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Assessment of clinical and MRI outcomes after mesenchymal stem cell implantation in patients with knee osteoarthritis: a prospective study

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SUMMARY

Objective: Cartilage regenerative procedures using the cell-based tissue engineering approach involving mesenchymal stem cells (MSCs) have been receiving increased interest because of their potential for altering the progression of osteoarthritis (OA) by repairing cartilage lesions.
The aim of this study was to investigate the clinical and magnetic resonance imaging (MRI) outcomes of MSC implantation in OA knees and to determine the association between clinical and MRI outcomes.
Design: Twenty patients (24 knees) who underwent arthroscopic MSC implantation for cartilage lesions in their OA knees were evaluated at 2 years after surgery. Clinical outcomes were evaluated according to the International Knee Documentation Committee (IKDC) score and the Tegner activity scale, and cartilage repair was assessed according to the MRI Osteoarthritis Knee Score (MOAKS) and Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score.
Results: The clinical outcomes significantly improved ($P < 0.001$ for both). The cartilage lesion grades (as described in MOAKS [grades for size of cartilage-loss area and percentage of full-thickness cartilage loss]) at follow-up MRI were significantly better than the preoperative values ($P < 0.001$ for both). The clinical outcomes at final follow-up were significantly correlated with the MOAKS and MOCART score at follow-up MRI ($P < 0.05$ for all).
Conclusions: Considering the encouraging clinical and MRI outcomes obtained and the significant correlations noted between the clinical and MRI outcomes, MSC implantation seems to be useful for repairing cartilage lesions in OA knees. However, a larger sample size and long-term studies are needed to confirm our findings.

Introduction

Osteoarthritis (OA) is characterized by degeneration of the articular cartilage and is accompanied by subchondral bone sclerosis and synovial inflammation[1]. Restoration of the diseased articular cartilage in patients with OA is a challenging problem for researchers and clinicians[2]. Recently, some clinical studies involving the use of mesenchymal stem cells (MSCs) as a potential cell-based treatment for OA have been reported[3–9]. MSCs could play a role in cartilage repair by generating new cartilage, releasing factors that stimulate cartilage formation by resident chondrocytes or other cells in the joint, and inhibiting joint inflammation[10].

For MSC-based therapies to emerge as a viable therapeutic alternative, the unique challenges associated with using MSCs to treat patients with OA must be identified and addressed. Therefore, the appropriate delivery of MSCs to the cartilage lesion site is crucial for durable cartilage repair in MSC-based treatment of OA[5]. In a previous recent study, Kim *et al.*[5] performed MSC implantation for cartilage lesions in patients with OA knees and found that the clinical and second-look arthroscopic outcomes of MSC implantation were encouraging. Furthermore, the second-look arthroscopic findings showed that cartilage repair was better in patients who underwent implantation of MSCs loaded in fibrin glue as a scaffold

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than in patients who underwent MSC implantation without a scaffold. However, a significant limitation of their prior research was that, using second-look arthroscopic evaluation, it is difficult to examine the full thickness of repaired cartilage and integration of repaired cartilage with adjacent native cartilage. Moreover, the second-look arthroscopy was performed at 1 year after surgery, and therefore it is not known how the repaired cartilage will behave after the first year. Therefore, we considered that another valid tool for evaluation of repaired cartilage for longer follow-up periods after MSC implantation is needed.

Magnetic resonance imaging (MRI) has rich image contrast variability, thereby providing the ability to discriminate articular tissues; therefore, it holds great potential as a tool for whole-organ imaging of the OA joint[11]. The relevance of MRI for evaluating structural changes during the development and progression of knee OA has been demonstrated[12]. Among several methods[13–16], MRI Osteoarthritis Knee Score (MOAKS), which has been used in several studies[16–18], is advocated to be the tool of choice for semiquantitative analyses of knee OA[17]. Therefore, we used the MOAKS for MRI evaluation of cartilage lesions after MSC implantation in this study. Furthermore, Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scoring system was also used for the evaluation of repaired cartilage.

The aims of this study were (1) to investigate the clinical outcomes of MSC implantation with fibrin glue as a scaffold in patients with OA knees, (2) to assess cartilage regeneration after MSC implantation by using MRI evaluation, and (3) to determine the association between clinical and MRI outcomes.

