Mesenchymal Stem Cell Implantation in Osteoarthritic Knees: Is Fibrin Glue Effective as a Scaffold?

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Mesenchymal Stem Cell Implantation in Osteoarthritic Knees

Is Fibrin Glue Effective as a Scaffold?

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Background: The cell-based tissue engineering approach that uses mesenchymal stem cells (MSCs) has addressed the issue of articular cartilage repair in osteoarthritic (OA) knees. However, to improve outcomes, an advanced surgical procedure with tissue-engineered scaffolds may be needed to treat patients with large cartilage lesions.

Purpose: To investigate the clinical and second-look arthroscopic outcomes of the implantation of MSCs loaded in fibrin glue as a scaffold in patients with OA knees and to compare these outcomes with those of MSC implantation without a scaffold.

Study Design: Cohort study; Level of evidence, 3.

Methods: This study retrospectively evaluated 54 patients (56 knees) who were examined with second-look arthroscopy after MSC implantation for cartilage lesions in their OA knees. Patients were divided into 2 groups: 37 patients (39 knees) were treated with MSC implantation without a scaffold (group 1), and 17 patients (17 knees) underwent implantation of MSCs loaded in fibrin glue as a scaffold (group 2). Clinical outcomes were evaluated according to the International Knee Documentation Committee (IKDC) score and the Tegner activity scale, and cartilage repair was assessed with the International Cartilage Repair Society (ICRS) grade. Statistical analyses were performed to identify various prognostic factors associated with the clinical and second-look arthroscopic outcomes.

Results: At final follow-up (mean, 28.6 months; range, 24-34 months), the mean IKDC score and Tegner activity scale in each group significantly improved: group 1, from 38.1 ± 7.7 to 62.0 ± 11.7 (IKDC) and from 2.5 ± 0.9 to 3.5 ± 0.8 (Tegner); group 2, from 36.1 ± 6.2 to 64.4 ± 11.5 (IKDC) and from 2.2 ± 0.8 to 3.8 ± 0.8 (Tegner) ($P < .001$ for all). According to the overall ICRS cartilage repair grades, 9 of the 39 lesions (23%) in group 1 and 12 of the 17 lesions (58%) in group 2 achieved a grade of I or II. There was a significant difference in ICRS grades between the groups ($P = .028$). Overweight (body mass index $\geq$ 27.5 kg/m^2) and large lesion size ($\geq$5.7 cm^2) were significant predictors of poor clinical and arthroscopic outcomes in group 1 ($P < .05$ for both). There was a similar trend in group 2, but the differences were not significant, possibly owing to the smaller sample size.

Conclusion: Clinical and arthroscopic outcomes of MSC implantation were encouraging for OA knees in both groups, although there were no significant differences in outcome scores between groups. However, at second-look arthroscopy, there were better ICRS grades in group 2.

Keywords: mesenchymal stem cell; implantation; osteoarthritis; knee; fibrin glue: scaffold

Osteoarthritis (OA) is the degenerative disease involving the whole joint, including the articular cartilage, subchondral bone, and periarticular tissue. The poor intrinsic healing potential of damaged cartilage, which results in progressive degradation of articular cartilage and subsequent widespread degeneration of the joint, is a major clinical problem in OA treatment.[14,33] Various surgical procedures—including abrasion arthroplasty or subchondral drilling[6,53] and microfracture,[2,30,35,36,51] which are not traditional treatment measures for OA—have been performed to regenerate articular cartilage. However, the outcomes of these procedures are often unfavorable because of the biomechanical insufficiency of the regenerative fibrocartilage compared with the hyaline cartilage. Therefore, controversy exists concerning the effectiveness

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of these procedures in OA knees. Nevertheless, although the precise mechanism and consensus by which these procedures improve the course of degenerative conditions of the knee have not been established, cartilage-regenerative procedures have become the focus of increased interest because of their potential to provide pain relief and alter the progression of OA.[32,60]

Recently, the cell-based tissue engineering approach has addressed the issue of articular cartilage repair by filling the cartilage lesion with a mechanically stable hyaline cartilage-like substance that will not deteriorate over time and will integrate well with the surrounding tissue.[49] In this approach, 2 candidate cell types—chondrocytes and mesenchymal stem cells (MSCs)—can be considered for transplantation into the cartilage lesion. Autologous chondrocyte implantation has been performed as a cell-based therapy for the treatment of cartilage defect, and encouraging clinical outcomes have been well established.[4,7,11,57] However, its use has been limited to focal cartilage defect, and generalized cartilage loss seen in OA is not an indication for autologous chondrocyte implantation.[7] Moreover, since autologous chondrocyte implantation is cell culture based, it is not cost-effective, and it requires 2 steps in surgery (harvesting healthy cartilage and implanting chondrocytes expanded from that sample).[29] Alternatively, MSCs are an attractive cell source for regenerative medicine. They can be harvested in a minimally invasive manner, and they are easily isolated and expanded, with multipotentiality including chondrogenesis.[46,47,52] Currently, MSCs have been proposed as a new treatment option for OA on the basis of their ability to differentiate into chondrocytes and their paracrine effects of secreted bioactive materials.[3,5,9,15,42] Since the first clinical study by Wakitani et al[58] on the MSC-based treatment of patients with OA, several clinical studies concerning the use of MSCs as a cell-based treatment for OA have been reported.[20,25,27,28,43]

