Original Article

Arthroscopic Implantation of Adipose-Derived Stromal Vascular Fraction Improves Cartilage Regeneration and Pain Relief in Patients With Knee Osteoarthritis

Yong Sang Kim, M.D., Sun Mi Oh, B.S., Dong Suk Suh, M.D., Dae Hyun Tak, M.D., Yoo Beom Kwon, M.D., and Yong Gon Koh, M.D.

Purpose: To compare the pain relief and cartilage repair status of patients with knee osteoarthritis who received arthroscopic treatment with or without stromal vascular fraction (SVF) implantation. **Methods:** We retrospectively evaluated the patients who were examined with 12-month follow-up magnetic resonance imaging (MRI) after arthroscopic treatment for knee osteoarthritis from September 2019 to April 2021. Patients were included in this study if they had grade 3 or 4 knee osteoarthritis according to the Outerbridge classification in MRI. The visual analog scale (VAS) was used for pain assessment over the follow-up period (baseline and at 1-, 3-, 6-, and 12-month follow-ups). Cartilage repair was evaluated using follow-up MRIs based on Outerbridge grades and the Magnetic Resonance Observation of Cartilage Repair Tissue scoring system. **Results:** Among 97 patients who received arthroscopic treatment, 54 patients received arthroscopic treatment alone (conventional group) and 43 received arthroscopic treatment along with SVF implantation (SVF group). In the conventional group, the mean VAS score decreased significantly at 1-month post-treatment compared with baseline ($P < .05$), and gradually increased from 3 to 12 months' post-treatment (all $P < .05$). In the SVF group, the mean VAS score decreased until 12 months post-treatment compared with baseline (all $P < .05$ except $P = .780$ in 1-month vs 3-month follow-ups). Significantly greater pain relief was reported in the SVF group than in the conventional group at 6 and 12 months' post-treatment (all $P < .05$). Overall, Outerbridge grades were significantly greater in the SVF group than in the conventional group ($P < .001$). Similarly, mean Magnetic Resonance Observation of Cartilage Repair Tissue scores were significantly greater ($P < .001$) in the SVF group (70.5 ± 11.1) than in the conventional group (39.7 ± 8.2). **Conclusions:** The results regarding pain improvement and cartilage regeneration and the significant correlation between pain and MRI outcomes at 12-months follow-up indicate that the arthroscopic SVF implantation technique may be useful for repairing cartilage lesions in knee osteoarthritis. **Level of Evidence:** III, retrospective comparative study.

Recently, cell-based therapies have emerged as a potential therapeutic option for the management of knee osteoarthritis.[1] Mesenchymal stem cells (MSCs) from various sources have been evaluated extensively for their ability to restore compromised articular cartilage and slow the progression of knee osteoarthritis.[2,3] Since the pathogenesis of osteoarthritis is based on degeneration and inflammation, the therapeutic properties of MSCs—including paracrine,[4,5] anti-inflammatory,[6] and immunomodulatory effects[7]—may help restore the intra-articular environment.[8] However, MSC culturing is expensive and involves a delay of a few weeks between isolation and application.

Adipose-derived stromal vascular fraction (SVF) cells have received attention as an alternative stem cell for the management of knee osteoarthritis at any stage. Lipoaspirates are easy to obtain using a minimally invasive procedure with a low complication rate and minimal donor-site morbidity.[9,10] Adipose-derived SVF comprises a heterogeneous cell population containing

From the Center for Stem Cell & Arthritis Research, Department of Orthopaedic Surgery, Yonsei Sarang Hospital, Seoul, Korea.

The authors report that they have no conflicts of interest in the authorship and publication of this article. Full ICMJE author disclosure forms are available for this article online, as supplementary material.

Received November 17, 2022; accepted March 31, 2023.

Address correspondence to Yong Gon Koh, M.D., Center for Stem Cell & Arthritis Research, Department of Orthopaedic Surgery, Yonsei Sarang Hospital, 10, Hyoryeong-ro, Seocho-gu, Seoul 06698, Republic of Korea. E-mail: yonggonkoh@gmail.com

2666-061X/221478

https://doi.org/10.1016/j.asmr.2023.03.013

regenerative cells (such as adipose-derived MSCs), macrophages, pericytes, fibroblasts, blood cells, and vessel-forming cells (including endothelial and smooth muscle cells) and their progenitors.[11] This cell population also includes cells with stem cell elements, which are thought to have a synergistic effect with adipose-derived MSCs.[12] Adipose-derived SVF cells and MSCs both result in comparable clinical improvement in patients with knee osteoarthritis.[10]