Materials and methods

Study subjects

In this prospective cohort study, the inclusion criterion was an isolated articular cartilage lesion in OA knees (Kellgren–Lawrence[19] grades 1–2) with symptoms of knee joint pain and/or functional limitations, despite a minimum of 3 months of nonsurgical treatments. Nonsurgical treatment options included rest, physical therapy, and nonsteroidal anti-inflammatory drugs. Intra-articular injections of viscosupplements or steroids were not given in all patients. 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 which might result in the mechanical symptom of knees or might be required the surgical treatment. We requested the patients to undergo follow-up MRI for evaluation of the cartilage lesion. From January 2012 to October 2012, 20 consecutive patients (24 knees) with cartilage lesions in the knees underwent arthroscopic MSC implantation with fibrin glue as a scaffold for cartilage regeneration. These patients underwent follow-up MRI at a mean of 24.2 months after surgery (range, 18–29 months). The study population included 11 men and 9 women, with a mean age of 57.9 years (SD, 5.9; range, 48–69). The mean follow-up period was 27.9 months (SD, 3.2; range, 24–34), and the mean preoperative body mass index (BMI) was 26.6 kg/m^2 (SD, 3.2; range, 22.2–31.2). This study was approved by the institutional review board of our hospital, and all patients provided written informed consent prior to treatment.

MSC preparation

Adipose-derived MSCs were isolated as described previously[5,9]. In brief, 1 day before arthroscopic surgery, adipose tissue was harvested from patient buttock. We aimed to collect 140 cc liposuctioned adipose tissue: 120 cc was used for the implantation, and the remaining 20 cc was analyzed to examine the plastic-adherent cells that form fibroblast colony-forming units (CFU–F) and confirm the multilineage differentiation of adipose-derived stem cells. In the operating room, 120 cc adipose tissue was suspended in phosphate-buffered saline (GIBCO BRL, Life Technologies, Carlsbad, CA, USA), 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 described by Zuk *et al.*[20] The remaining 20 cc adipose tissue was processed in the same manner and used for cell analysis.

Epitope profiles and multilineage differentiation were assessed to characterize the MSCs. We investigated the immunophenotype of the adipose-derived stem cells using CD14 (BD Biosciences), CD34 (BD Biosciences), CD90 (BD Biosciences), and CD105 (BD Biosciences) antibodies by flow cytometry (i.e., FACS) analysis, as described previously[21]. After culture expansion using specific inductive media, the adipogenic, osteogenic, and chondrogenic differentiation potentials of adipose-derived stem cells were assessed, as reported previously[21]. Adipogenic induction medium contained 100 nM dexamethasone (Sigma–Aldrich), 0.5 mM isobutyl-methylxanthine (Sigma–Aldrich), and 50 mM indomethacin (Sigma–Aldrich), osteogenic induction medium contained 1 nM dexamethasone, 10 mM b-glycerol phosphate (Sigma–Aldrich), and 50 mg/mL ascorbate-2-phosphate (Sigma–Aldrich), and chondrogenic induction medium contained 10 ng/mL transforming growth factor-β3 (TGF-β3) (Sigma–Aldrich, St. Louis, MO, USA), 1 × Insulin transferrin selenium + premix (Gibco BRL), and 100 nM dexamethasone (Sigma–Aldrich). The capacity of human subcutaneous adipose tissue to generate mesenchymal progenitors was evaluated according to CFU-F. The adipose-derived stem cells represented a mean of 9.0% of the stromal vascular fraction cells (range, 7.6–12.3) after isolation. After the stromal vascular fractions were isolated, a mean of 4.4×10^6 stem cells (9.0% of 4.9×10^7 stromal vascular fraction cells; range, $3.7–6.0 \times 10^6$) were prepared. A mean of 4.9×10^7 stromal vascular fraction cells, which contained a mean of 4.4×10^6 stem cells, were used for MSC implantation. FACS characterization indicated positive expression of the surface markers CD90 (97.83%) and CD105 (95.76%) and negative expression of CD34 (3.52%) and CD14 (2.14%), which represented the characteristics of MSCs. Adipose-derived stem cells treated with conditioned media exhibited adipogenic, osteogenic, and chondrogenic differentiation after staining.

Surgical technique

Before the MSC implantation, accurate debridement of all unstable and damaged cartilage in the lesion was performed [Fig. 1(A)]. We used the fibrin glue product from the commercially available Greenplast kit (Greencross, Seoul, Korea) as a scaffold. The product was administered using two syringes—one contained lyophilized human plasma fibrinogen (71.5–126.5 mg/mL) dissolved in 1 mL of aprotinin solution (1100 kallikrein inhibitor units [KIU/mL]) and the other contained thrombin (4.9–11.1 mg/mL) dissolved in 1 mL of 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 two solutions from each syringe are mixed. First, the cell suspension (stromal vascular fraction cells containing MSCs) was loaded into the thrombin solution in 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 in a 1:1 ratio by using 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 (i.e., MSCs mixed with the fibrin glue) was performed

