

Mesenchymal Stem Cell Implantation in Knee Osteoarthritis

Midterm Outcomes and Survival Analysis in 467 Patients

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Background: A cell-based tissue engineering approach that uses mesenchymal stem cells (MSCs) has addressed the issue of articular cartilage repair in knees with osteoarthritis (OA).

Purpose: To evaluate the midterm outcomes, analyze the survival rates, and identify the factors affecting the survival rate of MSC implantation to treat knee OA.

Study Design: Case series; Level of evidence, 4.

Methods: We retrospectively evaluated 467 patients (483 knees) who underwent MSC implantation on a fibrin glue scaffold for knee OA with a minimum 5-year follow-up. Clinical outcomes were determined based on the International Knee Documentation Committee (IKDC) and Tegner activity scale results measured preoperatively and during follow-up. Standard radiographs were evaluated using Kellgren-Lawrence grading. Statistical analyses were performed to determine the survival rate and the effect of different factors on the clinical outcomes.

Results: The mean IKDC scores (baseline, 39.2 ± 7.2 ; 1 year, 66.6 ± 9.6 ; 3 years, 67.2 ± 9.9 ; 5 years, 66.1 ± 9.7 ; 9 years, 62.8 ± 8.5) and Tegner scores (baseline, 2.3 ± 1.0 ; 1 year, 3.4 ± 0.9 ; 3 years, 3.5 ± 0.9 ; 5 years, 3.4 ± 0.9 ; 9 years, 3.2 ± 0.9) were significantly improved until 3 years postoperatively and gradually decreased from 3- to 9-year follow-up ($P < .05$ for all, except for Tegner score at 5 years vs 1 year [$P = .237$]). Gradual deterioration of radiological outcomes according to the Kellgren-Lawrence grade was found during follow-up. Survival rates based on either a decrease in IKDC or an advancement of radiographic OA with Kellgren-Lawrence scores were 99.8%, 94.5%, and 74.5% at 5, 7, and 9 years, respectively. Based on multivariate analyses, older age and the presence of bipolar kissing lesion were associated with significantly worse outcomes ($P = .002$ and $.013$, respectively), and a larger number of MSCs was associated with significantly better outcomes ($P < .001$) after MSC implantation.

Conclusion: MSC implantation provided encouraging outcomes with acceptable duration of symptom relief at midterm follow-up in patients with early knee OA. Patient age, presence of bipolar kissing lesion, and number of MSCs were independent factors associated with failure of MSC implantation.

Keywords: mesenchymal stem cell; implantation; knee osteoarthritis; survival analysis; articular cartilage

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Osteoarthritis (OA) is a highly prevalent, progressive, painful joint disease accompanied by decreasing joint function³⁶; with the increasing incidence of OA, it was anticipated to be the fourth leading cause of disability in 2020.⁴¹ The onset of OA is mainly characterized by gradual loss of articular cartilage due to impaired anabolic and/or catabolic balance, which progresses to full-thickness lesions with direct bone-to-bone contact, causing pain, swelling, stiffness, and impaired mobility.²¹ Additionally, OA follows a chronic cycle of aberrant attempts to repair the joints involved, leading to inflammation and tissue degradation.⁴⁵ According to the OA Research Society International, OA management should reduce pain and inflammation, slow cartilage degradation, improve function, and reduce disability.¹³ However, due to the limited intrinsic healing

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potential of cartilage secondary to its avascular, aneural, and alymphatic properties,^{3,21} restoration of the diseased articular cartilage in patients with OA is a challenging problem for researchers and clinicians.⁴² To solve these problems, various surgical procedures, including microfracture, drilling, abrasion arthroplasty, autologous chondrocyte implantation, and osteochondral autograft transfer, which are not traditional treatment measures for OA, have been performed to treat the diseased articular cartilage.³⁸ However, because of the limitations of these procedures, such as biomechanical insufficiency of the regenerative fibrocartilage compared with the hyaline cartilage, limited tissue availability, loss of phenotype from dedifferentiation of primary chondrocytes during expansion, and possible need for open surgery with donor-site morbidity, controversy exists concerning the effectiveness of these procedures in OA; thus, alternative techniques for cartilage regeneration remain a continuous quest.^{3,38}

Mesenchymal stem cells (MSCs) have emerged as a promising cell-based treatment modality for OA because they are known to 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.⁹ Recently, several clinical studies involving the use of MSCs have reported encouraging outcomes after MSC-based treatment for knee OA.[‡] However, despite the therapeutic effects of MSCs in cartilage repair, the most effective method for MSC application in the treatment of knee OA has not yet been established. 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.²⁸ Among the various procedures using MSCs for the treatment of knee OA, MSC implantation has been reported as an effective method for obtaining favorable outcomes,^{29,30} considering the cells' efficiency in traveling to target organs and tissues.²⁸ In previous studies, Kim et al^{29,30} performed MSC implantation using fibrin glue as a scaffold and reported that the improved cartilage regeneration was associated with the improved clinical outcomes; the investigators also identified the prognostic factors influencing the clinical outcomes. However, because the cohorts in these studies were small and restricted to 2-year follow-up, the long-term behavior of the repaired cartilage is unknown, and the changes in the influencing factors after the second year cannot be predicted.

Therefore, the aims of this study were to evaluate the midterm outcomes of MSC implantation, analyze survival rates, and identify the factors associated with survival rates in a large series of patients up to 9 years after surgery. We hypothesized that MSC implantation would provide acceptable clinical outcomes in patients with knee OA and that there would be factors affecting the outcomes.