Several authors have reported the use of adipose-derived SVF cells for the treatment of knee osteoarthritis.[10,13,14] In these studies, MSCs were administered to knee joints with osteoarthritis via a simple intra-articular injection, and favorable clinical outcomes were obtained. However, no studies have evaluated cartilage repair after SVF-based treatment. The appropriate delivery of SVF to the site of the cartilage lesion is crucial for durable cartilage repair in the SVF-based treatment of osteoarthritis. Therefore, we performed arthroscopic SVF cell implantation for more effective cartilage repair. We sought to compare the pain relief and cartilage repair status of patients with knee osteoarthritis who received arthroscopic treatment with or without SVF cell implantation. We hypothesized that arthroscopic SVF cell implantation would result in greater cartilage remodeling with better pain improvement than arthroscopic treatment without SVF cell application.

Methods

Study Design and Participants

This study was reviewed and approved by the institutional review board of Yonsei Sarang Hospital. The study is the result of analyzing the parts of participants among the all subjects who were participated in "Conditional Approval System of Health Technology" grant, funded by the Ministry of Health and Welfare. Informed consent was obtained from all participants before enrollment in this study. We retrospectively identified patients who were examined with 12-month follow-up magnetic resonance imaging (MRI) after arthroscopic treatment for knee osteoarthritis from September 2019 to April 2021. Patients were included in this study if they had grade 3 or 4 knee osteoarthritis according to the Outerbridge classification[15] on MRI at 12 months' follow-up with symptoms of knee joint pain and/or functional limitations, despite the nonoperative treatments. Patients were excluded if they had previous surgical treatment, knee instability, knee varus or valgus malalignment, and other pathologic diseases (including rheumatoid arthritis, hemophilia, and active knee infections).

Isolation of SVF From Subcutaneous Adipose Tissue

Subcutaneous adipose tissue samples were obtained through tumescent liposuction from the gluteal regions of patients 1 day before SVF cell implantation. We collected 140 mL of adipose tissue, and this was suspended in phosphate-buffered saline solution, placed in a sterile box, and transported to the laboratory. A 120-mL aliquot of the tissue was used for implantation. Mature adipocytes and connective tissues were separated from the SVF by centrifugation (Hanil Scientific Inc., Gyeonggi-do, South Korea).[16] Before implantation, bacteriologic tests including mycoplasma (iNtRON, Gyeonggi-do, South Korea), endotoxin (Associates of Cape Cod, MA), and gram stain kit (BD Biociences, Franklin Lakes, NJ) were performed to ensure that the samples were not contaminated, and cell viability was assessed using the methylene blue dye exclusion test (NanoEntek, Seoul, South Korea). The remaining 20 mL of adipose tissue was processed similarly and used for laboratory analysis to examine the plastic-adherent cells that form colony-forming unit fibroblasts (CFU-Fs) and to confirm the multilineage differentiation of adipose-derived stem cells.

Confirmation of MSC Characteristics

When plated at low densities, MSCs adhere to tissue culture plastic and generate colonies.[17,18] The CFU-F assay was used to confirm the generation of mesenchymal progenitors of adipose-derived stem cells. The cells were cultured in T25 flasks (16 cells/cm^2) to evaluate the frequency of mesenchymal-like progenitors. Colonies of $\geq$50-cell aggregates were scored under an optical microscope to assess their colony-forming ability. Cells regularly seeded at 50 cells/cm^2 were allowed to multiply and their flow cytometric immunophenotype was examined using fluorescence-activated cell sorting (FACS). MSC marker phenotyping was performed using CD14, CD34, CD90, and CD105 antibodies according to an established protocol.[19,20] FACS-based analysis of the flow cytometric immunophenotype requires 2×10^6 cells per CD marker. Therefore, we obtained 8×10^6 of cells for 4 CD markers through culture expansion. To confirm the multilineage differentiation of MSCs, adipose-derived stem cells were plated at 5×10^3 cells/cm^2 in Dulbecco's modified Eagle's medium (HyClone, Logan, UT) supplemented with 10% fetal bovine serum (HyClone), and 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.[21] We used CFU-F to evaluate the capacity of human subcutaneous adipose tissue to generate mesenchymal progenitors.