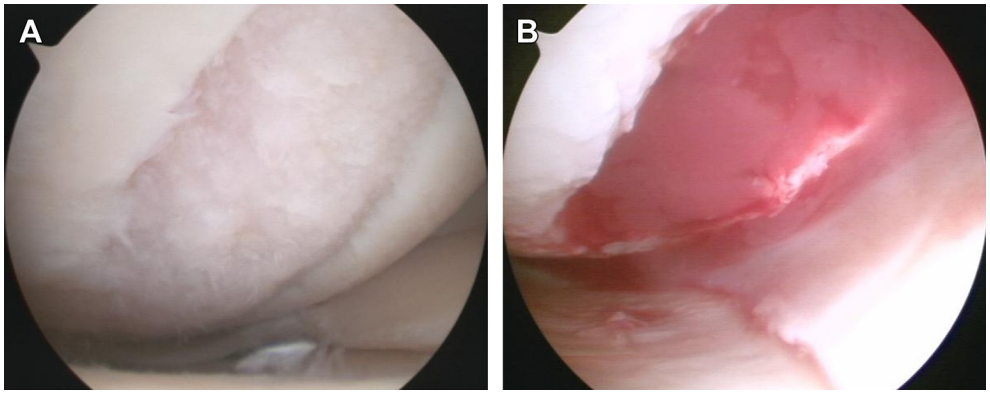

Fig. 1. (A) An articular cartilage lesion in the medial femoral condyle was noticed during arthroscopy. (B) Arthroscopic implantation of MSCs loaded in fibrin glue. The cartilage lesion was covered with the cell thrombin–fibrinogen suspension after manipulation with the probe.

under arthroscopic guidance after the arthroscopic fluid was extracted. The cell thrombin–fibrinogen suspension was applied and manipulated using the probe to be coated at the surface of the cartilage lesion [Fig. 1(B)]. No marrow-stimulation procedures such as microfracture, subchondral drilling, and abrasion arthroplasty were performed prior to this procedure.

After the arthroscopic procedure, the knee was immobilized for 2 weeks with a knee brace.Partial weight bearing and range-of-motion exercises were initiated at 2 weeks after arthroscopy, and full weight bearing was permitted at 4 weeks after surgery. 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.

Clinical evaluation

For clinical evaluation, the International Knee Documentation Committee (IKDC) score[22] and the Tegner activity scale[23] were used to determine joint function and sports activities. Furthermore, patients rated their overall satisfaction with the operation as excellent, good, fair, or poor.

MRI evaluation

Preoperative and follow-up MRI was performed using a 3.0 T MRI scanner (Achieva 3.0 T SE; Philips, Eindhoven, Netherlands) with a dedicated eight channel knee coil. The following sequences were utilized: (1) proton density (PD) spectral presaturation with inversion recovery (SPIR) transversal image (repetition time/echo time [TR/TE], 4000/15 ms): field of view (FOV), 150 × 150 mm; matrix, 308 × 249; slice thickness (SL), 3.5 mm with 0.35 mm gap; (2) PD SPIR coronal image (TR/TE, 3500/15 ms): FOV, 150 × 150 mm; matrix, 260 × 240; SL, 3.0 mm with 0.5 mm gap; (3) T2 SPIR sagittal image (TR/TE, 3200/70 ms): FOV, 150 × 150 mm; matrix, 240 × 192; SL, 3.0 mm with 0.3 mm gap; (4) turbo spin echo T1-weighted sagittal image (TR/TE, 600/20 ms): FOV, 150 × 150; matrix, 240 × 240; SL, 3.0 mm with 0.3 mm gap. To avoid potential bias, an independent observer, who was a musculoskeletal-trained radiologist not involved in the care of the patients and was blinded to the intention of this study, evaluated the MRI scans. On preoperative and follow-up MRI, evaluation of cartilage lesions was performed according to MOAKS, developed by Hunter *et al.*[15] Among seven independent criteria of MOAKS system[15], we used the articular cartilage grading system to evaluate the cartilage lesions before and after surgery. The rationale for the cartilage score was to provide separate scores for the size and depth of cartilage damage: each articular cartilage lesion was graded for the size of any cartilage loss (including partial and full thickness loss) in terms of the percentage of surface area and the percentage of full-thickness cartilage loss (Table I). In addition, on follow-up MRI, evaluation of repair tissue was performed according to MOCART score, according to Marlovits *et al.*[24] (Table II).