METHODS

Patient Enrollment

The study protocol was approved by the institutional review board of our hospital, and all patients provided written informed consent. We retrospectively reviewed the medical records of 534 consecutive patients (576 knees) with cartilage lesions in their knees who were treated using arthroscopic MSC implantation with fibrin glue as a scaffold for cartilage regeneration between November 2011 and January 2015. The inclusion criteria, determined by medical records, plain radiographs, and magnetic resonance imaging (MRI), were full-thickness cartilage lesion, Kellgren-Lawrence²⁶ OA grade 1 or 2, and symptoms of knee joint pain and/or functional limitations despite a minimum of 3 months of nonsurgical treatments. Only patients with a minimum of 5 years of follow-up were included. The exclusion criteria were multiple cartilage lesions or patellar lesions in the knees, previous surgical treatment, knee instability, knee varus or valgus malalignment $\geq 5^\circ$ evaluated using the tibial and femoral mechanical axis measured on a hip-to-ankle standing anterior-posterior (AP) radiograph, metabolic arthritis, joint infections, or large meniscal tears. Of the 534 patients (576 knees), 29 patients (49 knees) did not meet the inclusion criteria, and 38 patients (44 knees) were lost to follow-up; thus, 467 patients (483 knees) were ultimately included in this study. The characteristics of the patients are shown in Table 1. Among the categories of characteristics, we defined bipolar kissing lesion as combined cartilage lesion that affects both the contacting femoral condyle and tibial plateau. The lesion size was measured using preoperative MRI by an independent observer, who was a musculoskeletal-trained radiologist not involved in the care of patients and who was blinded to the intention of this study.

MSC Preparation

Sample collection and MSC isolation were performed as described previously.³⁰ Subcutaneous adipose tissue samples were obtained through liposuction from the gluteal regions of the patients 1 day before MSC implantation. The liposuction material was aspirated by gentle suction, the gluteal fat pad was collected, and the stromal vascular fraction (SVF) was separated through centrifugation, in accordance with a previously reported method.⁵³ MSCs were isolated from the lipoaspirate by enzymatic digestion and then cultured to determine the characteristics of the adipose-derived MSCs.³⁹ The adipose-derived MSC immunophenotype was investigated by analytical flow cytometry using cell markers, and the differentiation potentials of adipose-derived stem cells into adipogenic, osteogenic, and chondrogenic cell lineages were also assessed using specific inductive culture media.³⁹ These isolation and characterization procedures determined that the SVF contained adipose-derived MSCs, which made up 9.6% of this fraction. Consequently, an average of 8.45×10^8 cells in the SVF, which contained an average of 8.11×10^6 stem cells (9.6% of

‡References 15, 16, 19, 25, 29, 30, 32, 35, 37.

TABLE 1
Baseline Characteristics^a

Characteristic	
Knees/patients, n	483/467
Age, y	61.1 ± 6.6 (48-74)
Sex, male/female, n	150/333
Side of involvement, right/left, n	235/248
Body mass index	25.9 ± 2.9 (19.3-32.9)
Follow-up period, mo	86.3 ± 13.7 (60-110)
Location of cartilage lesion in femoral condyle, n (%)	
Medial femoral condyle	320 (66.3)
Lateral femoral condyle	148 (30.6)
Trochlea	15 (3.1)
Kissing lesion, n (%)	
Absence	278 (57.6)
Presence	205 (42.4)
Lesion size, cm ²	
Femoral condyle	7.0 ± 1.0 (4.8-9.1)
Medial femoral condyle	7.1 ± 0.9 (4.8-9.1)
Lateral femoral condyle	7.0 ± 0.9 (4.8-8.6)
Tibial plateau in cases of kissing lesion	6.1 ± 0.9 (4.0-8.3)
Medial tibial plateau	6.2 ± 0.9 (4.0-8.3)
Lateral tibial plateau	6.0 ± 0.9 (4.0-8.3)
Trochlea	5.4 ± 0.4 (4.8-5.9)
No. of mesenchymal stem cells (range)	8.11 × 10 ⁶ (3.02 × 10 ⁶ to 1.98 × 10 ⁷)

^aData are expressed as mean ± SD (range) unless otherwise indicated.

8.45 × 10⁸ cells in the SVF; range, 3.02 × 10⁶ to 1.98 × 10⁷ cells), were used for MSC implantation.

Surgical Procedures and MSC Application

The patients were placed in the supine position on the operating table, and thigh tourniquets were applied. MSC implantation was performed as described previously.³⁰ In brief, before MSC implantation, accurate debridement of all unstable and damaged cartilage in the lesion was performed. The prepared MSCs were loaded into the fibrin glue product (Greenplast kit W; Greencross), which was used as a scaffold for MSC implantation. After the arthroscopic fluid was extracted, MSCs mixed with fibrin glue were implanted into the lesion site under arthroscopic guidance. Then, the applied MSCs mixed with fibrin glue were manipulated using a probe to cover the surface of the cartilage lesion evenly (Figure 1). No marrow stimulation procedures, such as microfracture surgery, subchondral drilling, or abrasion arthroplasty, were performed before this procedure.

Postoperative Management

After the arthroscopic procedure, the knee was immobilized for 2 weeks using a knee brace; after the sutures were removed, the patients began active and passive range of motion 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 full return to normal sports or recreational activities was allowed according to individual recovery. Additional nonoperative treatments, including nonsteroidal anti-inflammatory drugs, physical therapy, and/or injections, were performed after MSC implantation in patients who needed or wanted the treatments; however, we considered that the outcomes should be mainly attributable to MSC implantation.

