical outcomes. The radiological evaluation with the last MRI scans were used for analysis.

Radiological Evaluation

Plain radiographs were assessed for knee osteoarthritic changes by Kellgren and Lawrence grade. MRI with a knee coil was used to analyze implanted tissue radiologically. The MRI sequences included T1 weighted, T2-weighted, and fat-suppressed proton density images. The slice thickness and interslice gap were 3.0 mm and 0.3 mm, respectively. The repaired tissues were scored using the Magnetic Resonance Observation of Cartilage Repair Tissue 2.0 (MOCART) scoring system at 2, 6, 12 months after the implantation and at a mean follow-up of 38 months (range 24-53 months) (10). Images were assessed by the experienced radiologist, who was blinded to the patient's clinical

information.

Second-look Arthroscopic Evaluation

Seven of 13 patients underwent second-look arthroscopy at a mean of 22 months (range, 8-41 months) after A-ACI procedure. Second-look arthroscopies were performed when the patient was symptomatic after A-ACI procedure or implants placed during combined surgery were removed. The arthroscopic results were graded by the operating surgeon according to the assessment scale for cartilage repair after ACI with the Oswestry Arthroscopy Score (OAS) and the International Cartilage Repair Society (ICRS) score (11, 12). The OAS is a 10-point scale with 5 parameters. The ICRS score is a 12-point scale and grade I (12 points) is defined as normal, grade II (8–11 points) nearly normal, grade III (4–7 points) abnormal, and grade IV (1–3 points) severely abnormal.

Histological Evaluation

Six of 13 patients underwent biopsy during the second-look arthroscopy at a mean of 24 months (range, 13-41 months) after A-ACI procedure. A biopsy was taken from the center of the implanted graft. The specimens were sectioned through their long axis and sections were stained with hematoxylin-eosin (H&E), and safranin-O or toluidine blue for evaluation of proteoglycan content. The histological sections were scored using the ICRS II scoring system (visual analog scale of 0-100 each of 14 parameters) according to the consensus of two experienced pathologists, who were blinded to the patient's clinical information (13).

Statistical Analysis

Statistical analysis was performed using the IBM SPSS Statistics version 26 (IBM corp., Armonk, NY, USA). Friedman test followed by Wilcoxon t-test with Bonferroni correction was used to compare the values at each time point with the preoperative scores in clinical outcomes and MOCART 2.0 scores. Wilcoxon rank sum analysis was utilized to compare the Tegner activity scores between preoperative and postoperative scores. Spearman rank test was used to assess the correlations between clinical outcomes and background factors, radiological and histological evaluations. Statistical significance was set at $p = 0.05$.

Results

Clinical Evaluation

The Lysholm score improved gradually over time and maintained at the mean final follow-up of 51 months (Fig. 2A). Although there was no significant difference among the preoperative, 6- and 12- month scores, the scores at 24 months after the implantation and at the final follow-up were significantly ($p=0.029$ and $p=0.008$, respectively) higher than that at preoperative. Each subscale in KOOS improved significantly ($p<0.001$) throughout the follow-up period (Fig. 2B). The KOOS subscale scores of ADL and QOL improved significantly ($p=0.003$ and $p=0.002$, respectively) from 12 months. The subscale scores of symptoms, pain, and Sport/Rec improved significantly ($p<0.003$, $p<0.001$, and $p=0.001$, respectively) from 24 months. There were no significant differences in the final clinical outcomes between patients with or without comorbidities ($p=0.56, 0.74, 0.90, 0.98, 0.98$, and 0.82 for Lysholm score, KOOS symptoms, pain, ADL, Sport/Rec, and QOL, respectively). Concerning the Tegner activity scale, the mean preinjury and postoperative scores were 6.2 ± 1.4 points and 5.7 ± 1.5 points, respectively,

with no significant difference ($p=0.08$). Ten patients (77%) achieved the same level of activity as prior to injury. The remaining 3 patients (23%) decreased their Tegner activity score by only one level. Although the number of cases enrolled in the present study was not sufficient to obtain a level of significance for some parameters, age had a tendency to negatively affect clinical outcomes and a significant negative correlation with the KOOS QOL subscale ($r=-0.557$, $p=0.048$) (Table 2). There was a positive significant correlation between KOOS Sport/Rec subscale and MOCART parameters “surface of the repair tissue” ($r=0.611$, $p=0.035$) and “subchondral changes” ($r=0.724$, $p=0.008$). There was no significant correlation between clinical outcomes and BMI, Tegner activity scale, defect size, and arthroscopic and histological evaluations.

Radiological Evaluation

The plain radiograph revealed osteoarthritis progression from Kellgren and Lawrence grade 1 to grade 2 in the tibiofemoral joint in two patients. The preoperative and postoperative MRI images of three cases with different radiological courses are shown in Figure 3 based on the total score of the MOCART 2.0 score. MRI scans were evaluated over time in all cases. The time course of the median MOCART 2.0 score is shown in Figure 2C. The highest score was obtained at the mean final follow-up of 38 months, which was significantly higher than that at 2 months postoperatively ($p=0.014$). The percentages and numbers of each parameter in MOCART 2.0 score are listed in Table 3. Regarding the subscale of “volume fill of cartilage defect”, 67% of grafts showed “complete filling or minor hypertrophy”, and 20% showed “major hypertrophy or 75-99% filling”. Homogenous repair tissue was not observed until 12 months after surgery and reached only 27% of cases at the final follow-up. All cases had some degree of bony

defect and 74% of cases had residual edema-like bone marrow signal under repair tissue even at the final follow-up. Four grafts (27%) had hypertrophy greater than 150% of the surrounding normal cartilage at some point (Fig. 4).

Arthroscopic Evaluation

The arthroscopic findings showed that the transplants were in line with the surrounding articular surface (Fig. 5A-F). They displayed a cartilage-like white color and were slightly fibrillated but soft in both the central and marginal areas, whereas the marginal areas were harder than the central areas. One case experienced the delamination of implanted graft (Fig. 5G). The mean OAS score was 7.9 ± 2.1 , and the mean ICRS score was 10.1 ± 3.6 . According to the ICRS grading scale, 3 knees were graded as normal, 3 knees as nearly normal, and 1 knee as severely abnormal.

Histological Evaluation

Histological evaluation revealed that the implanted tissues were strongly stained with safranin-O in 3 specimens (Fig. 6A, C, D) and partially stained in 2 specimens (Fig. 6B, E) in the mid to deep zone. Large round cells in the lacunae were scattered in a proteoglycan-rich matrix (Fig. 6 A4-E4). In the superficial layer, smooth fibrous tissues with scattered spindle-shaped cells were observed in each specimen (Fig. 6 A3-F3). One case consisted only of fibrous tissue with blood vessel formation without a proteoglycan-rich matrix (Fig. 6F). The integrations between the regenerated cartilage-like tissue and subchondral bone were observed in all cases with a slight artifactual cleft space (Fig. 6 A5-F5). The four-layer structure observed in normal cartilage tissue was not evident in all specimens. According to the ICRS II scale, the median overall assessment of the

repaired tissue was 75 (range, 10-90). ICRS II scores showed a considerable degree of variability across individual parameters in the biopsies analyzed (Table 4).