The MSC implantation route is associated with the cells' efficiency of travel to the target organs and tissues.[23] The direct intra-articular injection of MSCs into the OA knee has been performed[20,25,27,28,43] and is the most commonly used method. However, simple injection is insufficient to obtain the improved cell engraftment, because directly injected cells have limited cell retention and survival at the target site. In our previous study,[26] we performed MSC implantation under arthroscopic guidance, according to the local adherent technique reported by Koga et al,[24] to optimize implantation and prevent cell loss. Although the outcomes for MSC implantation in OA knees were encouraging, the large size of the cartilage lesion ($\geq$5.4 cm^2) was an important factor that resulted in the poor outcomes. We concluded that the development of an advanced surgical procedure with tissue-engineered scaffolds may be needed to treat patients with large cartilage lesions. Thus, we used fibrin glue as a scaffold in MSC implantation in this study. Fibrin glue is the most commonly used biomaterial because of its high biocompatibility, biodegradability, injectability, and ease of handling.[23] Moreover, fibrin glue facilitates cell attachment, proliferation, differentiation, and, ultimately, tissue formation and organization.[16,18]

From these viewpoints, we considered that fibrin glue can be applied as a scaffold in MSC implantation to induce better cell survival, proliferation, differentiation, and matrix synthesis, leading to the repair of cartilage lesion in OA knees. The purposes of the current study were (1) to investigate the clinical and second-look arthroscopic outcomes of implantation of MSCs loaded in fibrin glue as a scaffold in patients with OA knees and (2) to compare these outcomes with those of MSC implantation without a scaffold.

MATERIALS AND METHODS

Patient Selection

This study protocol was approved by the institutional review board of our hospital, and all patients provided written informed consent. We retrospectively reviewed 117 consecutive patients (127 knees) with cartilage lesions in the knees who were treated with MSC implantation for cartilage regeneration between October 2010 and April 2012. Patients were included in this study if they had an isolated full-thickness articular cartilage lesion in OA knees (Kellgren-Lawrence[22] grades 1-2) with symptoms of knee joint pain and/or functional limitations, despite a minimum of 3 months of nonsurgical treatments. Patients were excluded if they had a history of surgical treatments, as were patients with multiple cartilage lesions, knee instability, varus or valgus malalignment of 5° or more of the knee joint, metabolic arthritis, joint infections, or large meniscal tears. A total of 88 patients (93 knees) met the inclusion criteria. Of these, the first 58 patients (62 knees) were treated with MSC implantation without a scaffold from October 2010 to October 2011, and the next 30 patients (31 knees) underwent MSC implantation with a scaffold from November 2011 to April 2012. We suggested that all patients undergo second-look arthroscopy, and we explained its purpose to all patients before surgery (ie, the evaluation of the MSC-implanted site and the need for additional arthroscopic procedures such as debridement or synovectomy). Among the 88 patients (93 knees), second-look arthroscopies were performed at a mean of 12.3 months postoperatively (range, 9-16 months) in 56 of the 93 knees, and these 56 knees (54 patients) were ultimately included in the study (Figure 1). We divided these patients into 2 groups: 37 patients (39 knees) who were treated with MSC implantation without a scaffold (group 1) and 17 patients (17 knees) who underwent MSC implantation with a scaffold (group 2). Of the 37 patients (39 knees) in group 1, 35 patients (37 knees) were included in our prior study.[26] There were no significant differences in basic characteristics between the groups regarding age, sex, body mass index (BMI), follow-up period, preoperative clinical scores, or lesion size (Table 1).

Preparation of MSCs

The isolation of adipose-derived MSCs was performed as described previously.[26] One day before arthroscopic

TABLE 1
Comparison of Baseline Demographics in the Study Groups[a]

	Group 1	Group 2	Total	*P* Value
Knees:patients, n	39:37	17:17	56:54	
Age, y	57.5 ± 5.9	57.7 ± 5.8	57.5 ± 5.8	.674
Male:female, n	14:23	8:9	22:32	.436
Body mass index, kg/m^2	26.3 ± 3.2	27.3 ± 2.9	26.6 ± 3.1	.335
Follow-up period, mo	29.2 ± 4.1	27.3 ± 3.3	28.6 ± 3.9	.094
Preoperative IKDC score	38.1 ± 7.7	36.1 ± 6.2	37.5 ± 7.2	.368
Preoperative Tegner activity scale	2.5 ± 0.9	2.2 ± 0.8	2.4 ± 0.9	.411
Lesion size, cm^2	5.4 ± 1.6	6.4 ± 2.8	5.7 ± 2.1	.311

[a]Values are expressed as mean ± standard deviation unless otherwise indicated. IKDC, International Knee Documentation Committee.

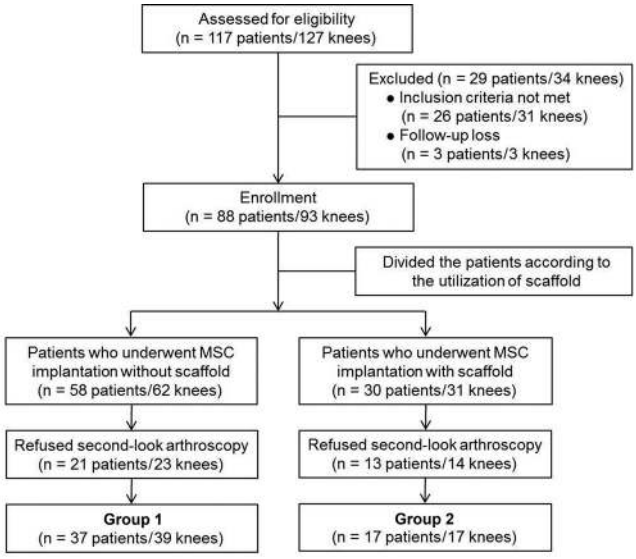

Figure 1. Flow diagram of patient involvement in the study.