Outcome Assessment

All patients were evaluated clinically and radiologically preoperatively and during follow-up. For the clinical evaluation, the International Knee Documentation Committee (IKDC)²³ and the Tegner activity scale⁴⁶ scores were used to determine joint function and sports activities. Preoperative and postoperative radiological evaluations consisted of a weight-bearing AP view, a true lateral view at 30° of knee flexion, and a hip-to-ankle standing AP radiograph on a long cassette. Kellgren-Lawrence grading was performed on the basis of AP radiographs taken preoperatively and during follow-up. To avoid potential bias, the same independent observer, who was a musculoskeletal-trained radiologist not involved in the care of patients and blinded to the intention of this study, evaluated the radiological outcomes.

Statistical Analysis

Descriptive statistics were calculated as mean ± SD for continuous variables and as frequencies and proportions for categorical variables. Paired-samples *t* tests were used to compare the preoperative and postoperative clinical values over the different time points of the follow-up period. Chi-square analysis was used to identify statistically significant changes in the proportion of the Kellgren-Lawrence grades over the different time points of the follow-up period. The associations among factors were also examined on the basis of the clinical outcomes. Differences between the groups were analyzed using 2-sample *t* test or 1-way analysis of variance for multiple comparisons. Kaplan-Meier survival curves were used for survival analyses, with failure of MSC implantation as the endpoint, followed by log-rank analysis. Multivariable Cox regression analysis was performed to assess the influence of the patients' characteristics on failure of MSC implantation while adjusting for covariates. The results of the Cox models are reported as hazards ratio (HR) and 95% CI to a chosen reference group. Statistical analyses were performed using SPSS Version 13.0 (IBM Corp), with significance defined as *P* < .05.

RESULTS

Clinical and Radiological Outcomes

The mean IKDC and Tegner scores were significantly improved at 1 year of follow-up compared with the preoperative baseline values (*P* < .05, respectively). Further

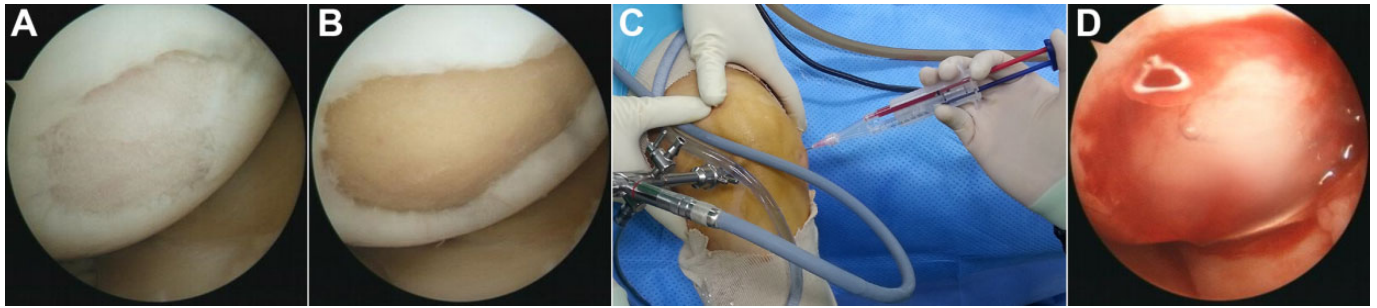


Figure 1. Arthroscopic implantation of mesenchymal stem cells loaded in fibrin glue. (A) An articular cartilage lesion in the medial femoral condyle was noticed. (B) An accurate debridement of all unstable and damaged cartilage in the lesion was performed. (C) The cell-thrombin-fibrinogen suspension was applied to the lesion. (D) The cartilage lesion was covered with the cell-thrombin-fibrinogen suspension after manipulation using the probe.

TABLE 2
Comparison of Preoperative and Postoperative Clinical and Radiological Outcomes^a

	Preoperative	Postoperative			
		1 y	3 y	5 y	9 y
IKDC score	39.2 ± 7.2	66.6 ± 9.6 ^b	67.2 ± 9.9 ^{b,c}	66.1 ± 9.7 ^{b,c,d}	62.8 ± 8.5 ^{b,c,d,e}
Tegner score	2.3 ± 1.0	3.4 ± 0.9 ^b	3.5 ± 0.9 ^{b,c}	3.4 ± 0.9 ^{c,d}	3.2 ± 0.9 ^{b,c,d,e}
KL grade					
Grade 1	189 (39.1)	184 (38.1)	173 (35.8)	164 (34.0) ^{b,c}	159 (32.9) ^{b,c,d}
Grade 2	294 (60.9)	299 (61.9)	310 (64.2)	305 (63.1) ^{b,c}	293 (60.7) ^{b,c,d}
Grade 3				12 (2.5) ^{b,c,d}	26 (5.4) ^{b,c,d,e}
Grade 4				2 (0.4) ^{b,c,d}	5 (1.0) ^{b,c,d,e}

^aData are expressed as mean ± SD or n (%). IKDC, International Knee Documentation Committee; KL, Kellgren-Lawrence.

^bSignificantly different from preoperative value.

^cSignificantly different from 1-year follow-up.

^dSignificantly different from 3-year follow-up.