Complications

Four transplanted grafts developed hypertrophic changes greater than 150% of the surrounding normal cartilage on MRI (Fig. 4). These patients had catching sensation and limited range of motion, which were resolved immediately after partial debridement of the hypertrophic tissue in second look arthroscopy.

One case treated with AOT for OCD at 8 years prior to A-ACI had partial detachment of the graft (Fig. 7). During the procedure of the A-ACI, the cartilage defect was covered by fibrous tissue with underlying sclerotic subchondral bone (Fig. 7A). The implanted tissue got hypertrophic and inhomogeneous 6 months after the implantation (Fig. 7B). Arthroscopic evaluation at 8 months after surgery revealed that half of the implanted tissue was detached (Fig. 5G). Arthroscopic removal of the detached graft and debridement of surrounding tissues were performed. Osteosclerosis of the subchondral bone under the detached graft was observed (Fig. 7C). Histologically, the detached graft fragment consisted of cartilage-like tissue stained with toluidine blue in the center (Fig. 7D).

Discussion

We evaluated the clinical, radiological, and histological outcomes of A-ACI for the treatment of large-sized and full-thickness cartilage defects of the knee. As a result, we found that the clinical scores based on Lysholm score and KOOS improved significantly at a minimum of 3 years follow-up. In MRI evaluation, MOCART 2.0 score was

significantly higher at the mean follow-up of 38 months than that at 2 months postoperatively. Arthroscopic assessment indicated that 86% of the patients had a “normal” or “nearly normal” appearance of transplanted grafts using the ICRS score. In addition, histological evaluation revealed that 83% of specimens contained cartilage-like tissue stained with safranin-O. Therefore, the findings of the present study suggest that transplanting chondrocytes embedded in a three-dimensional atelocollagen gel matrix (A-ACI) promotes restoration of the articular cartilage of the knee.

The present study showed that the A-ACI led to a significant improvement in clinical outcomes. Lysholm score improved significantly from 74.0 points to 94.0 points, and all subscales of KOOS improved significantly at the mean follow-up of 51 months. The Lysholm score in this study was comparable to the score of 89.8 to 96.7 points obtained in the previous studies on the A-ACI procedures with the mean lesion sizes less than 4 cm² at 2 to 8 years postoperatively (5-8). In the present study, the subjective clinical score, which had not been assessed in previous studies on A-ACI, improved significantly based on the KOOS. Zhang et al. reviewed the results of various ACI procedures for treating large-sized (> 4 cm²) cartilage defects, and referred to the effectiveness of matrix-assisted ACI (2). Our results also showed no significant correlation between cartilage defect size greater than 4 cm² and clinical outcomes. These results and reports support that A-ACI provides a favorable midterm clinical outcome for large-sized cartilage lesions of the knee. In cases where the lesion is large, extending into the subchondral bone or edge of the articular cartilage, reconstruction of the bone bed or wall should be considered (9, 14, 15). MRI and histology are commonly used to predict clinical outcomes; however, their utility is controversial (16-18). In this study, there was no significant correlation between clinical outcomes and most parameters of the MOCART

score, arthroscopic evaluation, and histological score. Age had a significant negative correlation with KOOS QOL subscale and only a slight tendency for negative correlation with the other clinical outcomes. Senescence of chondrocytes at the molecular level has been reported to occur with advancing age (19). In practice, a review article has reported a negative effect of increasing age on clinical outcomes in the ACI procedure (20). Although there is no age limit for patients undergoing A-ACI, the indication should be considered carefully.

In this study, comorbidities such as ACL tear and meniscal tear were addressed to provide a favorable environment for transplanted tissue-engineered cartilage to engraft. The additional high tibial osteotomy procedure has been reported to reduce revision rate and improve the longevity of ACI in patients with varus deformity (21). ACI combined with ACL reconstruction has been reported to have good short-term clinical outcomes similar to those after isolated ACI in the knee with intact ligament (9, 22-24). Pike et al. stated that ACL insufficiency with relatively large chondral defects could be effectively managed with concomitant ACL reconstruction and ACI (24). These reports support the necessity of managing addressable comorbidities.

In this study, the median MOCART 2.0 score gradually improved over time postoperatively and the highest score was given at the mean final follow-up of 38 months with 67.5 points. The MOCART scores after ACI surgery have been reported to range from 60 to 72.5 at 1 to 10 years (7, 8, 15-17). MOCART scores after A-ACI were reported to be 70 and 70.7 for mean lesion sizes of 3.6 cm² and 3.1 cm², respectively (7, 8). The results of our study and these reports suggest that A-ACI is qualitatively comparable even for lesions larger than 4.0 cm². A review article for ACI demonstrated that 58.9-82% of grafts showed a complete filling or minor hypertrophy, 21.7% of graft showed

homogenous structure, 87% of grafts had bone marrow edema (2). These reports support that A-ACI in this study shows a similar pattern of postoperative MRI changes as the other ACIs.

The present study arthroscopically evaluated 7 of 13 cases (54%) at 1-2 years after the implantation; the mean OAS and ICRS scores were 7.9 and 10.1, respectively. Our arthroscopic finding is consistent with the previous reports on A-ACI, in which 87-93% were graded as “normal” or “nearly normal” in the ICRS score (6-8).

In this study, the overall assessment of ICRS II score averaged 75 and three of six engrafted tissue exhibited strong staining with safranin-O. ICRS II scores showed high variability in all parameters of biopsies analyzed as previously reported by McCarthy et al. (17). Adachi et al. reported an ICRS II score of 70.4 after A-ACI with a mean defect size of 3.6 cm² and observed cartilage-like tissues; however, the normal layer composition was not well constructed (7). This result is consistent with our histological findings, suggesting that A-ACI could yield qualitatively identical tissue for small- and large-sized cartilage defects.

The reported failure and reoperation rates after ACI were 7-26% and 13-68%, respectively (25, 26). Three studies have reported complications and failures after A-ACI ranging from 11 to 30% (5-7). In the present study, five patients (38%) had to undergo revision arthroscopy because of symptomatic graft hypertrophy or partial graft detachment. Hypertrophy of implanted graft is a common complication and the rate range is reported from 9% to 40% (27). Although most hypertrophic grafts have been described to decline in thickness after 24 months and have no effect on symptoms and quality of repaired cartilage, hypertrophy greater than 150% is a risk of surgical intervention (28-30). Indeed, our case that required surgical intervention had a greater than 150%

hypertrophy. The risk of hypertrophy is reported to be higher in periosteum-covered ACI compared to membrane-covered ACI (25, 29). Future studies are needed to compare the effectiveness of collagen and periosteal membrane for A-ACI. In the present study, we experienced partial graft detachment. Although favorable outcomes of ACI for OCD have been reported, 6 of 8 patients who required revision ACI had previous surgical treatment (31). The ACI after a failed BMS was reported to have inferior outcomes compared to primary ACI, which was attributed to the underlying subchondral bone violation (32, 33). Our case had previously taken AOT for OCD and the sclerotic change was observed at the site of implantation. The detachment of the graft might be owing to the altered sclerotic bone bed after the procedure of AOT. A few authors reported the combination procedure of autologous bone grafts and ACI to address the sclerotic subchondral bone for OCD (14, 15). This combined surgical procedure might be a possible alternative to address both cartilage lesion and altered bone plate simultaneously.