Surgical Procedures and SVF Application

The patients were positioned supine on the operating table under spinal anesthesia, and a thigh tourniquet was applied. All procedures in both groups were performed by an experienced senior orthopaedic surgeon

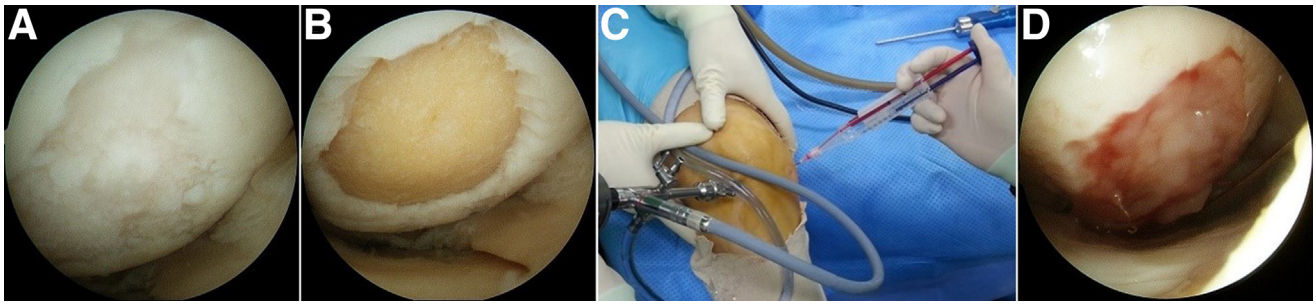

Fig 1. Arthroscopic implantation of stromal vascular fraction (SVF) cells loaded in fibrin glue. (A) An articular cartilage lesion in the medial femoral condyle. (B) Acute debridement of all unstable and damaged cartilage in the lesion. (C) Application of the SVF–thrombin–fibrinogen suspension to the lesion. (D) The SVF–thrombin–fibrinogen suspension is manipulated with a probe to cover the cartilage lesion.

using the same technique. The surgeon performed arthroscopic debridement of the damaged or undermined cartilage to obtain a smooth surface of the cartilage lesion and firm edges facing the surrounding cartilage. No marrow-stimulation procedures (such as microfracture, subchondral drilling, or abrasion arthroplasty) were performed. The fibrin glue product from the Greenplast kit (Greencross, Seoul, South Korea) was used as a scaffold for SVF implantation. The product was administered in 2 syringes: 1 containing lyophilized human plasma fibrinogen dissolved in the aprotinin solution, and 1 containing thrombin dissolved in calcium chloride solution in sterile packaging. The fibrin glue product is designed to form a gel instantaneously when the 2 solutions in the syringes are mixed. First, the SVF suspension was loaded into the thrombin solution at a 1:1 ratio (v/v, SVF suspension:thrombin solution). Next, the SVF–thrombin suspension was mixed with the fibrinogen solution at a 1:1 ratio via a Duploject syringe support system (included in the Greenplast kit), and the solutions were simultaneously added to each well on the surface of the cartilage lesion. This implantation was performed under arthroscopic guidance after the arthroscopic fluid had been extracted. The implanted SVF–thrombin–fibrinogen suspension was manipulated with a probe to provide even coverage to the surface of the cartilage lesion (Fig 1).

Outcome Assessments

All patients were evaluated clinically before surgery and postoperatively at 1-, 3-, 6-, and 12-month follow-up visits. The visual analog scale (VAS; range, 0-100) was used for pain assessment over the follow-up period. Adverse events were recorded for safety evaluation. Preoperative and follow-up MRIs was performed using a 3.0 T MRI scanner (Achieva 3.0 T SE; Philips, Eindhoven, Netherlands) with a dedicated 8-channel knee coil. The following sequences were used: (1) proton density (PD) spectral presaturation with inversion recovery (SPIR) transverse images (repetition time/echo time [TR/TE], 4,000/15 milliseconds): field of view (FOV), 150 × 150 mm; matrix, 308 × 249; slice thickness (SL), 3.5 mm (gap, 0.35 mm); (2) proton density SPIR coronal images (TR/TE, 3,500/15 milliseconds): FOV, 150 × 150 mm; matrix, 260 × 240; SL, 3.0 (gap, 0.5 mm); (3) T2 SPIR sagittal images (TR/TE, 3200/70 milliseconds): FOV, 150 × 150 mm; matrix, 240 × 192; SL, 3.0 mm (gap, 0.3 mm); and (4) turbo spin echo T1-weighted sagittal images (TR/TE, 600/20 milliseconds): FOV, 150 × 150; matrix, 240 × 240; SL, 3.0 mm (gap, 0.3 mm). To avoid potential bias, an independent musculoskeletal-trained radiologist not involved in the care of participants and blinded to the intention of this study evaluated the MRI scans. During preoperative MRI, the Outerbridge grade[15] was used to select patients for inclusion in this study. Follow-up MRIs were performed at 12 months postoperatively for all patients, and tissue repair was evaluated according to the Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scoring system as described by Marlovits et al.[22] (Table 1).