^eSignificantly different from 5-year follow-up.

significant improvements of the mean IKDC and Tegner scores were found at 3-year follow-up compared with those preoperatively and at 1-year follow-up ($P < .05$). However, the mean IKDC and Tegner scores gradually decreased from 3- to 9-year follow-up, with statistically significant differences ($P < .05$), except for the Tegner score at the 5-year follow-up compared with that at the 1-year follow-up ($P = .237$) (Table 2). According to Kellgren-Lawrence OA grade, the gradual deterioration of radiological outcomes was found at 1 to 9 years of follow-up (Table 2).

Association Between Patient Characteristics and Clinical Outcomes

To assess characteristics (age, sex, side of involvement, body mass index [BMI], location of the cartilage lesion, presence of bipolar kissing lesion, cartilage lesion size, and number of MSCs) that may influence the clinical outcomes, the factors were divided into each individual group. The patients were divided according to age (<50, 50-59, 60-69, and ≥70 years), sex, side of involvement (right and left),

BMI (<20.0, 20.0-24.9, 25-29.9, and ≥30.0), location of the cartilage lesion (medial femoral condyle, lateral femoral condyle, and trochlea), cartilage lesion size (<6.0, 6.0-6.9, 7.0-7.9, and ≥8.0 cm² for the femoral condyle and <5.0, 5.0-5.9, 6.0-6.9, and ≥7.0 cm² for the tibial plateau), and number of MSCs (<5 × 10⁶, 5 × 10⁶ to 10 × 10⁶, 10 × 10⁶ to 15 × 10⁶, and ≥15 × 10⁶ cells). There were significant differences in IKDC scores at 1 year, 3 years, 5 years, and 9 years after surgery among the groups divided according to age, presence of kissing lesion, and number of MSCs ($P < .05$ for all). The bivariate correlation showed a statistically significant association between age and number of MSCs (correlation coefficient = -0.215; $P < .001$). However, no significant differences were found among the groups divided according to sex, side of involvement, BMI, location of the cartilage lesion, or cartilage lesion size (Table 3).

We analyzed the lesion size and number of MSCs according to the presence of bipolar kissing lesion to assess whether it influenced the outcomes. We noted significant differences in the lesion size of the femoral condyle and the total lesion size (defined as the size of the femoral condyle alone or the trochlea in unipolar lesions or the sum of the

TABLE 3
Preoperative and Postoperative IKDC Scores by Patient Characteristics^a

	IKDC Score				
	Preoperative	Postoperative			
		1 y	3 y	5 y	9y
Age					
<50 y	41.0 ± 7.0	70.4 ± 7.8	71.3 ± 8.9	70.0 ± 8.0	65.2 ± 8.9
50-59 y	38.3 ± 7.4	68.8 ± 7.3	69.3 ± 8.1	68.2 ± 7.6	63.8 ± 7.7
60-69 y	37.0 ± 7.2	65.1 ± 10.3	65.8 ± 10.2	64.5 ± 10.2	62.2 ± 8.9
≥70 y	38.2 ± 7.2	64.1 ± 11.8	64.1 ± 11.8	63.6 ± 11.7	61.6 ± 8.7
<i>P</i> value ^b	.279	<.001	<.001	<.001	.014
Sex					
Male	39.2 ± 7.6	66.3 ± 9.8	67.0 ± 10.1	65.8 ± 9.8	62.7 ± 8.3
Female	39.2 ± 6.9	66.3 ± 9.6	67.3 ± 10.0	66.2 ± 9.7	62.9 ± 8.6
<i>P</i> value ^c	.349	.849	.936	.884	.608
Side of involvement					
Right	39.8 ± 7.1	66.7 ± 9.3	67.2 ± 9.8	66.1 ± 9.4	62.5 ± 9.0
Left	38.6 ± 7.1	66.5 ± 10.0	67.2 ± 10.2	66.1 ± 10.1	63.1 ± 8.6
<i>P</i> value ^c	.823	.324	.464	.299	.072
Body mass index					
<20.0	38.6 ± 10.9	61.6 ± 10.8	63.5 ± 11.3	61.5 ± 10.7	63.4 ± 9.4
20.0-24.9	39.0 ± 7.5	66.5 ± 9.7	67.1 ± 9.6	65.9 ± 9.8	64.4 ± 8.6
25.0-29.9	39.0 ± 6.5	66.9 ± 9.4	67.4 ± 10.1	66.4 ± 9.4	63.8 ± 8.1
≥30.0	40.0 ± 4.2	68.4 ± 10.7	69.1 ± 10.8	68.4 ± 10.7	62.5 ± 6.4
<i>P</i> value ^b	.127	.104	.313	.114	.246
Location of cartilage lesion					
Medial FC	39.3 ± 7.2	66.7 ± 9.6	67.5 ± 10.0	66.3 ± 9.6	63.0 ± 8.3
Lateral FC	38.7 ± 7.0	66.3 ± 9.8	66.7 ± 9.8	65.8 ± 9.8	62.6 ± 8.8
Trochlea	42.3 ± 7.0	65.5 ± 11.0	66.1 ± 12.0	64.8 ± 11.1	59.5 ± 8.0
<i>P</i> value ^b	.177	.825	.682	.770	.327
Bipolar kissing lesion					
Absence	40.1 ± 7.0	68.1 ± 8.4	69.3 ± 9.1	68.3 ± 8.6	64.1 ± 8.8
Presence	38.6 ± 7.1	64.8 ± 10.4	65.9 ± 10.5	64.5 ± 10.3	61.8 ± 7.9
<i>P</i> value ^c	.475	.002	<.001	.012	.034
Lesion size					
FC					
<6.0 cm ²	39.3 ± 6.7	67.6 ± 7.9	68.3 ± 8.3	67.0 ± 8.0	62.1 ± 7.7
6.0-6.9 cm ²	39.7 ± 7.1	66.9 ± 10.0	67.7 ± 10.2	66.4 ± 10.1	63.1 ± 9.3
7.0-7.9 cm ²	38.7 ± 7.5	66.6 ± 9.3	67.0 ± 9.7	66.2 ± 9.4	63.3 ± 8.1
≥8.0 cm ²	39.1 ± 6.9	65.4 ± 10.9	65.8 ± 11.3	64.8 ± 10.9	62.2 ± 8.4
<i>P</i> value ^b	.711	.449	.353	.441	.689
TP in cases of kissing lesion					
<5.0 cm ²	39.0 ± 6.8	67.7 ± 7.4	67.8 ± 7.9	67.1 ± 7.6	61.3 ± 8.7
5.0-5.9 cm ²	40.5 ± 6.9	68.1 ± 9.6	68.9 ± 10.3	67.6 ± 9.8	64.5 ± 9.0
6.0-6.9 cm ²	39.9 ± 7.6	68.8 ± 7.3	68.7 ± 8.6	68.2 ± 7.6	63.4 ± 8.3
≥7.0 cm ²	40.1 ± 6.3	65.8 ± 7.9	66.4 ± 7.9	65.2 ± 8.0	60.2 ± 8.7
<i>P</i> value ^b	.859	.368	.565	.389	.089
No. of MSCs					
<5 × 10 ⁶	39.3 ± 6.6	63.3 ± 12.6	63.4 ± 13.3	62.9 ± 12.5	61.2 ± 9.2
5 × 10 ⁶ to 10 × 10 ⁶	39.1 ± 7.4	66.9 ± 9.1	67.6 ± 9.1	66.4 ± 9.2	62.9 ± 8.5
10 × 10 ⁶ to 15 × 10 ⁶	38.9 ± 6.7	67.1 ± 8.1	67.6 ± 8.9	66.6 ± 8.3	63.8 ± 8.3
≥ 15 × 10 ⁶	40.6 ± 7.1	70.2 ± 8.0	71.7 ± 8.2	69.9 ± 8.3	65.6 ± 7.4
<i>P</i> value ^b	.663	.002	<.001	.002	.041