This study has several limitations. The first limitation is that the number of patients enrolled in the present study was small. Second, the minimum follow-up period of the present study was 36 months. Further follow-up is needed for all patients in this study, as the clinical results after ACI are reported to deteriorate over the long term (16, 34). Third, performance rates of the second-look arthroscopy and biopsy were low since they were not routinely performed in all case, resulting in sampling bias. Fourth, we did not evaluate the mechanical characteristics of the grafted tissues (5). Fifth, we did not evaluate biopsy specimens immunohistochemically for type I and type II collagen. Despite these limitations, we believe that the present study has provided important information for the treatment of large-size and full-thickness cartilage defects in the knee joint.

In conclusion, the present study of A-ACI showed a significant subjective and objective clinical improvement in the repair of large cartilage defects of the knee at a minimum follow-up period of 3 years. Our study is the first report of A-ACI for knee cartilage defects larger than 4.0 cm² excluding knee osteoarthritis after the marketing approval.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from the patient for publication.

Consent to publish

Written informed consents was obtained from the patients for publication.

Competing interests

The authors declare no conflicts of interest associated with this manuscript.

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References

1. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994 Oct 6;331(14):889-95.
2. Zhang C, Cai YZ, Lin XJ. Autologous chondrocyte implantation: Is it likely to become a saviour of large-sized and full-thickness cartilage defect in young adult knee. *Knee Surg Sports Traumatol Arthrosc* 2016 May;24(5):1643-50.
3. Ochi M, Uchio Y, Tobita M, Kuriwaka M. Current concepts in tissue engineering technique for repair of cartilage defect. *Artif Organs* 2001 Mar;25(3):172-9.
4. Krill M, Early N, Everhart JS, Flanigan DC. Autologous chondrocyte implantation (ACI) for knee cartilage defects: a review of indications, technique, and outcomes. *JBJS Rev* 2018 Feb;6(2):e5.
5. Ochi M, Uchio Y, Kawasaki K, Wakitani S, Iwasa J. Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defects of the knee. *J Bone Joint Surg Br* 2002 May;84(4):571-8.
6. Tohyama H, Yasuda K, Minami A, Majima T, Iwasaki N, Muneta T, et al. Atelocollagen-associated autologous chondrocyte implantation for the repair of chondral defects of the knee: a prospective multicenter clinical trial in Japan. *J Orthop Sci* 2009 Sep;14(5):579-88.
7. Adachi N, Ochi M, Deie M, Nakamae A, Kamei G, Uchio Y, et al. Implantation of tissue-engineered cartilage-like tissue for the treatment for full-thickness cartilage defects of the knee. *Knee Surg Sports Traumatol Arthrosc* 2014 Jun;22(6):1241-8.
8. Takazawa K, Adachi N, Deie M, Kamei G, Uchio Y, Iwasa J, et al. Evaluation of magnetic resonance imaging and clinical outcome after tissue-engineered cartilage

- implantation: prospective 6-year follow-up study. J Orthop Sci 2012 Jul;17(4):413-24.
9. Kaibara T, Kondo E, Matsuoka M, Iwasaki K, Onodera T, Momma D, et al. Large osteochondral defect in the lateral femoral condyle reconstructed by atelocollagen-associated autologous chondrocyte implantation combined with anterior cruciate ligament reconstruction. BMC Musculoskelet Disord 2020 Jul 27;21(1):494.
10. Schreiner MM, Raudner M, Marlovits S, Bohndorf K, Weber M, Zalaudek M, et al. The MOCART (magnetic resonance observation of cartilage repair tissue) 2.0 knee score and atlas. Cartilage 2019 Aug 17:1947603519865308.
11. Smith GD, Taylor J, Almqvist KF, Erggelet C, Knutsen G, Garcia Portabella M, et al. Arthroscopic assessment of cartilage repair: a validation study of 2 scoring systems. Arthroscopy 2005 Dec;21(12):1462-7.
12. Van Den Borne MPJ, Raijmakers NJH, Vanlauwe J, Victor J, De Jong SN, Bellemans J, et al. International cartilage repair society (ICRS) and oswestry macroscopic cartilage evaluation scores validated for use in autologous chondrocyte implantation (ACI) and microfracture. Osteoarthritis and Cartilage 2007;15(12):1397-402.
13. Mainil-Varlet P, Van Damme B, Nesic D, Knutsen G, Kandel R, Roberts S. A new histology scoring system for the assessment of the quality of human cartilage repair: ICRS II. Am J Sports Med 2010 May;38(5):880-90.
14. Steinhagen J, Bruns J, Deuretzbacher G, Ruether W, Fuerst M, Niggemeyer O. Treatment of osteochondritis dissecans of the femoral condyle with autologous bone grafts and matrix-supported autologous chondrocytes. Int Orthop 2010 Aug;34(6):819-25.
15. Ochs BG, Müller-Horvat C, Albrecht D, Schewe B, Weise K, Aicher WK, et al. Remodeling of articular cartilage and subchondral bone after bone grafting and matrix-

associated autologous chondrocyte implantation for osteochondritis dissecans of the knee.
Am J Sports Med 2011 Apr;39(4):764-73.

16. Aldrian S, Zak L, Wondrasch B, Albrecht C, Stelzeneder B, Binder H, et al.
Clinical and radiological long-term outcomes after matrix-induced autologous
chondrocyte transplantation: a prospective follow-up at a minimum of 10 years. Am J
Sports Med 2014 Nov;42(11):2680-8.

17. McCarthy HS, McCall IW, Williams JM, Mennan C, Dugard MN, Richardson
JB, et al. Magnetic resonance imaging parameters at 1 year correlate with clinical
outcomes up to 17 years after autologous chondrocyte implantation. Orthop J Sports Med
2018 Aug;6(8):2325967118788280.

18. de Windt TS, Welsch GH, Brittberg M, Vonk LA, Marlovits S, Trattinig S, et al.
Is magnetic resonance imaging reliable in predicting clinical outcome after articular
cartilage repair of the knee? A systematic review and meta-analysis. Am J Sports Med
2013 Jul;41(7):1695-702.

19. Toh WS, Brittberg M, Farr J, Foldager CB, Gomoll AH, Hui JH, et al. Cellular
senescence in aging and osteoarthritis. Acta Orthop 2016 Dec;87(sup363):6-14.

20. Jeuken RM, van Hugten PPW, Roth AK, Timur UT, Boymans T, van Rhijn LW,
et al. A systematic review of focal cartilage defect treatments in middle-aged versus
younger patients. Orthop J Sports Med 2021 Oct;9(10):23259671211031244.

21. Bode G, Schmal H, Pestka JM, Ogon P, Südkamp NP, Niemeyer P. A non-
randomized controlled clinical trial on autologous chondrocyte implantation (ACI) in
cartilage defects of the medial femoral condyle with or without high tibial osteotomy in
patients with varus deformity of less than 5°. Arch Orthop Trauma Surg 2013
Jan;133(1):43-9.