Statistical analysis

All analyses were conducted using SPSS Version 13.0 (IBM Corporation, Armonk, NY), with significance defined as P value < 0.05. The Wilcoxon signed-rank test was used to evaluate differences between the preoperative and final follow-up values, and Fisher exact test was used to compare categorical data. The Spearman rank-order correlation test was used to analyze correlations between MOCART score and clinical outcomes at final follow-up. To determine the associations between the various factors (patient characteristics [age, sex, and BMI] and cartilage lesion variables [size and location of cartilage lesion]) and) and satisfaction with clinical results, we used multivariate logistic regression analyses and reported odds ratios with 95% confidence intervals (CIs) relative to a chosen reference group. We defined satisfactory clinical results as an IKDC score of more than 68 points (mean IKDC score at final follow-up, 67.3), a Tegner activity scale of more than four (mean Tegner activity scale at final follow-up, 3.9), and good or excellent satisfaction with the surgery at the final follow-up. P values and 95% CIs are presented for each results, and categorical data are presented as n (%).

Results

Clinical outcomes

Before MSC implantation, the mean IKDC score was 38.7 (SD, 7.0), and the mean Tegner activity scale score was 2.5 (SD, 0.9). At the final follow-up (mean, 27.9 months; range, 24–34), the mean IKDC and Tegner activity scale scores both significantly improved to 67.3 (SD, 11.6) and 3.9 (SD, 0.7) (P < 0.001 for both), respectively. With regard to overall patient satisfaction with the surgery, 12 patients reported their satisfaction as excellent (50%), eight as good (33.3%), three as fair (12.5%), and one as poor (4.2%).

MRI outcomes

The cartilage lesion grades (as described in MOAKS) before surgery and at final follow-up are summarized in Table III.

Table I
Delineation of grading for cartilage lesions, as described in MOAKS

Grade	Size of cartilage loss area	Percentage of full-thickness cartilage loss
0	None	None
1	<10% of region of cartilage surface area	<10% of region of cartilage surface area
2	10–75% of region of cartilage surface area	10–75% of region of cartilage surface area
3	>75% of region of cartilage surface area	>75% of region of cartilage surface area

Abbreviations: MOAKS, magnetic resonance imaging osteoarthritis knee score.

Table II
MOCART scores at follow-up MRI

Variables	Score	*n* (%)	Mean (SD)	95% confidence interval
1. Degree of defect repair and filling of the defect			14.6 (6.9)	11.67–17.50
Complete	20	14		
Hypertrophy	15	0		
Incomplete				
>50% of the adjacent cartilage	10	5		
<50% of the adjacent cartilage	5	4		
Subchondral bone exposed	0	1		
2. Integration to border zone			10.6 (4.3)	8.83–12.42
Complete	15	9		
Incomplete				
Demarcating border visible	10	10		
Defect visible				
<50% of the length of the repair tissue	5	4		
>50% of the length of the repair tissue	0	1		
3. Surface of the repair tissue			7.9 (3.3)	6.54–9.30
Surface intact	10	16		
Surface damaged				
<50% of repair tissue depth or total degeneration	5	6		
>50% of repair tissue depth or total degeneration	0	2		
4. Structure of the repair tissue			4.4 (1.7)	3.66–5.09
Homogenous	5	19		
Inhomogenous or cleft formation	0	3		
5. Signal intensity of the repair tissue			22.5 (7.7)	19.26–25.74
Normal (identical to adjacent cartilage)	30	12		
Nearly normal (slight areas of signal alteration)	15	12		
Abnormal (large areas of signal alteration)	0	0		
6. Subchondral lamina			3.8 (2.2)	2.82–4.68
Intact	5	18		
Not intact	0	6		
7. Subchondral bone			3.5 (2.3)	2.56–4.52
Intact	5	17		
Not intact	0	7		
8. Adhesions			3.8 (2.2)	2.82–4.68
No	5	18		
Yes	0	6		
9. Effusion			2.9 (2.5)	1.85–3.98
No	5	14		
Yes	0	10		
Total			69.8 (14.3)	63.71–75.88

Abbreviations: MOCART, magnetic resonance observation of cartilage repair tissue; MRI, magnetic resonance imaging.

Table III
Grades of cartilage lesions, as described in MOAKS, at preoperative and follow-up MRI

Grade	Size of cartilage loss area			Percentage of full-thickness cartilage loss		
	Preoperative	Follow-up	*P* value*	Preoperative	Follow-up	*P* value*
			<0.001			<0.001
0	0	11 (45.8)		0	14 (58.3)	
1	3 (12.5)	8 (33.3)		1 (4.1)	5 (20.8)	
2	14 (58.3)	3 (12.5)		13 (54.2)	5 (20.8)	
3	7 (29.2)	2 (8.3)		10 (41.7)	2 (8.3)	

Data are presented as number (%).
Abbreviations: MOAKS, magnetic resonance imaging osteoarthritis knee score; MRI, magnetic resonance imaging.
* Fisher's exact test for comparison of grades of cartilage lesions, as described in MOAKS, between groups.