surgery, adipose tissue was harvested from the patient's buttock through tumescent liposuction. We aimed to routinely collect 140 mL of liposuctioned adipose tissue, of which 120 mL was used for the injection and the remaining 20 mL was analyzed to examine the plastic-adherent cells that form colony-forming units–fibroblast and to confirm the multilineage differentiation of adipose-derived stem cells. In the operating room, 120 mL of adipose tissue was suspended in phosphate-buffered saline, placed in a sterile box, and transported to the laboratory. Mature adipocytes and connective tissues were separated from the stromal vascular fraction by centrifugation, as reported by Zuk et al.[61] The remaining 20 mL of adipose tissue was processed by the same method and used for cell analysis. To evaluate the frequency of mesenchymal-like progenitors in the stromal vascular fraction, cells were cultured in T-25 flasks at a final concentration of 16 cells/cm^2. Colonies consisting of ≥50-cell aggregates were scored under an optical microscope, and the immunophenotype of the adipose-derived stem cells was analyzed by flow cytometry (FACS) analysis. MSC marker phenotyping was performed with CD14, CD34, CD90, and CD105 antibodies as previously described.[34] Adipose-derived stem cells were plated at cells/cm^2 in Dulbecco's modified Eagle's medium, containing 10% fetal bovine serum and were allowed to adhere for 24 hours. The culture medium was then replaced with specific inductive media to determine the adipogenic, osteogenic, and chondrogenic differentiation potential, as previously reported.[34] We evaluated the capacity of human subcutaneous adipose tissue to generate mesenchymal progenitors using the colony-forming units–fibroblast. Thus, after isolation, the adipose-derived stem cells represented a mean of 9.2% of the stromal vascular fraction cells (range, 8.1%-10.9% of the stromal vascular fraction cells). After the stromal vascular fractions were isolated, a mean of 3.9×10^6 stem cells (9.2% of 4.2×10^7 stromal vascular fraction cells; range, 3.0-4.6 $\times 10^6$) were prepared. Accordingly, we used an average of 4.2×10^7 stromal vascular fraction cells, which contained an average of 3.9×10^6 stem cells for MSC implantation. The FACS characterization indicated a positive expression of the surface markers CD90 and CD105 and a negative expression of CD34 and CD14. Adipose-derived stem cells treated with conditioned media demonstrated characteristics of adipogenic, osteogenic, and chondrogenic differentiation after staining.

Surgical Procedures and MSC Application

Before the implantation of MSCs, accurate debridement of all unstable and damaged cartilage in the lesion was performed. In group 1, the articular cartilage lesion was filled with MSCs under arthroscopic guidance, according to the local adherent technique reported by Koga et al,[24] as described in our previous study.[26] Briefly, the knee was positioned so that the femoral condyle faced upward; then the cartilage lesion was filled with the cell suspension (stromal vascular fraction cells contained MSCs) and held in a stationary position for 10 minutes for adherence of the cell suspension with the defect facing upward. The degree of flexion of knee joints, as to face the femoral condyle upward, was determined according to the location of lesions on the femoral condyle. In group 2, the fibrin glue product from the commercially available Greenplast kit (Greencross) was used as a scaffold. The product was administered in 2 syringes—1 contained lyophilized human plasma fibrinogen (71.5-126.5 mg/mL) dissolved in 1 mL of the aprotinin

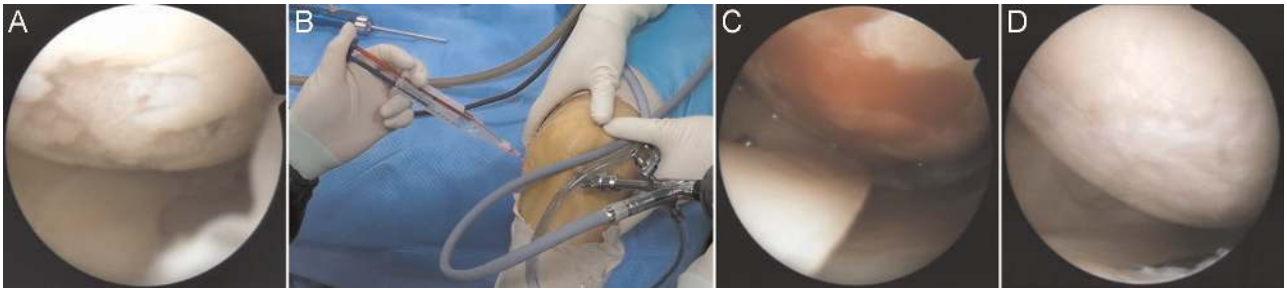

Figure 2. Arthroscopic implantation of mesenchymal stem cells loaded in fibrin glue. (A) An articular cartilage lesion in the medial femoral condyle was noticed. (B) The cell-thrombin-fibrinogen suspension was applied to the lesion. (C) The cartilage lesion was covered with the cell-thrombin-fibrinogen suspension after manipulation with the probe. (D) Second-look arthroscopic finding showing complete coverage of the lesion site with cartilage.

solution (1100 Kallikrein inhibitor units per milliliter), and 1 contained thrombin (4.9-11.1 mg/mL) dissolved in 1 mL of the calcium chloride solution (13.9-15.6 mg/mL) in sterile packaging. In general, the fibrin glue product is designed to form a gel instantaneously when the 2 solutions of each syringe are mixed. First, the cell suspension (stromal vascular fraction cells contained MSCs) was loaded into the thrombin solution at a 1:1 mixture ratio (volume of cell suspension to the volume of thrombin solution); then the cell-thrombin suspension was mixed with the fibrinogen solution at a 1:1 ratio via a Duploject syringe support system (included in the Greenplast kit), which were simultaneously added to each well on the surface of the cartilage lesion. Implantation of this cell-thrombin-fibrinogen suspension (ie, MSCs mixed with the fibrin glue) was performed under arthroscopic guidance after the arthroscopic fluid was extracted (Figure 2). The cell-thrombin-fibrinogen suspension was applied and manipulated with the probe to be coated at the surface of cartilage lesion. No marrow stimulation procedures, such as microfracture, subchondral drilling, and abrasion arthroplasty, were performed before this procedure.