22. Mehl J, Feucht M, Achtnich A, Imhoff AB, Niemeyer P, Schmitt A, et al. Autologous chondrocyte implantation combined with anterior cruciate ligament reconstruction: similar short-term results in comparison with isolated cartilage repair in ligament intact joints. *Knee Surg Sports Traumatol Arthrosc* 2022 Oct;30(10):3249-57.
23. Amin AA, Bartlett W, Gooding CR, Sood M, Skinner JA, Carrington RW, Briggs TW, Bentley G. The use of autologous chondrocyte implantation following and combined with anterior cruciate ligament reconstruction. *Int Orthop* 2006 Feb;30(1):48-53.
24. Pike AN, Bryant T, Ogura T, Minas T. Intermediate- to long-term results of combined anterior cruciate ligament reconstruction and autologous chondrocyte implantation. *Orthop J Sports Med* 2017 Feb;5(2):2325967117693591.
25. Harris JD, Siston RA, Brophy RH, Lattermann C, Carey JL, Flanigan DC. Failures, re-operations, and complications after autologous chondrocyte implantation: a systematic review. *Osteoarthritis Cartilage* 2011 Jul;19(7):779-91.
26. Pareek A, Carey JL, Reardon PJ, Peterson L, Stuart MJ, Krych AJ. Long-term outcomes after autologous chondrocyte implantation: a systematic review at mean follow-up of 11.4 years. *Cartilage* 2016 Oct;7(4):298-308.
27. Niethammer TR, Loitzsch A, Horng A, Baur-Melnyk A, Bendiks M, Gülecyyüz MF, et al. Graft hypertrophy after third-generation autologous chondrocyte implantation has no correlation with reduced cartilage quality: matched-pair analysis using T2-weighted mapping. *Am J Sports Med* 2018 Aug;46(10):2414-21.
28. Niemeyer P, Pestka JM, Kreuz PC, Erggelet C, Schmal H, Suedkamp NP, et al. Characteristic complications after autologous chondrocyte implantation for cartilage defects of the knee joint. *Am J Sports Med* 2008 Nov;36(11):2091-9.
29. Zeifang F, Oberle D, Nierhoff C, Richter W, Moradi B, Schmitt H. Autologous

chondrocyte implantation using the original periosteum-cover technique versus matrix-associated autologous chondrocyte implantation: a randomized clinical trial. *Am J Sports Med* 2010 May;38(5):924-33.

30. Kreuz PC, Steinwachs M, Erggelet C, Krause SJ, Ossendorf C, Maier D, et al. Classification of graft hypertrophy after autologous chondrocyte implantation of full-thickness chondral defects in the knee. *Osteoarthritis Cartilage* 2007 Dec;15(12):1339-47.

31. Carey JL, Shea KG, Lindahl A, Vasiliadis HS, Lindahl C, Peterson L. Autologous chondrocyte implantation as treatment for unsalvageable osteochondritis dissecans: 10- to 25-year follow-up. *Am J Sports Med* 2020 Apr;48(5):1134-40.

32. Minas T, Gomoll AH, Rosenberger R, Royce RO, Bryant T. Increased failure rate of autologous chondrocyte implantation after previous treatment with marrow stimulation techniques. *Am J Sports Med* 2009 May;37(5):902-8.

33. Pestka JM, Bode G, Salzmänn G, Südkamp NP, Niemeyer P. Clinical outcome of autologous chondrocyte implantation for failed microfracture treatment of full-thickness cartilage defects of the knee joint. *Am J Sports Med* 2012 Feb;40(2):325-31.

34. Bentley G, Biant LC, Vijayan S, Macmull S, Skinner JA, Carrington RW. Minimum ten-year results of a prospective randomised study of autologous chondrocyte implantation versus mosaicplasty for symptomatic articular cartilage lesions of the knee. *J Bone Joint Surg Br* 2012 Apr;94(4):504-9.

Figure legends

Figure 1. The surgical procedure of the atelocollagen-associated ACI in the medial femoral condyle. (A) The affected lesion was exposed with a minimal arthrotomy. (B) Debridement of the calcified cartilage until normal surrounding healthy cartilage and subchondral plate appeared. (C) The half side of the periosteal flap was sutured to the surrounding rim of the normal cartilage using the pull-out technique and atelocollagen-associated ACI was placed on the prepared lesion bed. (D) The atelocollagen-associated ACI was covered by suturing the remaining side of the periosteal flap. ACI, autologous chondrocyte implantation.

Figure 2. Box-and-whisker plots of clinical outcomes and MOCART 2.0 score. (A) Lysholm score, (B) KOOS score, (C) MOCART 2.0 score. Boxes are drawn between the first and third quartile. The whiskers represent minimum and maximum values, the bands inside box indicate the median. Outliers are plotted with small circles. Friedman test followed by Wilcoxon t-test with Bonferroni correction was used to compare the values at each time point with the preoperative scores. * $p < 0.05$; ** $p < 0.01$. KOOS, Knee Injury and Osteoarthritis Outcome Score; Sport/Rec, function in sport and recreation; QOL, knee-related quality of life; MOCART, Magnetic Resonance Observation of Cartilage Repair Tissue.

Figure 3. The pre- and postoperative MRI images of the three cases. The MRI images of Case 6 showed over time improvement of the grafted tissue in the integration into adjacent cartilage, signal intensity, and subchondral bone lamina. The MRI images of Case 11 exhibited hypertrophy of the grafted tissue, however, the signal intensity was normal. The

MRI images of Case 7 demonstrated hypertrophy and high signal intensity of the grafted tissue, consequently requiring a partial debridement. MRI, magnetic resonance imaging.

Figure 4. Hypertrophic change of atelocollagen-associated autologous chondrocyte implantation observed by magnetic resonance imaging. Hypertrophy of the implanted grafts greater than 150% of the adjacent cartilage was seen in 4 cases. Medial femoral condyle in coronal view (A), lateral femoral condyle in sagittal view (B), and femoral trochleas in axial view (C, D). The white arrows indicate hypertrophic grafts.

Figure 5. Second-look arthroscopic findings of atelocollagen-associated autologous chondrocyte implantation. The transplanted grafts appeared to be the same color as the surrounding normal cartilage although they showed a fibrillated surface.

Figure 6. Histological results of biopsies taken from implanted grafts during second-look arthroscopy in six patients (A-F). Histological sections of samples were stained with hematoxylin-eosin (1, 3, 5) and Safranin-O (2, 4). Three specimens (A, C, D) were strongly stained and two specimens (B, E) were partially stained with safranin-O in the mid to deep zone of regenerated tissue. In the superficial layer, smooth fibrous tissues with scattered spindle-shaped cells were observed (A3-F3). Large round cells in lacunae were scattered in a proteoglycan-rich matrix (A4-E4). One case consisted only of fibrous tissue with blood vessel formation (F). Black scale bar, 500 μm ; yellow scale bar, 200 μm ; white scale bar, 50 μm ; blue scale bar 20 μm .

Figure 7. Partial detachment of the implanted graft after the A-ACI in a patient of OCD

previously treated with AOT. (A) The cartilage defect was covered by fibrous tissue with underlying sclerotic subchondral bone during the procedure of A-ACI. (B) Implanted tissue got hypertrophic and inhomogeneous on MRI at 6 months postoperatively. (C) Arthroscopic removal of the detached graft and debridement of surrounding tissues were performed. Osteosclerosis of the subchondral bone under the detached graft was observed. (D) Histologically, the center of the detached graft was composed of cartilage-like tissue stained with toluidine blue. The black scale bar indicates 200 μ m. A-ACI, atelocollagen-associated autologous chondrocyte implantation; OCD, osteochondritis dissecans; AOT, autologous osteochondral transplantation; MRI, magnetic resonance imaging.