According to the grades for the size of the cartilage-loss area, 21 lesions (87.5%) were grade 2 or 3 before surgery and only five lesions (20.8%) were grade 2 or 3 at follow-up MRI. According to the grades for percentage of full-thickness cartilage loss, 23 lesions (95.9%) were grade 2 or 3 before surgery and only five lesions (20.8%) were grade 2 or 3 at follow-up MRI [Figs. 2 and 3]. The cartilage lesion grades (as described in MOAKS; grades for size of the cartilage-loss area and percentage of full-thickness cartilage

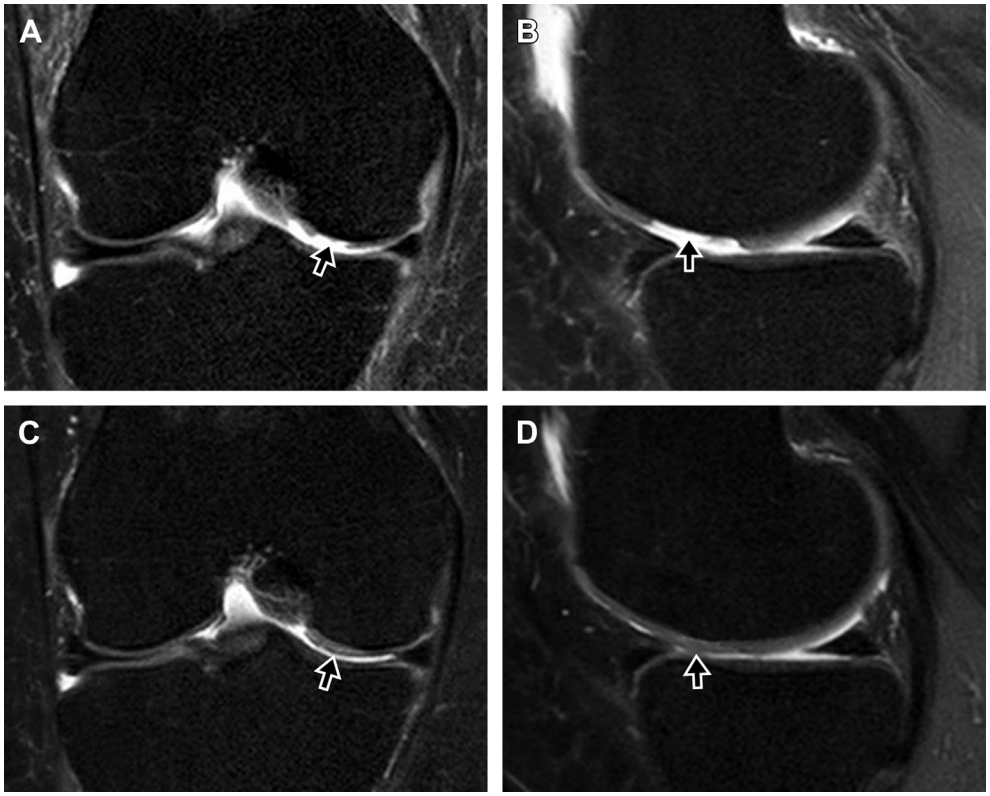

Fig. 2. (A and B) Preoperative fat-saturated proton density coronal and sagittal images of the right knee of a 58-year-old female patient. Cartilage loss (grade 2 for size of cartilage loss as a percentage of surface area and grade 3 for percentage of full-thickness cartilage loss of the region according to the MOAKS) was observed in the medial femoral condyle (arrows). (C and D) Follow-up fat-saturated proton density coronal and sagittal images. Complete filling of the defect along with complete integration with the adjacent native cartilage (grade 0 for size of cartilage loss as a percentage of surface area and grade 0 for percentage of full-thickness cartilage loss of the region according to MOAKS) with 75 points of MOCART score was observed (arrows).

loss) at follow-up MRI were significantly better than the preoperative values ($P < 0.001$ for both). The MOCART scores at follow-up MRI are summarized in Table II. At the follow-up, mean MOCART score was 69.8 (SD, 14.3).

Association between clinical and MRI outcomes

The IKDC score and Tegner activity scale at final follow-up, according to the cartilage lesion grades (as described in MOAKS) at follow-up MRI, are shown in Table IV. As the grades for the size of the cartilage-loss area and percentage of full-thickness cartilage loss decreased (quality of repaired cartilage increased), the IKDC score and Tegner activity scale increased ($P < 0.05$ for all). In addition, significant correlations of MOCART score with clinical outcomes were found (Spearman rho = 0.933, $P < 0.001$ for IKDC score and Spearman rho = 0.782, $P < 0.001$ for Tegner activity scale, respectively).