^aData are expressed as mean ± SD. Bolded *P* values indicate statistical significance (*P* < .05). FC, femoral condyle; IKDC, International Knee Documentation Committee; MSC, mesenchymal stem cell; TP, tibial plateau.

^bOne-way analysis of variance.

^cTwo-sample *t* test.

lesion sizes of the femoral condyle and tibial plateau in bipolar kissing lesions) in accordance with the presence of bipolar kissing lesions (*P* < .05) (Table 4). Although no

significant difference was seen in the total number of MSCs by the presence of bipolar kissing lesion, the number of MSCs per total lesion size was significantly larger in

TABLE 4
Lesion Size and Number of MSCs According to the Presence of Bipolar Kissing Lesion^a

	n	Bipolar Kissing Lesion		P Value
		Absence	Presence	
Lesion size, cm ²				
FC		6.8 ± 0.9 (4.8-8.8)	7.1 ± 1.0 (5.0-9.1)	<.001
Medial FC	329	6.8 ± 0.9 (5.0-8.8)	7.2 ± 0.8 (5.8-9.1)	<.001
Lateral FC	139	6.9 ± 0.9 (4.8-8.5)	7.3 ± 0.8 (5.0-8.6)	.005
TP			6.1 ± 0.9 (4.0-8.3)	
Medial TP	136		6.2 ± 0.9 (4.0-8.3)	
Lateral TP	69		6.0 ± 0.9 (4.0-8.3)	
Trochlea	15		5.4 ± 0.4 (4.8-5.9)	
Total (FC alone, trochlea, or FC+TP)	483	6.8 ± 0.9 (4.8-8.8)	12.9 ± 1.5 (9.5-16.2)	<.001
No. of MSCs		7.94 × 10 ⁶	8.33 × 10 ⁶	.224
No. of MSCs per total lesion size, n/cm ²		1.14 × 10 ⁶	6.51 × 10 ⁵	<.001

^aData are expressed as mean ± SD (range) unless otherwise indicated. Bolded *P* values indicate statistical significance (*P* < .05). FC, femoral condyle; MSC, mesenchymal stem cell; TP, tibial plateau.

unipolar lesions than in bipolar kissing lesions (*P* < .001) (Table 4).

Survival and Risk Factor Analyses

No patient went on to have high tibial osteotomy or knee arthroplasty during the study period. To assess the survival rate of MSC implantation, we defined the failure of MSC implantation as IKDC score <40 (mean preoperative IKDC score, 39.2) during the follow-up period or deterioration of radiological outcomes from Kellgren-Lawrence grade 1 or 2 to 3 or 4 during the follow-up period. Of the 483 knees, 49 (10.1%) had an IKDC score <40 points, and 31 (6.4%) knees with an IKDC score <40 points had deterioration of radiological outcomes from Kellgren-Lawrence grade 1 or 2 to 3 or 4 during the follow-up period. Using the Kaplan-Meier survival estimates of failure, we found that the probabilities of survival after MSC implantation were 99.8% at 5 years, 94.5% at 7 years, and 74.5% at 9 years postoperatively (Figure 2).