1 Table 1. Patient Demographics

Case	Age, y	Sex	BMI (kg/m ²)	Cause	Lesion site	Defect size, cm ²	Previous surgery	Combined surgery	Period at second look arthroscopy, mo	Follow-up period, mo
1	38	M	24.6	Trauma	MFC	6	BMS, MCL repair	ACLR + bone graft transplantation	13	49
2	28	M	19.4	OCD	LFC	4	AOT	BMS	8	47
3	41	M	24.9	Trauma	MFC	5	-	AOT	-	84
4	42	M	19.8	Trauma	LFC	4	-	Meniscal reconstruction	20	67
5	47	M	25.7	Trauma	MFC	5	-	ACLR	24	36
6	16	M	17.9	OCD	LFC	4	AOT	-	24	45
7	15	F	20.0	Trauma	LFC	5	-	Meniscal resection	24	60
8	43	M	27.0	Trauma	Trochlea	10.5	-	AOT	41	52
9	39	M	22.9	Trauma	MFC, Trochlea	5, 4	-	OWHTO, meniscal repair	-	47
10	16	M	26.0	OCD	MFC	5	-	-	-	39
11	26	M	19.3	Trauma	Trochlea	8	BMS	-	-	36
12	52	F	21.5	Trauma	Trochlea	5	-	AOT	-	36
13	40	M	33.6	Trauma	LFC, Trochlea	4, 5	AOT	-	-	39

2 BMI, body mass index; OCD, osteochondritis dissecans; MFC, medial femoral condyle; LFC, lateral femoral condyle; BMS, bone marrow
3 stimulation; MCL, medial collateral ligament; AOT, autologous osteochondral transplantation; ACLR, anterior cruciate ligament
4 reconstruction; OWHTO, open-wedge high tibial osteotomy

5

6 Table 2. Spearman correlation coefficient and two-tailed *p*-values between clinical outcomes and demographic data, MOCART 2.0 score,
7 arthroscopic evaluations, and histological evaluation

	Lysholm	KOOS				
		Symptoms	Pain	ADL	Sport/Rec	QOL
Demographic data						
Age	-0.420 (0.153)	-0.412 (0.162)	-0.288 (0.339)	-0.294 (0.329)	-0.257 (0.397)	-0.557 (0.048)
BMI	-0.143 (0.641)	-0.265 (0.382)	-0.205 (0.502)	-0.191 (0.532)	0.227 (0.455)	-0.141 (0.646)
Preoperative Tegner activity scale	0.058 (0.850)	0.319 (0.288)	0.305 (0.310)	0.076 (0.804)	0.504 (0.079)	0.119 (0.700)
Postoperative Tegner activity scale	-0.028 (0.928)	0.194 (0.526)	0.184 (0.548)	-0.012 (0.968)	0.290 (0.337)	-0.028 (0.928)
Defect size	0.063 (0.838)	-0.195 (0.523)	0.085 (0.782)	-0.018 (0.953)	-0.220 (0.470)	0.257 (0.397)
MOCART 2.0 score						
Volume fill of cartilage defect	-0.370 (0.237)	-0.187 (0.560)	-0.533 (0.074)	-0.439 (0.153)	-0.137 (0.671)	-0.221 (0.491)
Integration into adjacent cartilage	0.219 (0.495)	0.312 (0.324)	0.397 (0.201)	0.187 (0.561)	0.310 (0.328)	0.484 (0.111)
Surface of the repair tissue	0.080 (0.804)	0.374 (0.231)	0.392 (0.208)	0.112 (0.730)	0.611 (0.035)	0.336 (0.286)
Structure of the repair tissue	0.125(0.683)	0.488 (0.091)	0.212 (0.488)	-0.014 (0.963)	0.372 (0.210)	0.214 (0.482)
Signal intensity of the repair tissue	-0.021 (0.947)	0.210 (0.512)	0.232 (0.468)	0.029 (0.928)	0.349 (0.267)	0.018 (0.957)
Bony defect or bony overgrowth	-0.389 (0.211)	-0.424 (0.169)	-0.348 (0.267)	-0.427 (0.166)	-0.26 (0.937)	-0.290 (0.361)
Subchondral changes	0.288 (0.364)	0.366 (0.242)	0.572 (0.052)	0.464 (0.129)	0.724 (0.008)	0.397 (0.201)
Total score	-0.066 (0.832)	0.260 (0.390)	0.209 (0.493)	-0.031 (0.920)	0.474 (0.102)	0.136 (0.658)
Arthroscopic evaluation						
OAS	-0.383 (0.454)	-0.552 (0.256)	0.147 (0.781)	-0.224 (0.670)	-0.702 (0.120)	-0.471 (0.346)
ICRS	-0.098 (0.854)	-0.198 (0.707)	0.488 (0.326)	0.000 (1.000)	-0.396 (0.437)	-0.098 (0.854)
Histological evaluation						
ICRS II overall assessment	0.004 (0.994)	0.000 (1.000)	0.719 (0.107)	0.230 (0.661)	0.122 (0.818)	0.467 (0.350)

8 Values are expressed as Spearman's correlation coefficient (p -value). KOOS, Knee Injury and Osteoarthritis Outcome Score; Sport/Rec,
9 function in sport and recreation; QOL, knee-related quality of life; BMI, body mass index; MOCART, Magnetic Resonance Observation
10 of Cartilage Repair Tissue; OAS, Oswestry Arthroscopy Score; ICRS, International Cartilage Repair Society II.

11

12 Table 3. The percentages and numbers of each parameter in MOCART 2.0 score

MOCART 2.0 Parameter	Postoperative			
	2 months	6 months	12 months	Last scan
1 Volume fill of cartilage defect				
Complete filling Or minor hypertrophy: 100% to 150% filling of total defect volume	40% (6)	53% (8)	67% (10)	67% (10)
Major hypertrophy $\geq 150\%$ Or 75% to 99% filling of total defect volume	40% (6)	20% (3)	13% (2)	20% (3)
50% to 74% filling of total defect volume	13% (2)	20% (3)	13% (2)	13% (2)
25% to 49% filling of total defect volume	7% (1)	0% (0)	7% (1)	7% (1)
<25% filling of total defect volume Or complete delamination in situ	0% (0)	7% (1)	0% (0)	0% (0)
2 Integration into adjacent cartilage				
Complete integration	60% (9)	87% (13)	73% (11)	93% (14)
Split-like defect at repair tissue and native cartilage interface ≤ 2 mm	40% (6)	7% (1)	27% (4)	7% (1)
Defect at repair tissue and native cartilage interface >2 mm, but $< 0\%$ of repair tissue length	0% (0)	0% (0)	0% (0)	0% (0)
Defect at repair tissue and native cartilage interface $\geq 50\%$ of repair tissue length	0% (0)	7% (1)	0% (0)	0% (0)
3 Surface of the repair tissue				
Surface intact	13% (2)	20% (3)	20% (3)	47% (7)
Surface irregular $< 50\%$ of repair tissue diameter	40% (6)	33% (5)	47% (7)	33% (5)
Surface irregular $\geq 50\%$ of repair tissue diameter	47% (7)	47% (7)	33% (5)	20% (3)
4 Structure of the repair tissue				
Homogeneous	0% (0)	0% (0)	0% (0)	27% (4)
inhomogeneous	100% (15)	100% (15)	100% (15)	73% (11)
5 Signal intensity of the repair tissue				
Normal	7% (1)	20% (3)	20% (3)	46% (6)
Minor abnormal—minor hyperintense Or minor hypointense	80% (12)	73% (11)	80% (12)	54% (7)
Severely abnormal—almost fluid like Or close to subchondral plate signal	13% (2)	7% (1)	0% (0)	0% (0)
6 Bony defect or bony overgrowth				
No bony defect or bony overgrowth	7% (1)	7% (1)	7% (1)	0% (0)