Postoperative Rehabilitation

After the arthroscopic procedure, the knee was immobilized for 2 weeks with a knee brace, and after the sutures were removed, the patients began range of motion exercises, including active and passive exercises of the knee joint. Partial weightbearing was initiated at 2 weeks after arthroscopy, and full weightbearing was permitted at 4 weeks postoperatively. Sports or high-impact activities were allowed after 3 months, and subsequently, full return to normal sports or recreational activities was allowed according to individual recovery.

Outcome Assessment

All patients were evaluated clinically before surgery and during follow-up. For clinical evaluation, the International Knee Documentation Committee (IKDC) score[19] and the Tegner activity scale[55] were used to determine joint function and sports activities. Additionally, patients rated their overall satisfaction with the operation as excellent, good, fair, or poor and were asked whether they would undergo the procedure again.

During the second-look arthroscopy performed by a single senior surgeon (Y.G.K.), the cartilage lesions were evaluated macroscopically with the International Cartilage Repair Society (ICRS)[8,45] grading system (see the Appendix, available in the online version of this article at http://ajsm.sagepub.com/supplemental). The ICRS grading system is a reliable and relevant means of macroscopically evaluating cartilage repair after microfracture or autologous chondrocyte implantation.[56] It consists of 3 criteria: (1) the degree to which a defect was filled by repair tissue, (2) the degree of integration of repair tissue with the adjacent articular cartilage, and (3) the macroscopic surface appearance of the repair site. Each subjective arthroscopic evaluation criterion was assigned a maximum score of 4 points, which were then combined to obtain an overall grade (Appendix, available online).

Statistical Analysis

The principal dependent variables of clinical outcomes were the IKDC score and Tegner activity scale at the second-look arthroscopy and final follow-up. Descriptive statistics were calculated as mean ± standard deviation. The Wilcoxon signed-rank test was used to evaluate differences between the preoperative and second-look arthroscopy values and between the second-look arthroscopy and final follow-up values. Differences between groups were analyzed with the Mann-Whitney *U* test or the Kruskal-Wallis test for multiple comparisons. The Fisher exact test was used to compare categorical data. The associations among factors—patient characteristics (age, sex, and BMI) and cartilage lesion variables (size and location of lesion and the existence of subchondral cysts)—were examined on the basis of the clinical outcomes for each group. Median values were used as standard values for dividing patients according to age and lesion size, and 27.5 kg/m^2 was used as the cutoff BMI value[59] in this study. According to a previous study that evaluated BMI in more than 1.1 million persons recruited in 19 cohorts in Asia, persons with a BMI <27.5 kg/m^2 in the cohorts of East Asian, including Chinese, Japanese, and Koreans, had the lowest

risk of death.[59] The associations between the ICRS cartilage repair grades and the clinical outcomes of the second-look arthroscopy were also analyzed according to the Kruskal-Wallis test for each group. Stepwise multivariate linear regression was used to assess the correlations between lesion size and the ICRS score in both groups. Statistical analysis was performed with SPSS 12.0.1 (SPSS Inc), with significance defined as $P < .05$.

RESULTS

Clinical Outcomes at Follow-up

Clinical outcomes from preoperative to final follow-up in each group (mean, 29.2 ± 4.1 months [group 1] and 27.3 ± 3.3 months [group 2]) are summarized in Table 2. The mean IKDC score in each group significantly improved from 38.1 ± 7.7 to 61.0 ± 11.3 in group 1 and from 36.1 ± 6.2 to 62.3 ± 10.4 in group 2 at the time of the second-look arthroscopy ($P < .001$ for both groups). The mean Tegner activity scale in each group also significantly improved, from 2.5 ± 0.9 to 3.4 ± 1.0 in group 1 and from 2.2 ± 0.8 to 3.5 ± 1.0 in group 2 at the second-look arthroscopy ($P < .001$ for both groups). At the final follow-up, compared with the values at the second-look arthroscopy, the mean IKDC score and Tegner activity scale in each group improved further to 62.0 ± 11.7 and 3.5 ± 0.8, respectively, in group 1 (P = .078 and .096) and to 64.4 ± 11.5 and 3.8 ± 0.8 in group 2 (P = .005 and .025) (Figure 3, A and B). The improvements of the IKDC score and Tegner activity scale from the second-look arthroscopy to final follow-up were statistically significant only in group 2. However, there were no significant differences in the mean IKDC score and Tegner activity scale between the groups at serial follow-up ($P > .05$ for all). Among 34 patients (37 knees) who refused the second-look arthroscopy, 21 patients (23 knees) underwent MSC implantation without scaffold, and 13 patients (14 knees) underwent MSC implantation with scaffold (Figure 1). The mean IKDC score and Tegner activity scale were significantly improved for both these patient groups, from a respective 35.7 ± 6.6 and 2.1 ± 0.9 preoperatively to 63.6 ± 12.6 and 3.7 ± 0.9 at the final follow-up in the former 21 patients ($P < .001$ for both) and from 37.4 ± 5.1 and 2.4 ± 0.6 preoperatively to 64.1 ± 11.0 and 3.6 ± 0.6 at the final follow-up in the latter 13 patients ($P < .001$ for both). There were no significant differences in clinical outcomes preoperatively and at the final follow-up between the patients who underwent MSC implantation with or without scaffold ($P > .05$ for both sets of patients; Kruskal-Wallis test).