Based on the multivariate Cox regression analysis, patient age (HR = 1.948 for age 50-59 years, HR = 4.656 for 60-69 years, and HR = 6.726 for ≥70 years, when compared with <50 years), presence of bipolar kissing lesion (HR = 2.626), and number of MSCs (HR = 0.856 for 5 × 10⁶ to 10 × 10⁶, HR = 0.470 for 10 × 10⁶ to 15 × 10⁶, and HR = 0.144 for ≥15 × 10⁶, when compared with <5 × 10⁶) were the risk factors associated with failure of MSC implantation (*P* = .002, *P* = .013, and *P* < .001, respectively) (Table 5; Figure 3). However, sex (*P* = .828), side of involvement (*P* = .827), BMI (*P* = .787), location of the cartilage lesion (*P* = .911), and cartilage lesion size (*P* = .137 for the femoral condyle and *P* = .785 for the tibial plateau) were not associated with failure of MSC implantation.

DISCUSSION

To our knowledge, this is the first study to report midterm results of MSC implantation in a large cohort of patients

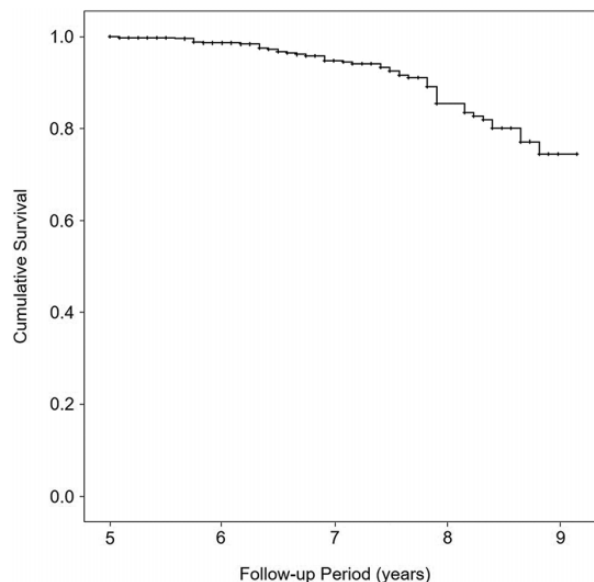


Figure 2. Kaplan-Meier survival curve at a minimum 5-year follow-up after mesenchymal stem cell implantation.

with early knee OA. In this retrospective review of 467 patients (483 knees) who underwent MSC implantation for knee OA and had a minimum of 5-year follow-up (mean, 7.2 years), results of MSC implantation showed improved clinical outcomes and survival rates of 99.8% at 5 years, 94.5% at 7 years, and 74.5% at 9 years postoperatively. Therefore, we believe that MSC implantation can be an effective procedure for the treatment of early knee OA. Additionally, we found that patient age, presence of bipolar kissing lesion, and number of MSCs were independent factors associated with failure of MSC implantation.

MSCs have been suggested for the treatment of knee OA because of their ability to differentiate into chondrocytes, which can repair cartilage tissue, and their homing characteristics, which make them ideal seed cells for gradual

TABLE 5
Influence of Different Factors on Survival^a

Factor	n (%)	Hazard Ratio	95% CI	P Value
Age				.002
<50 y	31 (6.4)	1.0		
50-59 y	180 (37.3)	1.948	0.927-4.095	
60-69 y	189 (39.1)	4.656	0.551-39.354	
≥70 y	83 (17.2)	6.726	2.346-19.283	
Sex				.828
Male	150 (31.1)	1.0		
Female	333 (68.9)	0.966	0.477-1.955	
Side of involvement				.827
Right	235 (48.7)	1.0		
Left	248 (51.3)	1.076	0.552-2.095	
Body mass index				.787
<20.0	19 (3.9)	1.0		
20.0-24.9	207 (42.9)	1.438	0.211-9.789	
25.0-29.9	232 (48.0)	2.142	0.481-9.547	
≥30.0	25 (5.2)	2.216	0.496-9.898	
Location of cartilage lesion				.911
Medial FC	320 (66.3)	1.0		
Lateral FC	148 (30.6)	0.854	0.416-1.753	
Trochlea	15 (3.1)	0.687	0.094-5.029	
Bipolar kissing lesion				.013
Absence	278 (57.6)	1.0		
Presence	205 (42.4)	2.626	1.222-5.646	
Lesion size				.137
FC				
<6.0 cm ²	81 (16.7)	1.0		
6.0-6.9 cm ²	151 (31.3)	1.616	0.668-3.907	
7.0-7.9 cm ²	153 (31.7)	1.958	0.802-4.778	
≥8.0 cm ²	98 (20.3)	3.278	0.994-10.805	
TP in cases of kissing lesion				.785
<5.0 cm ²	21 (10.2)	1.0		
5.0-5.9 cm ²	76 (37.1)	0.597	0.118-3.035	
6.0-6.9 cm ²	73 (35.6)	1.045	0.182-6.000	
≥7.0 cm ²	35 (17.1)	1.212	0.103-14.243	
No. of MSCs				<.001
<5 × 10 ⁶	79 (16.4)	1.0		
5 × 10 ⁶ to 10 × 10 ⁶	289 (59.8)	0.856	0.101-7.262	
10 × 10 ⁶ to 15 × 10 ⁶	80 (16.6)	0.470	0.049-4.501	
≥15 × 10 ⁶	35 (7.2)	0.144	0.016-1.267	

^aBolded *P* values indicate statistical significance (*P* < .05). FC, femoral condyle; MSC, mesenchymal stem cell; TP, tibial plateau.