Association between various factors and outcomes

We used logistic regression models to assess the independent effects of various factors on clinical outcomes. We defined satisfactory clinical results as an IKDC score of more than 68 points (mean IKDC score at final follow-up, 67.3), a Tegner activity scale of more than four (mean Tegner activity scale at final follow-up, 3.9), and good or excellent satisfaction with the surgery at the final follow-up. The final model, as shown in Table V, controls for age, sex, BMI, and size and location of cartilage lesion. When compared with patients younger than 57.9 years of age, age was not an independent risk factor for poor clinical outcomes after MSC implantation ($P = 0.114$). Similarly, sex ($P = 0.345$), BMI ($P = 0.114$), cartilage lesion size ($P = 0.394$), and lesion location ($P = 0.178$) did not independently predict clinical outcomes.

Discussion

Recently, several methods for semiquantitative assessment of knee OA have been developed[13–15,25], and MRI-based semiquantitative scoring of knee OA has proven to be a valuable method for performing multifeature joint assessment[11,26]. Although many joint structures are affected in OA, articular cartilage is one of the main tissues involved in the OA disease process[16]. In the MOAKS system[15], the articular cartilage grading system provides separate scores for the size and depth of cartilage damage (Table I). In this study, we used the articular cartilage grading system, as described in MOAKS, to focus on evaluation of the cartilage lesions before and after MSC implantation. In addition, MOCART scoring system was also used for the evaluation of repaired cartilage. To our knowledge, this is the first study to present MRI outcomes after MSC implantation for the treatment of OA knees.

The source of the MSCs is a very important factor affecting the quality of the results. In this study, subcutaneous adipose tissue, composed of two main cell populations (mature adipocytes and the stromal vascular fraction cells), was used as the stem cell source. Compared with bone marrow-derived stem cells, adipose-derived MSCs have several advantages: Adipose-derived MSCs can easily be purified after digestion of fat and selection by adhesion onto plastic from the very heterogeneous crude stromal fraction.

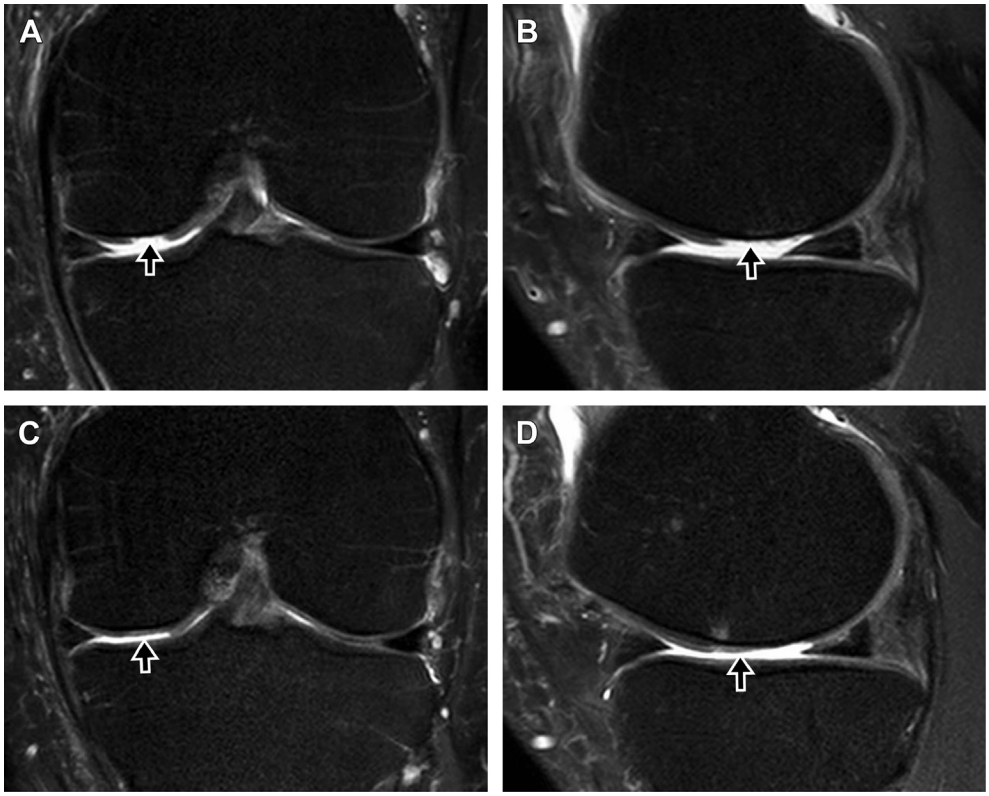

Fig. 3. (A and B) Preoperative fat-saturated proton density coronal and sagittal images of the right knee of a 60-year-old male patient. Cartilage loss (grade 2 for size of cartilage loss as a percentage of surface area and grade 3 for percentage of full-thickness cartilage loss of the region according to the MOAKS) was observed in the medial femoral condyle (arrows). (C and D) Follow-up fat-saturated proton density coronal and sagittal images. Incomplete filling of the defect along with incomplete integration with the adjacent native cartilage (grade 1 for size of cartilage loss as a percentage of surface area and grade 2 for percentage of full-thickness cartilage loss of the region according to MOAKS) with 60 points of MOCART score was observed (arrows).