Associations Between Patient Characteristics and Clinical Outcomes

The Mann-Whitney U test was used to assess the independent effects of patient characteristics, including age, sex, and BMI, on clinical outcomes (Table 3). The median value was used to divide patients according to age (<57 or ≥57 years), and no significant differences were found between the 2 age groups in either group 1 or 2 ($P > .05$). The patients' sex did not have a significant influence on the clinical outcomes ($P > .05$). To analyze the association between BMI and clinical outcomes, the patients were divided according to BMI (high, ≥27.5 kg/m^2; low, <27.5 kg/m^2). In group 1, patients with a high BMI had significantly worse outcomes, according to the IKDC score and Tegner activity scale, than did patients with a low BMI (P = .022 and P = .005, respectively). However, these associations were not significant in group 2 ($P > .05$ for both).

TABLE 2
Comparison of Clinical Outcomes at Preoperation, Second-Look Arthrosocopy, and Final Follow-up in Both Groups[a]

	Group 1	Group 2	P Value[b]
IKDC score			
Preoperation	38.1 ± 7.7	36.1 ± 6.2	.411
Second-look arthroscopy	61.0 ± 11.3	62.3 ± 10.4	.624
Final follow-up	62.0 ± 11.7	64.4 ± 11.5	.304
P value[c]	**<.001**	**<.001**	
P value[d]	.078	**.005**	
Tegner activity scale			
Preoperation	2.5 ± 0.9	2.2 ± 0.8	.311
Second-look arthroscopy	3.4 ± 1.0	3.5 ± 1.0	.808
Final follow-up	3.5 ± 0.8	3.8 ± 0.8	.303
P value[c]	**<.001**	**<.001**	
P value[d]	.096	**.025**	

[a]Values are expressed as mean ± SD. Boldface indicates statistical significance ($P < .05$). IKDC, International Knee Documentation Committee.
[b]Mann-Whitney U test.
[c]Wilcoxon signed-rank test for comparison of clinical outcomes at preoperation and second-look arthroscopy.
[d]Wilcoxon signed-rank test for comparison of clinical outcomes at second-look arthroscopy and final follow-up.

Associations Between Cartilage Lesion Variables and Clinical Outcomes

The mean size of the cartilage lesions was 5.4 ± 1.6 cm^2 (range, 2.3-9.4 cm^2) in group 1 and 6.4 ± 2.8 cm^2 (range, 2.8-13.8 cm^2) in group 2. There was no significant difference in lesion size between the groups (P = .368). The mean size of cartilage lesions in both groups was 5.7 ± 2.1 cm^2, and this value was used as a standard value for dividing the patients to analyze the association of the lesion size with clinical outcomes. In group 1, patients with lesions larger than 5.7 cm^2 had significantly worse outcomes, as determined from the IKDC score and Tegner activity scale, than patients with lesions smaller than 5.7 cm^2 (P = .012 and .024, respectively). However, these associations were not significant in group 2 (Table 3). With regard to lesion location in group 1, 18 knees had lesions in the medial femoral condyle, 15 had lesions in the lateral femoral condyle, and 6 had lesions in the trochlea. In group 2, there were 9 knees with lesions in the medial femoral condyle, 6 with lesions in the lateral femoral condyle, and 2 with lesions in the trochlea. Subchondral cysts, investigated through preoperative magnetic resonance imaging, were found in 16 knees of group 1 and in

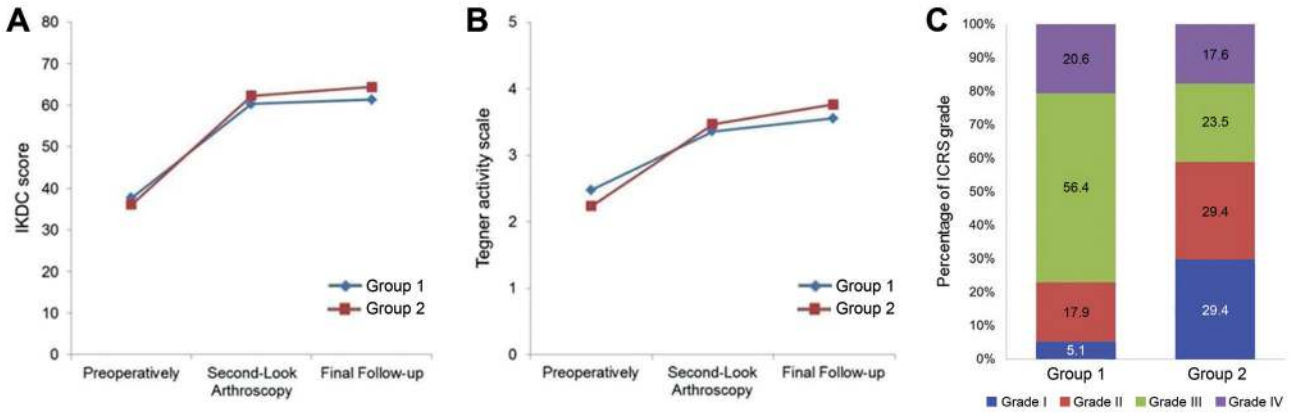

Figure 3. (A and B) Time courses of the postoperative International Knee Documentation Committee (IKDC) score and Tegner activity scale and (C) percentage of International Cartilage Repair Society (ICRS) grade in both groups.