Statistical Analysis

The principal dependent variables were the VAS score at final follow-up and the postoperative MOCART score. Descriptive statistics were calculated as mean ± standard deviation (continuous variables) or frequencies and proportions (categorical variables). The Wilcoxon signed-rank test was used to compare pre- and postoperative values over the follow-up period, and the Mann–Whitney *U* test was used for intergroup comparisons. The Fisher exact test was used to compare categorical data. The Spearman rank-order correlation test was used to analyze correlations between MOCART and VAS scores at final follow-up. Statistical analyses were performed using SPSS, version 13.0 (SPSS Inc., Chicago, IL), and statistical significance was indicated at $P < .05$.

Table 1. MOCART Scores at Follow-up MRIs

Variables	Score	Conventional (n = 54) n	Conventional (n = 54) Mean	SVF (n = 43) n	SVF (n = 43) Mean	*P* Value
1. Degree of defect repair and filling of the defect			6.0 ± 3.7		17.9 ± 3.1	<.001
Complete	20	0		27		
Hypertrophy	15	3		15		
Incomplete						
>50% of the adjacent cartilage	10	12		0		
<50% of the adjacent cartilage	5	32		1		
Subchondral bone exposed	0	7		0		
2. Integration to border zone			1.9 ± 2.5		7.7 ± 2.7	<.001
Complete	15	0		1		
Incomplete						
Demarcating border visible	10	0		21		
Defect visible						
<50% of the length of the repair tissue	5	21		21		
>50% of the length of the repair tissue	0	33		0		
3. Surface of the repair tissue			3.4 ± 4.2		2.6 ± 3.2	.463
Surface intact	10	13		3		
Surface damaged						
<50% of repair tissue depth or total degeneration	5	11		26		
>50% of repair tissue depth or total degeneration	0	30		24		
4. Structure of the repair tissue			4.7 ± 1.2		4.9 ± 0.7	.429
Homogenous	5	51		42		
Inhomogeneous or cleft formation	0	3		1		
5. Signal intensity of the repair tissue			9.4 ± 7.3		21.6 ± 7.5	<.001
Normal (identical to adjacent cartilage)	30	0		19		
Nearly normal (slight areas of signal alteration)	15	34		24		
Abnormal (large areas of signal alteration)	0	20		0		
6. Subchondral lamina			4.8 ± 1.0		4.9 ± 0.8	.698
Intact	5	52		42		
Not intact	0	2		1		
7. Subchondral bone			4.8 ± 1.0		4.8 ± 1.1	.817
Intact	5	52		41		
Not intact	0	2		2		
8. Adhesions			3.2 ± 2.4		3.5 ± 2.3	.608
No	5	35		30		
Yes	0	19		13		
9. Effusion			1.5 ± 2.3		1.9 ± 2.4	.433
No	5	16		26		
Yes	0	38		27		
Total	100		39.7 ± 8.2		70.5 ± 11.1	<.001

NOTE. Values are expressed as mean ± standard deviation unless otherwise indicated.
MOCART, magnetic resonance observation of cartilage repair tissue; MRI, magnetic resonance imaging; SVF, stromal vascular fraction.

Results

Study Subjects and General Characteristics

A total of 198 patients with grade 3 or 4 knee osteoarthritis according to the Outerbridge classification[15] underwent the arthroscopic procedure. Among these, 54 were excluded and 144 were enrolled in this study. Of the 144 patients, 82 received only the arthroscopic procedure (conventional group), and 62 underwent the arthroscopic procedure with SVF application (SVF group). All patients were advised to undergo follow-up MRI, and its purpose (to evaluate the cartilage lesion and the other pathologic conditions) was explained before surgery. Of the 82 patients in the conventional group, 17 patients dropped out and 9 were lost to follow-up. Of the 62 patients in the SVF group, 14 patients dropped out and 5 were lost to follow-up. Therefore, 54 patients (conventional group) and 43 patients (SVF group) were finally enrolled in this study (Fig 2). The baseline demographics of the patients are summarized in Table 2. There were no significant differences inter-group with respect to age, sex, body mass index, side of involvement, preoperative Outerbridge grade, or lesion size. Lesion size was measured on preoperative MRIs by an independent observer. No clinically significant adverse events were noted during the 12-month follow-up. Although 2 patients in the SVF group reported mild stiffness with swelling of the knee joints, the condition resolved without intervention.