Table IV
Clinical outcomes at final follow-up according to the grade of cartilage lesions, as described in MOAKS, at follow-up MRI

		IKDC score		Tegner activity scale	
Grade described in MOAKS	*n* (%)	Mean (SD)	*P* value*	Mean (SD)	*P* value*
Size of cartilage loss area			0.019		0.026
0	11 (45.8)	72.4 (10.7)		4.2 (0.6)	
1	8 (33.3)	69.1 (7.7)		4.0 (0.5)	
2	3 (12.5)	56.3 (6.8)		3.3 (0.6)	
3	2 (8.3)	48.0 (1.4)		2.5 (0.7)	
Percentage of full-thickness cartilage loss			0.018		0.015
0	14 (58.3)	73.1 (8.0)		4.2 (0.4)	
1	5 (20.8)	64.4 (11.3)		3.8 (0.8)	
2	3 (12.5)	56.7 (10.0)		3.3 (0.6)	
3	2 (8.3)	49.0 (2.8)		2.5 (0.7)	

Abbreviations: MOAKS, magnetic resonance imaging osteoarthritis knee score; MRI, magnetic resonance imaging; IKDC, International Knee Documentation Committee.
* Kruskal–Wallis test for differences in IKDC score and Tegner activity scale according to grade, as described in MOAKS.

Adipose tissue contains a substantially higher number of MSCs than the bone marrow[27]; therefore, a large number of cells can be obtained without culture expansion, which poses a risk of culture-induced chromosomal abnormality or senescence[28]. Adipose-derived MSCs have been shown to maintain their differentiation potential even in the later stages of life and may have better chondrogenic potential than bone marrow-derived MSCs[29]. The pain and morbidity associated with the harvesting of adipose tissue are considerably lesser than those associated with bone marrow harvesting[30]. In addition, the stromal vascular fraction cells containing adipose-derived MSCs are promising candidates from a broad range of innovative therapies ranging from those involving regenerative medicine to those involving tissue engineering, because of their immunoregulatory and anti-inflammatory activities[31]. Therefore, we chose the adipose tissue as the stem cell source.

The ideal scaffold should be biocompatible and biodegradable upon tissue healing, conducive to cell attachment and migration, permit appropriate extracellular matrix formation and the transmission of signaling molecules, and be adaptable to the mechanical environment[2,32,33]. Fibrin glue has been used widely in the development of articular cartilage repair strategies as a cell delivery matrix for generating a new cartilage matrix[34–38]. According to recent studies[39–41], fibrin glue promotes the proliferation and gene expression of MSCs and improves the therapeutic effect of adipose-derived MSCs for cartilage repair by sustaining survival and paracrine function. Therefore, we used fibrin glue as a scaffold in the present study because we believed that it could function as a

Table V
Associations between various factors and unsatisfactory clinical outcomes

Factors	*n* (%)	Unsatisfactory clinical outcomes	
		Odds ratio (95% confidence interval)	*P* value
Age*, y			0.114
<57.9	13 (54.2)	1.00	
≥57.9	11 (45.8)	0.25 (0.05–1.39)	
Sex			0.345
Male	9 (37.5)	1.00	
Female	15 (62.5)	0.44 (0.08–2.44)	
Body mass index*, kg/m^2			0.114
<26.6	11 (45.8)	1.00	
≥26.9	13 (54.2)	0.25 (0.05–1.39)	
Size of cartilage lesion*, cm^2			0.394
<6.2	11 (45.8)	1.00	
≥6.2	13 (54.2)	0.49 (0.01–2.53)	
Location of cartilage lesion			0.178
Medial femoral condyle	15 (62.5)	1.00	
Lateral femoral condyle	6 (25)	1.43 (0.20–1.43)	
Trochlea	3 (12.5)	0.20 (0.20–20.44)	

* Median values are used as standard values for dividing the groups.

scaffold in MSC implantation to induce better cell survival, proliferation, differentiation, and matrix synthesis, leading to the repair of cartilage lesions in OA knees.