TABLE 3
Associations Between Various Factors and Clinical Outcomes at Second-Look Arthroscopy in Both Groups[a]

	Group 1					Group 2				
		IKDC score		Tegner Activity Scale			IKDC score		Tegner Activity Scale	
Factors	n	Mean ± SD	*P* Value	Mean ± SD	*P* Value	n	Mean ± SD	*P* Value	Mean ± SD	*P* Value
Age,[b] y			.333		.566			.740		.813
<57	17	62.8 ± 10.4		3.5 ± 0.9		10	61.8 ± 10.6		3.4 ± 1.2	
≥57	22	59.5 ± 12.1		3.3 ± 1.0		7	63.0 ± 10.8		3.6 ± 0.8	
Sex			.592		.377			.606		.321
Male	14	58.9 ± 13.4		3.2 ± 1.1		8	63.3 ± 11.5		3.8 ± 1.0	
Female	25	62.1 ± 10.1		3.5 ± 0.9		9	58.9 ± 13.4		3.2 ± 1.0	
Body mass index,[c] kg/m^2			**.022**		**.005**			.180		.350
<27.5	16	66.4 ± 11.1		3.9 ± 0.9		6	66.7 ± 8.8		3.8 ± 1.0	
≥27.5	23	57.1 ± 10.0		3.0 ± 0.8		11	59.9 ± 10.7		3.3 ± 1.0	
Lesion size,[b] cm^2			**.012**		**.024**			.277		.370
<5.7	19	62.5 ± 8.4		3.7 ± 0.7		9	65.3 ± 9.8		3.7 ± 0.9	
≥5.7	20	56.6 ± 12.2		3.1 ± 1.0		8	58.9 ± 10.5		3.3 ± 1.2	
Location			.213		.532			.721		.673
MFC	18	61.3 ± 13.1		3.4 ± 1.0		9	61.3 ± 10.4		3.4 ± 0.9	
LFC	15	58.1 ± 10.5		3.2 ± 0.9		6	64.7 ± 12.1		3.7 ± 1.4	
Trochlea	6	66.8 ± 4.1		3.7 ± 0.5		2	59.5 ± 7.8		3.0 ± 0.0	
Subchondral cyst			.662		.703			.799		.442
Existence	16	59.8 ± 10.7		3.3 ± 0.9		5	64.9 ± 8.9		3.8 ± 0.8	
Nonexistence	23	61.7 ± 12.0		3.4 ± 1.0		12	61.6 ± 12.1		3.3 ± 1.1	

[a]Boldface indicates statistical significance ($P < .05$). IKDC, International Knee Documentation Committee; LFC, lateral femoral condyle; MFC, medial femoral condyle.
[b]Median values are used as standard values for dividing the groups.
[c]A value of 27.5 was used as a cutoff for dividing the groups.[59]

5 knees of group 2. The location of the cartilage lesions and presence of subchondral cysts did not significantly influence the clinical outcomes in either group ($P > .05$ for both) (Table 3).

Second-Look Arthroscopic Findings

Second-look arthroscopies were performed at a mean of 12.3 months postoperatively (range, 9-16 months). According to the ICRS overall repair grades, 2 of the 39 lesions in group 1 (5%) showed grade I repair (normal); 7 (18%), grade II (near normal); 22 (56%), grade III (abnormal); and 8 (21%), grade IV (severely abnormal). In group 2, of 17 lesions, 5 (29%) showed grade I; 5 (29%), grade II; 4 (24%), grade III; and 3 (18%), grade IV (Figure 3C). There was a significant difference in ICRS grades between the groups ($P = .028$, according to the Fisher exact test) (Table 4). The IKDC score and Tegner activity scale, according to

TABLE 4
Clinical Outcomes According to the ICRS Repair Grades at Second-Look Arthroscopy in Both Groups[a]

	Group 1					Group 2				
		IKDC Score		Tegner Activity Scale			IKDC Score		Tegner Activity Scale	
	n (%)	Mean ± SD	*P* Value[b]	Mean ± SD	*P* Value[b]	n (%)	Mean ± SD	*P* Value[b]	Mean ± SD	*P* Value[b]
Grade			**< .001**		**< .001**			**.002**		**.003**
I	2 (5)	73.0 ± 1.4		4.2 ± 0.1		5 (29)	74.0 ± 1.6		4.6 ± 0.5	
II	7 (18)	62.1 ± 6.2		3.8 ± 0.3		5 (29)	65.8 ± 2.6		3.6 ± 0.5	
III	22 (56)	53.6 ± 4.5		3.0 ± 0.7		4 (24)	53.8 ± 3.9		3.0 ± 0.0	
IV	8 (21)	35.3 ± 3.7		2.1 ± 0.1		3 (18)	48.3 ± 3.1		2.0 ± 0.0	

[a]Boldface indicates statistical significance ($P < .05$). Fisher exact test for comparison of ICRS repair grades between groups, P = **.028**. ICRS, International Cartilage Repair Society; IKDC, International Knee Documentation Committee.
[b]Kruskal-Wallis test.

the ICRS repair grades during the second-look arthroscopic evaluation, are shown in Table 4. As the quality of repaired cartilage increased, the IKDC score and Tegner activity scale increased in both groups ($P < .05$ for all). As illustrated in the scatter plots, significant correlations between lesion size and ICRS score were found in both groups (P = .001 for group 1, P = .048 for group 2) (Figure 4).