Bony defect: depth < thickness of adjacent cartilage Or overgrowth < 50% of adjacent cartilage	47% (7)	27% (4)	27% (4)	33% (5)
Bony defect: depth ≥ thickness of adjacent cartilage Or overgrowth ≥ 50% of adjacent cartilage	47% (7)	67% (10)	67% (10)	67% (10)
7 Subchondral changes				
No major subchondral changes	7% (1)	20% (3)	20% (3)	27% (4)
Minor edema-like marrow signal—maximum diameter <50% of repair tissue diameter	13% (2)	40% (6)	33% (5)	27% (4)
Severe edema-like marrow signal—maximum diameter ≥ 50% of repair tissue diameter	80% (12)	40% (6)	47% (7)	40% (6)
Subchondral cyst ≥ 5 mm in longest diameter Or osteonecrosis-like signal	0% (0)	0% (0)	0% (0)	7% (1)
Median total score (points)	55.0	65.0	60.0	67.5

14 Table 4. International Cartilage Repair Society (ICRS) II score

ICRS II Parameter	ICRS II score
	Median (range)
Tissue morphology	50 (15-70)
Matrix Staining	65 (10-90)
Cell Morphology	70 (5-80)
Chondrocyte clustering	100 (100)
Architecture of Surface	97.5 (70-100)
Basal Integration	90 (30-100)
Calcification Front/Tidemark	45 (0-60)
Subchondral Bone Abnormalities	95 (0-100)
Abnormal Calcification	100 (100)
Inflammation	100 (100)
Vascularization in Repair Tissue	100 (5-100)
Surface/Superficial Assessment	30 (10-90)
Mid//Deep Zone Assessment	50 (10-80)
Overall Assessment	75 (10-90)