The relationship between structural assessment and pain and/or functional limitation in OA is critical in determining the clinical relevance of MRI[26]. According to a systemic review and meta-analysis reported by Blackman *et al.*[42], the MRI findings do correlate with clinical outcomes after cartilage repair surgery for the knee, although the specific parameters that correlate best vary according to the type of procedure performed. Structural success is usually evaluated by description of the repair tissue based on morphologic semiquantitative or compositional MRI approaches[43–45]. Kreuz *et al.*[46] performed repair surgery for cartilage lesions of the knee and found a strong correlation between MRI parameters and the IKDC score. Ochs *et al.*[47] studied autologous chondrocyte implantation for chondral defects of the knee and reported that there were significant correlations between increase in the grade of defect filling and increase in the IKDC score ($P < 0.02$) and Tegner activity scale ($P < 0.001$). Although the present study evaluated joints with OA, which differ from joints with localized chondral defects, a similar correlation between clinical and MRI outcomes was found. In this study, as the quality of repaired cartilage increased, the IKDC score and Tegner activity scale increased ($P < 0.05$ for all; Table IV). In addition, significant correlations of MOCART score with clinical outcomes were found. We considered that the improvement in clinical outcomes might be due to the repair process of cartilage lesions mediated by implanted MSCs.

Identifying the prognostic factors associated with the clinical outcomes of MSC implantation would be useful for treating patients with OA knees. Patient characteristics or cartilage lesion variables may serve as important selection criteria for stem cell–based repair strategies. In the current study, we assessed the independent effects of various factors on clinical outcomes. The results showed that age, sex, BMI, and size and location of the cartilage lesion did not play a role as an independent predictor for clinical outcomes of MSC implantation. Therefore, we considered that MSC implantation with fibrin glue as a scaffold is an effective treatment method that can be widely used for patients with OA knees. However, further study identifying the prognostic factors with more precise verification would be required owing to the small sample size of this study.

The present study has some limitations. First, the number of cases was small, and the follow-up period was relatively short. For more accurate evaluation of MSC implantation for OA knees, a study with a larger number of cases and a longer follow-up period are required. Second, in the current study, the articular cartilage grading system was only used to evaluate the cartilage lesions before and after the MSC implantation for MRI evaluation because the indication for MSC implantation was an isolated articular cartilage lesion in OA knees (Kellgren–Lawrence[19] grades 1–2). For more precise evaluation of the MRI outcomes after MSC implantation at an advanced stage in OA knees, further study assessing the other criteria described in MOAKS, in addition to the articular cartilage grading system, would be necessary. Future research specifically analyzing the correlation between the clinical outcomes and the criteria of MOAKS after MSC implantation could be of great importance to the field. In addition, in this study, the cartilage repair was evaluated by one observer. Therefore we could not determine the inter-observer variability in the articular cartilage grading system. For the more precise evaluation of the arthroscopic findings, a power analysis with inter-observer variability in the articular cartilage grading system would be useful in future studies. Third, reduction of pain is an important factor in clinical scores used for evaluating clinical outcomes. In a randomized placebo-controlled trial, Moseley *et al.*[48] found that in patients with knee OA the outcomes after arthroscopic lavage or arthroscopic debridement were not better than after a placebo procedure. In addition, van Buul *et al.*[49] reported that intra-articular injection of MSCs reduced pain but not degenerative changes in an OA model. Therefore, the role of placebo effect in MSC therapy needs to be evaluated in the future studies. Fourth, the ratio of the mixture of the cell suspension of MSCs and fibrin glue was determined arbitrarily. 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[50–52]. Therefore, further study is required to determine the optimal mixture ratio between the MSCs and fibrin glue for achieving a suitable matrix environment, for example, with respect to temporary retention space, cytoprotective effects, and cell-matrix interactions provided by the fibrin glue. Fifth, the follow-up MRI was performed at 2 year after surgery. It is not known how the repaired cartilage will behave over time, and changes in the property of repaired cartilage after the second year cannot be predicted. Sixth, the optimal number of MSCs to be applied remains unknown.

Conclusion

This study showed encouraging clinical outcomes of MSC implantation with fibrin glue as a scaffold in OA knees. The cartilage

regeneration, as determined via MRI evaluation, was also encouraging after MSC implantation and correlated with the clinical outcomes. Therefore, MSC implantation with fibrin glue as a scaffold seems to be effective for repairing cartilage lesions in OA knees. However, a larger sample size and long-term studies are needed to confirm our findings.

Author contributions

Y.S. Kim: Conception and design, collection and assembly of data, analysis and interpretation of data, drafting of the article, critical revision of article, final approval of the article.

Y.J. Choi: Conception and design, analysis and interpretation of data.

S.W. Lee: Collection and assembly of data.

O.R. Kwon: Conception and design, final approval of the article.

D.S. Suh: Analysis and interpretation of data.

D.B. Heo: Collection and assembly of data, analysis and interpretation of data.

Y.G. Koh: Conception and design, analysis and interpretation of data, critical revision of the article, final approval of the article.

Conflict of interest

The authors certify that we have no commercial associations that might pose a conflict of interest in connection with this article.

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