DISCUSSION

Cartilage primarily serves a biomechanical function; therefore, tissue engineering strategies must ultimately produce a construct that is able to recapitulate the most essential mechanical properties of native cartilage.[14] The appropriate delivery of MSCs to the site of the cartilage lesion is crucial for durable cartilage repair in the MSC-based treatment of OA. Recently, direct intra-articular injection of MSCs into the OA knee has been performed in several studies.[20,25,27,28,43] However, simple injection is insufficient for obtaining an improved cell engraftment, because directly injected cells have limited cell retention and survival at the target site. In our previous study,[26] we performed MSC implantation under arthroscopic guidance, according to the local adherent technique reported by Koga et al,[24] to further optimize implantation and prevent cell loss. However, we found that large cartilage lesions ($\geq$5.4 cm^2) showed significantly worse outcomes, and we concluded that the development of an advanced surgical procedure with tissue-engineered scaffolds was needed to treat patients with large cartilage lesions. Compared with direct intra-articular injection, seeding MSCs into a scaffold, such as a biodegradable template, for proliferation and matrix production offers the advantage of providing an accessible, easy-to-manipulate, self-renewing source of progenitor cells.[41] Moreover, resurfacing the joint with a biologic implant can be addressed by seeding MSCs in scaffolds that provide initial mechanical integrity, as the scaffold provides sufficient functional properties at the time of implantation.[38,39] The ideal scaffold should be biocompatible and biodegradable upon tissue healing, highly porous to permit cell penetration and tissue impregnation, sufficiently permeable to allow nutrient delivery and gas exchange, and adaptable to the mechanical environment. Additionally, the scaffold should be conducive to cell attachment and migration, permitting appropriate extracellular matrix formation and the transmission of signaling molecules.[41,48,54] Various biomaterials are used as scaffolds to deliver MSCs for cartilage repair, but few fulfill all the requirements described above.[40]

Fibrin is a tissue-derived natural material that can be used as a 3-dimensional scaffold. It is a protein involved in the clotting of blood, and it is formed by polymerization of fibrin glue in the presence of thrombin. Fibrin glue has been used widely during the development of articular cartilage repair strategies as a cell delivery matrix for generating a new cartilage matrix.[1,10,13,21,44] Moreover, fibrin glue promotes the proliferation and gene expression of MSCs.[18] In an in vitro human study, Kim et al[23] demonstrated that adipose-derived MSCs in fibrin glue sustained functional survival and paracrine function, and they suggested further development of the MSCs with fibrin glue for clinical treatment. Haleem et al[17] transplanted culture-expanded MSCs, which were loaded on platelet-rich fibrin glue into full-thickness cartilage defects of the femoral condyles and covered with an autologous periosteal flap. In their pilot study of 5 patients, all patients with osteochondral defects who were reconstructed with MSCs transplanted on platelet-rich fibrin glue experienced significant improvement in their functional knee scores and magnetic resonance imaging findings in as early as 6 months, which were maintained over 12 months postoperatively. Therefore, we used fibrin glue as a scaffold in the present study because we believed that it could function as a scaffold in MSC implantation to induce better cell survival, proliferation, differentiation, and matrix synthesis, leading to the repair of cartilage lesion in OA knees. To our knowledge, this is the first in vivo clinical study using fibrin glue as a scaffold in MSC implantation for the treatment of OA knees. As a result, this study showed encouraging clinical and second-look arthroscopic outcomes of implantation of MSCs loaded in fibrin glue as a scaffold in patients with OA knees compared with the outcomes of those who underwent MSC implantation without a scaffold. The mean IKDC score and Tegner activity scale significantly

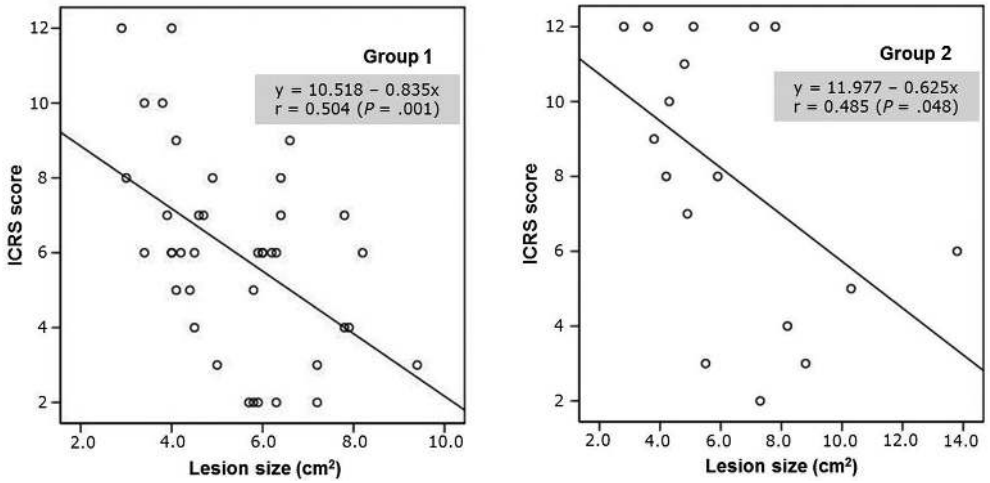

Figure 4. Correlations between lesion size and International Cartilage Repair Society (ICRS) score in both groups.

improved at the time of the second-look arthroscopy in both groups ($P < .001$, respectively). At final follow-up, although the mean IKDC score and Tegner activity scale improved further compared with the values at the second-look arthroscopy in both groups, the statistical significances in improvements of the IKDC score and Tegner activity scale from the second-look arthroscopy to final follow-up were found only in group 2 ($P = .005$ and .025, respectively) (Table 2). In patients treated with MSC implantation with a scaffold, the fibrin glue may facilitate better cell survival, proliferation, differentiation, and matrix synthesis. Subsequently, the biomechanical properties of repaired cartilage may be more similar to those of native cartilage in patients who underwent MSC implantation with scaffold compared with patients who underwent MSC implantation without a scaffold. Accordingly, our results showed that clinical outcomes at final follow-up further improved significantly compared with the values at second-look arthroscopy in group 2 (Figure 3, A and B).