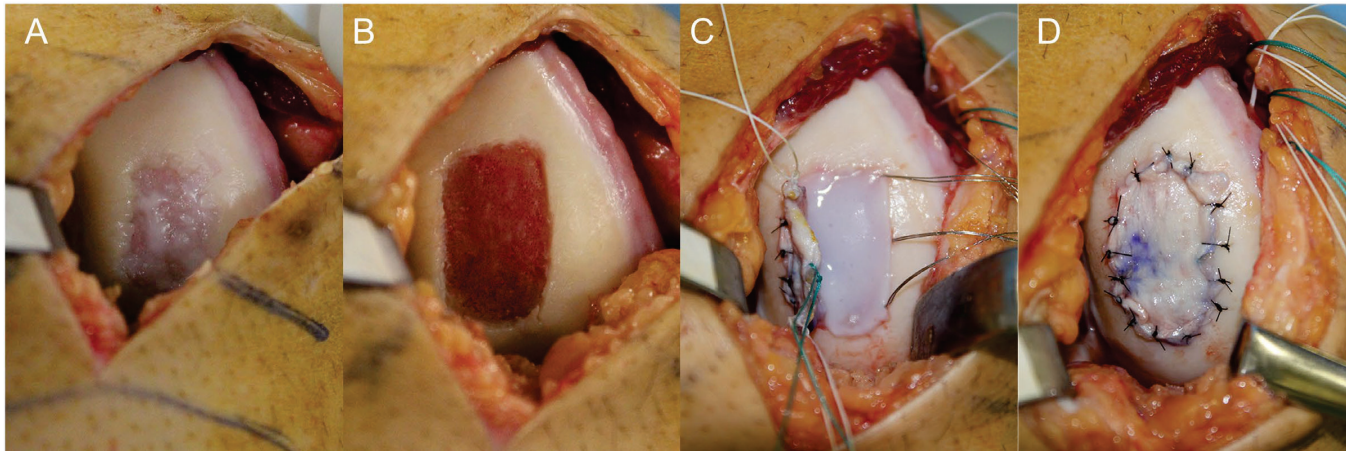


Fig. 1

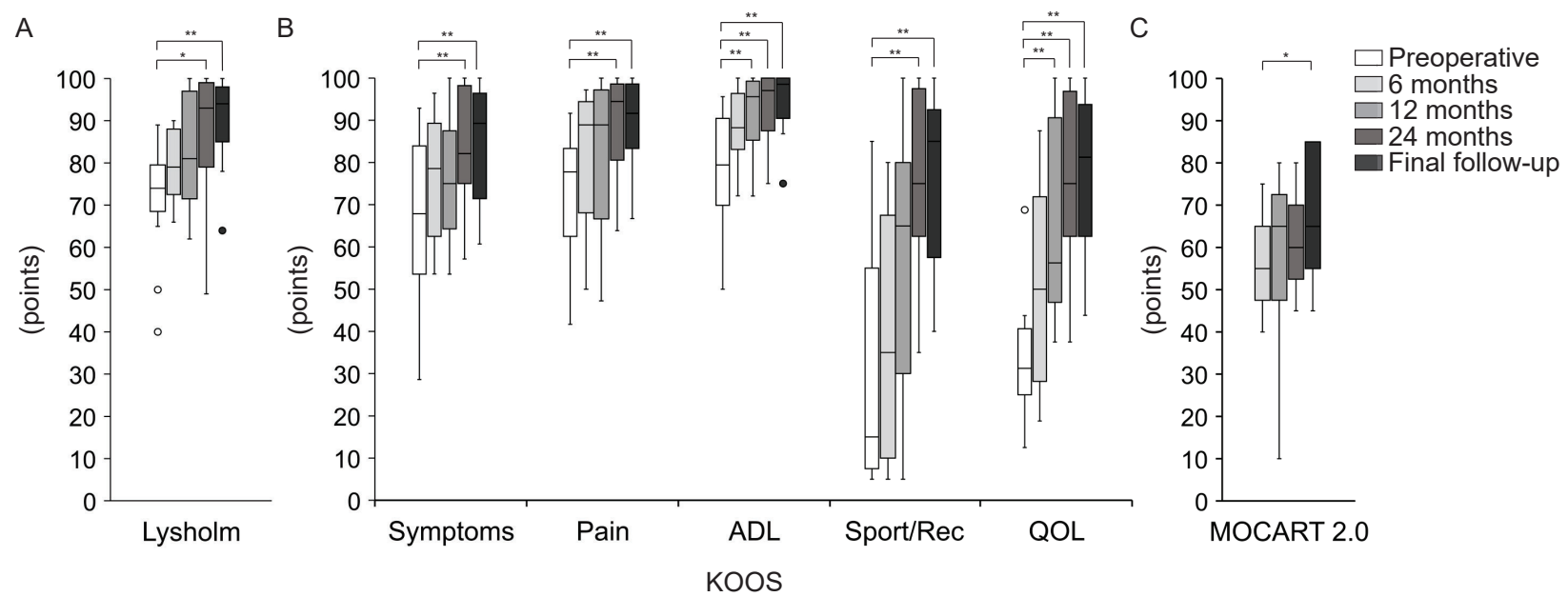
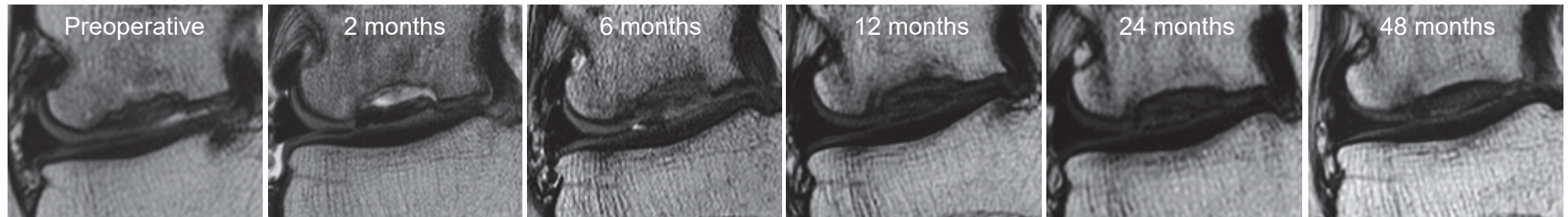
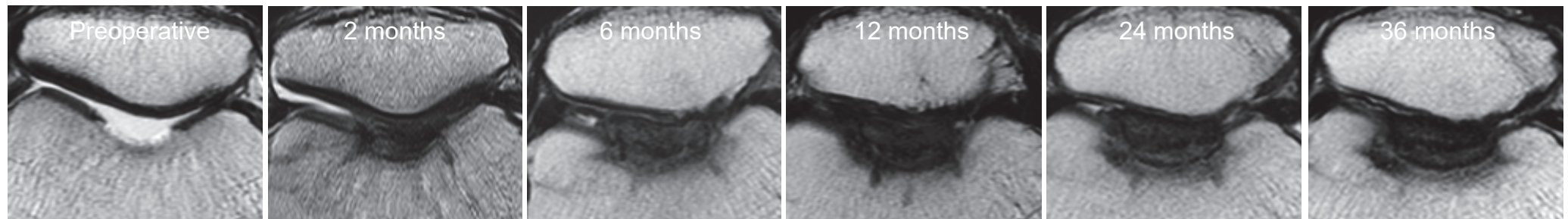