OA treatment.^{4,14,50} Furthermore, considering the pathogenesis of OA, which is based on both degeneration and inflammation, the therapeutic properties of MSCs, including the paracrine,^{4,20} anti-inflammatory,⁴⁸ and immunomodulatory effects,^{24,52} would contribute to the improvement of the intra-articular environment by modifying OA progression.⁴⁴ Therefore, several clinical studies using MSCs for the treatment of knee OA have reported improvement of clinical outcomes.[§] However, evaluation of the efficacy of MSC therapy for knee OA is difficult because various methods of MSC application as well as different types of cell sources were used in these previous studies. In our study, we performed MSC implantation as described in a previous study reported by Kim et al.,³⁰

which achieved significant improvement in clinical outcomes after the implantation of MSCs loaded in fibrin glue as a scaffold, and these results were confirmed in a matched-pair analysis.³¹ A review of the literature revealed that only a few studies have reported the midterm clinical outcomes after MSC treatment for knee OA,^{11,34} and we considered that our midterm results were comparable with and/or superior to those of these previous studies. The clinical outcomes in this study were significantly improved until 3 years postoperatively and gradually decreased from 3- to 9-year follow-up, and gradual deterioration of radiological outcomes was found concurrently (see Table 2). Given that no similar studies of this size have been published, we believe that these data are valuable for predicting the outcomes of MSC implantation in patients with knee OA.

[§]References 12, 15, 25, 27, 29-32, 37, 47.

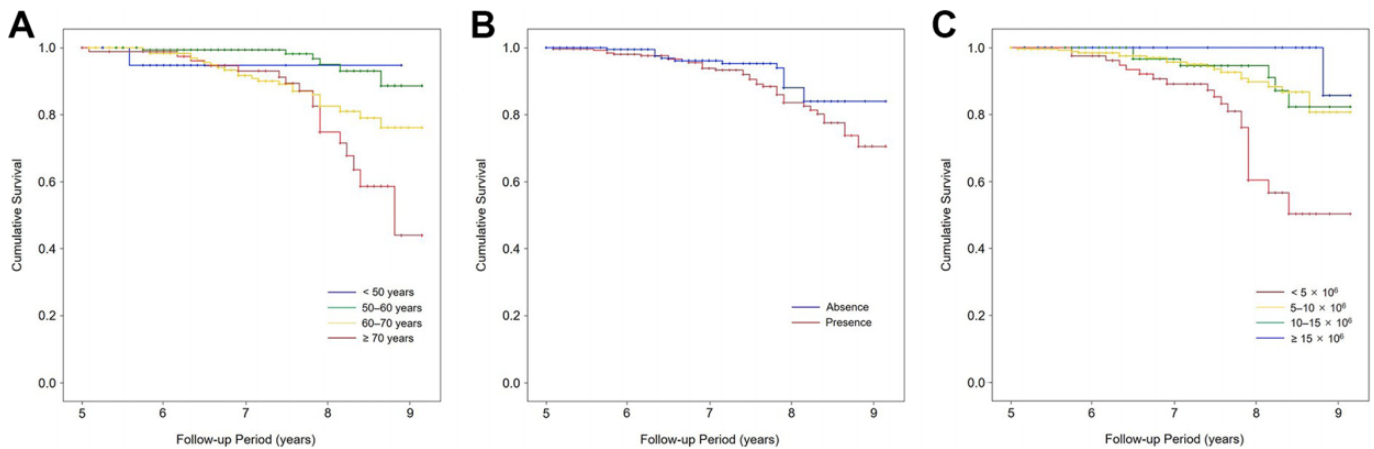


Figure 3. Kaplan-Meier survival curves. Survival rate of groups divided according to (A) age, (B) presence of bipolar kissing lesion, and (C) number of mesenchymal stem cells.

Identifying the prognostic factors associated with clinical outcomes will help patients with knee OA to have more realistic expectations after undergoing MSC implantation. Older age is a significant risk factor for OA that may affect the quality of MSCs,⁹ and several studies have described an age-dependent effect on the properties of MSCs.^{6,7,10} Chang et al⁶ reported that the number and function of MSCs in the articular cartilage gradually decreased as the patients' age increased and the chondrogenic differentiation of MSCs was lower in elderly patients. Choudhery et al⁷ found that the viability, proliferation, and differentiation potential of MSCs were reduced in older donors compared with young donors. Additionally, Dos-Anjos Vilaboa et al¹⁰ demonstrated a statistically significant decline in MSC yield with increasing age. In the current study, we found significant differences in IKDC scores at 1 year, 3 years, 5 years, and 9 years after surgery among the age groups ($P < .05$ for all) (Table 3). In addition, we found that age was an independent predictor of failure of MSC implantation ($P = .002$) (Table 5), and the bivariate correlation showed a statistically significant association between age and number of MSCs (correlation coefficient = -0.215 ; $P < .001$). We considered that these findings would have resulted from a less favorable quality of MSCs from older patients.