It would be useful to identify the prognostic factors associated with the clinical outcomes of MSC implantation in the treatment of patients with OA knees. In our previous study,[26] we found that a high BMI ($\geq$27.5 kg/m^2) was a poor prognostic factor in MSC implantation. In the current study, patients' BMI also influenced the clinical outcomes of MSC implantation in group 1, because patients with a high BMI had significantly worse outcomes, according to the IKDC score and Tegner activity scale, than patients with a low BMI ($<$27.5 kg/m^2). However, this association was not found in group 2 (Table 3). Roldan et al[50] reported that MSCs from obese patients showed a reduced proliferation rate, greater cell senescence, and reduced differentiation to multiple lineages, including chondrogenesis. These negative effects in patients with a high BMI can be overcome by using fibrin glue as a scaffold. Furthermore, according to our previous study,[26] we found that MSC implantation based on the local adherent technique for cartilage regeneration in OA knees was effective for small cartilage lesions but not for large ones. This result was also found in the current study. Compared with patients with small lesions, those with large lesions ($\geq$5.4 cm^2 in our previous study and $\geq$5.7 cm^2 in group 1 of the current study) had significantly worse outcomes, according to the IKDC score and Tegner activity scale. However, this association was not found in group 2 of the current study (Table 3). In group 2, there were fewer patients and, thus, lower power to detect a difference between subgroups. As the same tendencies toward worse outcomes in patients with higher BMI or larger lesions were found in group 2 and at similar magnitudes, it is likely that high BMI and larger lesion size are also negative prognostic factors when a fibrin scaffold is used, but we were unable to verify it statistically owing to the smaller sampler size in group 2.

With regard to the ICRS overall repair grades during the second-look arthroscopy, there were significant correlations between clinical outcomes (IKDC score and Tegner activity scale) and the ICRS repair grades in both groups ($P < .05$ for all). Additionally, there was a significant difference in ICRS grades between the groups ($P = .028$); 23% of the lesions in group 1 and 58% in group 2 had achieved a normal or near-normal state (ICRS grade I or II) (Table 4 and Figure 3C). Moreover, there were significant correlations between lesion size and ICRS overall repair grades in both groups (Figure 4). These results show that the lesion size is an important prognostic factor in MSC implantation in OA knees. Although it is still in the early stages of application, we believe that fibrin glue is an effective scaffold in MSC implantation and that implantation of MSCs loaded in fibrin glue can be considered as a treatment for OA knees. Moreover, there were no complications associated with the implantation of MSCs loaded in fibrin glue, including infection, fever, hematoma, tissue hypertrophy, adhesion formation, or other major adverse events. Therefore, we consider fibrin glue an effective and safe scaffold in MSC implantation for the treatment of OA knees.

The present study does have some limitations. First, the number of patients was relatively small, especially in group 2; the follow-up period was short; and data were

collected retrospectively. For more accurate evaluation of the fibrin glue as a scaffold in MSC implantation for OA knees, a prospective study with a matched control group and a larger series of cases with a longer follow-up period is required. Second, the mixture ratio between the cell suspension of MSCs and fibrin glue was determined arbitrarily. The cell suspension was loaded into the thrombin solution at a 1:1 mixture ratio; the cell-thrombin suspension was mixed with the fibrinogen solution at a 1:1 ratio; and the cell-thrombin-fibrinogen suspension (MSCs mixed in the fibrin glue) was implanted on the cartilage lesion. According to the literature, when fibrin glue is used as a scaffold in the cell-based tissue engineering approach, the fibrinogen concentration is important for cell morphology, proliferation, and migration.[12,16,31] Therefore, a future study is required to determine the optimal mixture ratio between the MSCs and fibrin glue for achieving a suitable matrix environment, such as temporary retention space, cytoprotective effects, and cell-matrix interactions provided by the fibrin glue. Third, we used the IKDC score and the Tegner activity scale to evaluate clinical outcomes and the ICRS score to investigate the second-look arthroscopic outcomes of MSC implantation. It is important to examine the mechanical properties and biological functions of native cartilage and to compare the properties of the current cell localization approaches with those of native cartilage. Moreover, weak mechanical properties, shrinkage of the gel, and early degradation of fibrin have been problematic.[44] Therefore, further studies with histologic evaluation correlated with clinical and arthroscopic outcomes and power analysis are necessary to identify the effect of fibrin glue in MSC implantation. Fourth, reduction of pain is an important factor in clinical scores evaluating clinical outcome. Moseley et al[37] performed a randomized placebo-controlled trial to evaluate the efficacy of arthroscopy for OA knees and found that the outcomes after arthroscopic lavage or arthroscopic debridement were no better than those after a placebo procedure. Therefore, future study evaluating the placebo effect in MSC implantation is needed. Fifth, the second-look arthroscopy was performed at 1 year postoperatively. It is unknown how the repaired cartilage will behave over time, and changes in the influencing factors after the first year cannot be predicted. Last, the number of MSCs to be applied to achieve the optimal response remains unknown.

CONCLUSION

Our findings indicate that MSC implantation is a promising option for OA knee treatment. According to the overall ICRS cartilage repair grades at second-look arthroscopy, 9 of the 39 lesions (23%) in group 1 and 12 of the 17 lesions (58%) in group 2 achieved a grade I or II. There was a significant difference in ICRS grades between the groups (P = .028). Therefore, we recommend fibrin glue as an effective scaffold in MSC implantation for OA knees. However, a larger sample size and long-term prospective randomized studies are needed to confirm our findings.

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