Fig. 2

Case 6



Case 11



Case 7

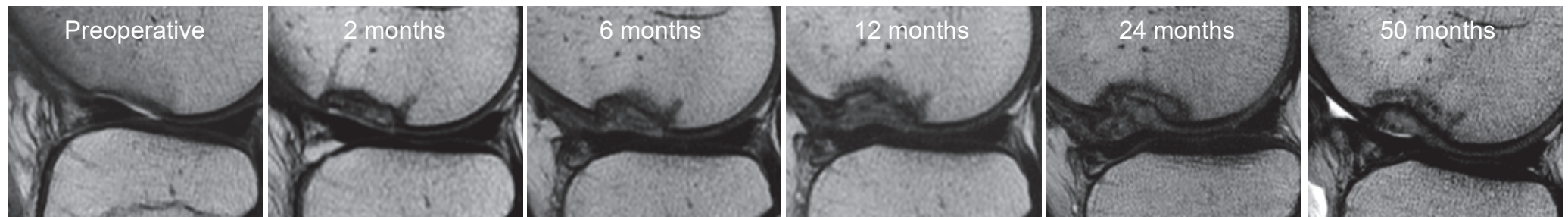


Fig. 3

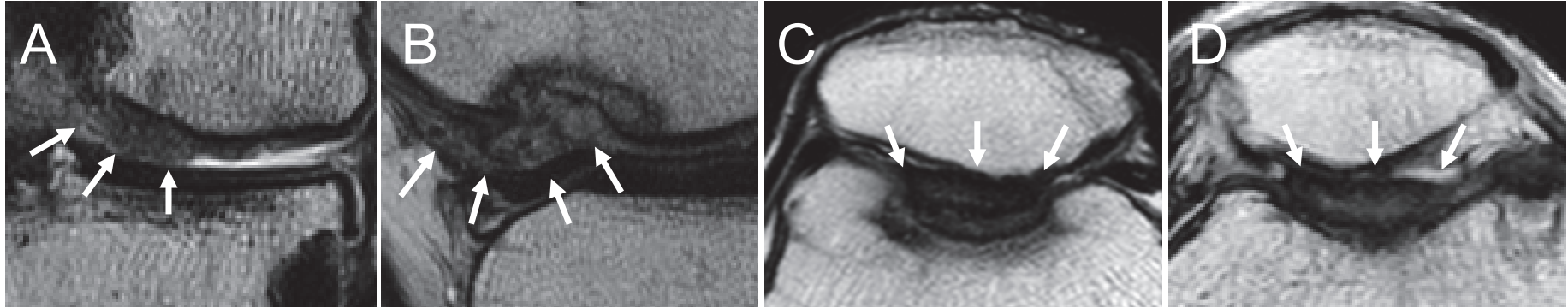


Fig. 4

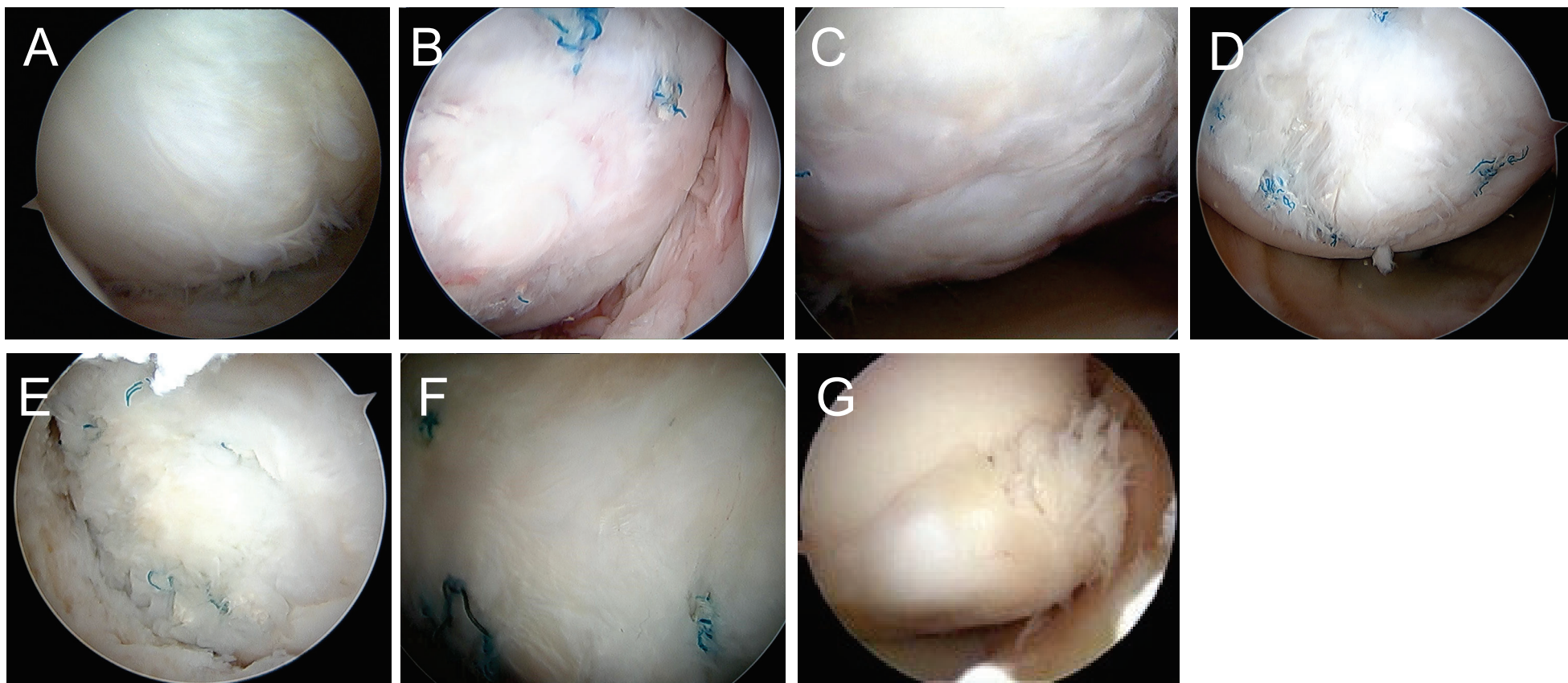


Fig. 5

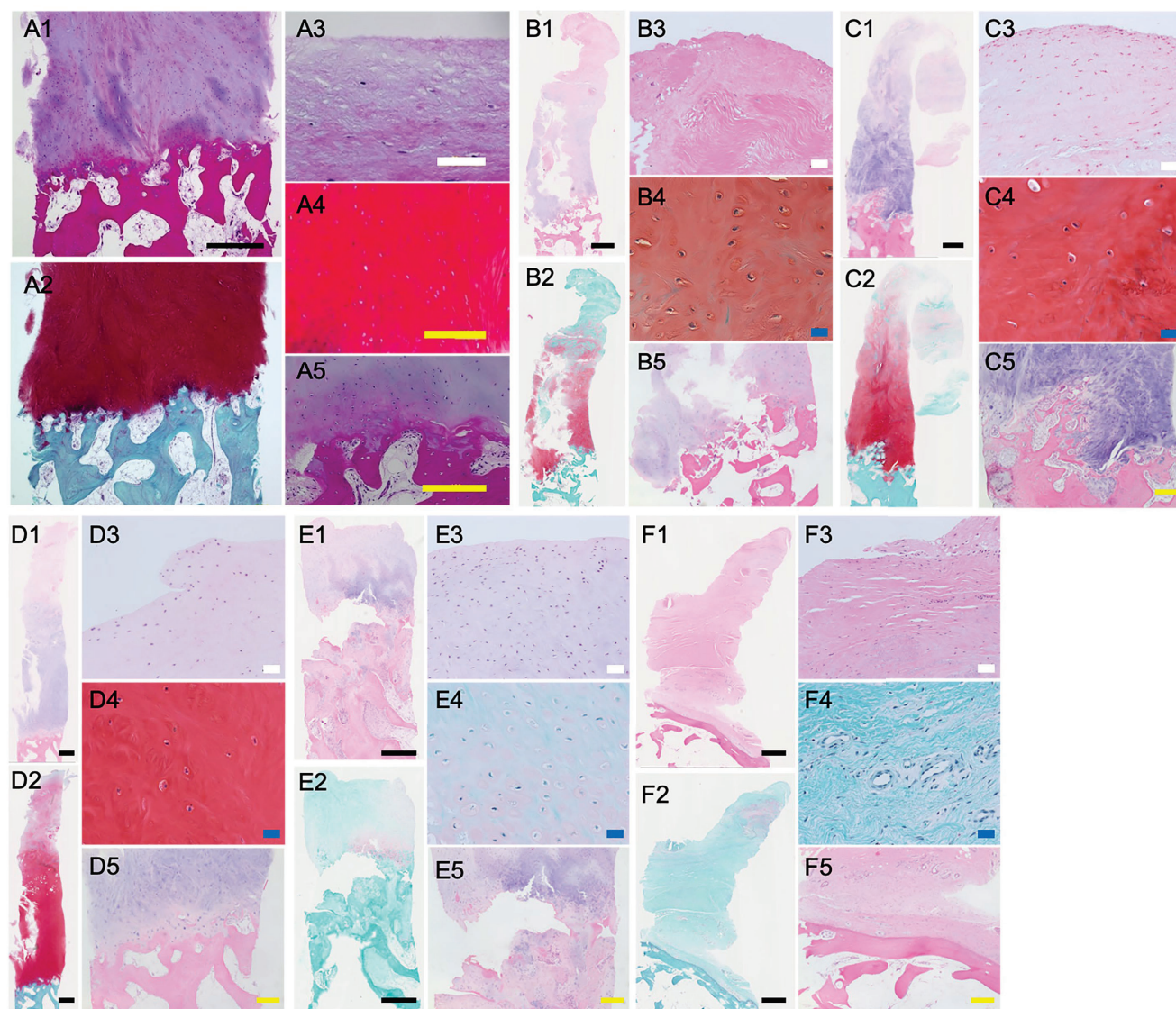


Fig. 6

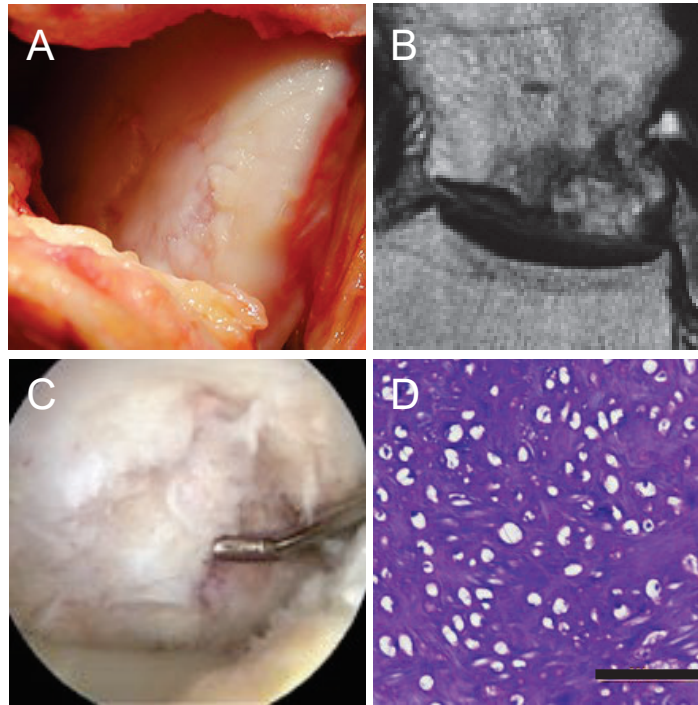


Fig. 7