A review of the literature revealed that the number of MSCs used for the treatment of OA is another important prognostic factor of the outcomes.^{22,33} Afizah and Hui¹ reviewed studies using bone marrow-derived MSCs, which ranged from 8×10^6 to 4×10^7 cells,^{8,12,43,49,51} for OA treatment and concluded that $>1 \times 10^7$ bone marrow-derived MSCs are required to achieve significantly better repair. Meanwhile, Jo et al²⁵ performed intra-articular injection of adipose-derived MSCs using 3 different amounts of MSCs (1×10^7 , 5×10^7 , and 1×10^8 cells) for knee OA and compared the outcomes between the different dosage groups. Those investigators found that only the patient group injected with 1×10^8 MSCs demonstrated a decrease in articular cartilage defects through regeneration of hyaline-like articular cartilage. Kim et al³⁰ performed MSC implantation using 3.9×10^6 cells for knee OA and reported

improved cartilage regeneration with encouraging clinical outcomes. Another study reported encouraging clinical outcomes after MSC implantation using 4.3×10^6 cells in patients with knee OA.²⁹ In the current study, an average of 8.11×10^6 cells (range, 3.02×10^6 to 1.98×10^7 cells) were used for MSC implantation, and we assessed whether the number of MSCs influenced the clinical outcomes. We found significant differences in IKDC scores at 1 year, 3 years, 5 years, and 9 years after surgery among the groups divided according to the number of MSCs ($P < .05$ for all) (Table 3). Furthermore, we found that the number of MSCs was an independent predictor of failure of MSC implantation ($P < .001$) (Table 5). Although the optimal number of MSCs to be applied remains unknown, we believe that a larger number of MSCs would be more helpful for adequate cartilage regeneration. Therefore, development of a technique to obtain a larger number of MSCs or concentrate MSCs will be necessary for better outcomes.

Compared with simple, unipolar lesions, cartilage lesions involving reciprocal femoral and tibial articular surfaces (bipolar kissing lesion) are known to be difficult to treat and have inferior outcomes after surgical treatment.^{2,40} Moreover, whether treatment of both reciprocal lesions or treatment of only 1 surface lesion is sufficient is unknown.^{5,17} Meric et al⁴⁰ indicated that bipolar kissing lesions usually involve more surface area and are considerably larger, a finding that is similar to our results. In our study, the lesion size was significantly larger in bipolar kissing lesions than in unipolar lesions, regardless of the location of lesions ($P < .05$) (Table 4). The presence of bipolar kissing lesion significantly influenced the IKDC scores at 1 year, 3 years, 5 years, and 9 years after surgery (Table 3) and was also an independent predictor of failure of MSC implantation ($P = .013$) (Table 5). We speculated that these results originated from the number of MSCs implanted on the lesion site. In this study, if a bipolar kissing lesion were present, MSC implantation was performed in the cartilage lesion of the tibial plateau along with the femoral condyle. Accordingly, the implanted MSCs would be insufficient for achieving adequate cartilage

regeneration in the bipolar kissing lesion because the cartilage lesion size was certainly larger in bipolar kissing lesions than in unipolar lesions; thus, the number of MSCs implanted in the lesion site was lower in bipolar kissing lesions than in unipolar lesions (Table 4).

This study has certain limitations that are worth considering. First, the retrospective nature of the study involving a large patient population has inherent limitations. Because 44 (7.6%) of the 576 cases were lost to follow-up, incomplete data were collected. In addition, this study included patients with localized lesions, with early OA according to Kellgren-Lawrence grades, and without obesity, and these results might not be duplicated in patients with more advanced OA, malalignment, or obesity. Second, the current study was a retrospective case series that lacked any comparative cohort or control. A comparative study of MSC implantation versus nonoperative management or other operative treatment is required to identify the exact effects of MSC implantation for knee OA. In addition, we did not control for or measure other nonoperative treatments that patients received (eg, nonsteroidal anti-inflammatory drugs, physical therapy, injections). Third, because of the large patient population studied, we did not extensively evaluate the clinical data to support our results. In this study, the IKDC score and the Tegner activity scale, which were the only 2 scoring methods that have been recorded since the beginning of MSC implantation, were used for the clinical evaluation, and only the IKDC score was used to assess the prognostic factors influencing clinical outcomes. Therefore, power analyses with another scoring system are necessary to identify prognostic factors more confidently. We also defined failure as an IKDC score <40; however, we are not aware of any other study that has used a specific IKDC score to define failure of knee OA treatment. Fourth, the lack of structural imaging to confirm the status of the articular cartilage at the midterm follow-up period is an area of future interest. MRI or second-look arthroscopy with histological evaluation would be helpful to assess the quality of regenerated cartilage. Fifth, because MSCs are a heterogeneous population of cells with variable growth potential and distinct morphologic and functional characteristics,¹⁸ the quality of MSCs needed to achieve adequate cartilage regeneration should be identified to predict the outcomes of MSC implantation. In this study, we found that the number of MSCs was an important factor influencing clinical outcomes; however, further study is needed to estimate other qualities of MSCs that influence the clinical outcomes of MSC implantation in order to more accurately assess influential prognostic factors. Moreover, the nonhomogeneity of the number of MSCs (ie, the number of MSCs had a relatively wide range of distribution) could render the definition of the clinical efficacy of MSCs difficult. Sixth, the definition of failure of MSC implantation was determined arbitrarily. Generally, failure of MSC implantation would be defined in terms of conversion to total knee arthroplasty. However, the inclusion criterion of this study was Kellgren-Lawrence²⁶ grade 1 or 2; therefore, total knee arthroplasty rarely could be performed. Despite the study's limitations, the strength of this study lies in its detailed analysis of midterm survival

rates of MSC implantation and identification of the prognostic factors associated with survival rates. Because this study is ongoing, it can be strengthened further in the future, as the number of patients who undergo MSC implantation will increase over time.

CONCLUSION

The current study showed encouraging midterm clinical outcomes with acceptable duration of symptom relief after MSC implantation in patients with early knee OA. Furthermore, patient age, presence of bipolar kissing lesion, and number of MSCs were independent factors associated with failure of MSC implantation. Identifying these factors may provide a more accurate screening tool for surgeons to better assess which patients are good candidates for MSC implantation and who will have a better chance at successful clinical outcomes.

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