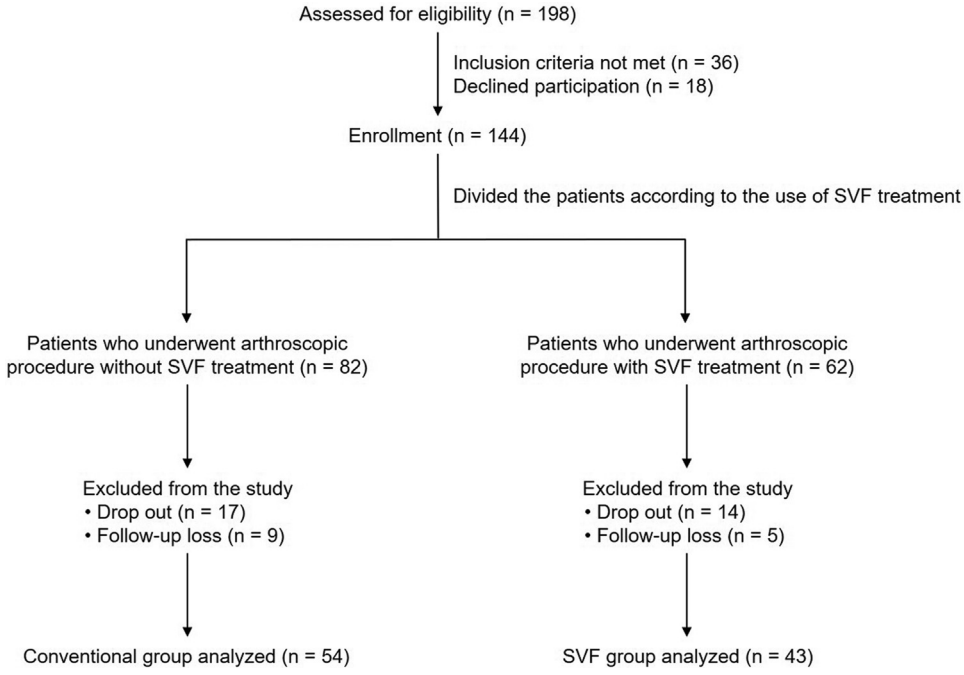

Fig 2. Patient enrollment in this study. (SVF, stromal vascular fraction.)

Isolation and Characterization of Cells

Adipose-derived stem cells comprised 9.5% (range 8.6%-11.2%) of the SVF cells. The SVF cells contained an average of 7.0×10^6 stem cells (range 6.4-8.1 $\times$ 10^6 cells), and an average of 7.4×10^7 SVF cells were used for implantation. FACS analysis indicated positive expressions of CD90 (99.35%) and CD105 (94.23%) and negative expressions of CD34 (5.37%) and CD14 (2.74%). The treated stem cells exhibited adipogenic, osteogenic, and chondrogenic differentiation potential, as revealed by staining assays.

Pain Scores

The mean VAS scores at baseline and at 1-, 3-, 6-, and 12-month follow-ups are summarized in Table 3. Compared with the baseline, the mean VAS score in the SVF group decreased progressively until the 12-month follow-up (all $P < .05$ except $P = .780$ in 1-month vs 3-month follow-up; Fig 3). In contrast, the mean VAS score in the conventional group decreased at 1-month post-treatment compared with the baseline ($P < .05$), and gradually increased from 3-month to 12-month post-treatment (all $P < .05$) (Fig 3). Significantly greater pain relief was reported in the SVF group than in the conventional group at 6- and 12-month follow-ups (47.3 $\pm$ 5.9 vs 40.2 $\pm$ 8.8 at 6 months and 50.8 $\pm$ 6.3 vs 35.9 $\pm$ 7.1 at 12 months, $P < .05$ for both; Table 3). Although pain relief was more pronounced in the conventional group during early follow-ups, the SVF group eventually exhibited superior pain relief at the 12-month follow-up.

MRI Outcomes

The Outerbridge grades before surgery and at final follow-up are summarized in Table 4. Overall, Outerbridge grades improved in the SVF group, and significant intergroup differences were found in follow-up MRIs ($P < .001$). At the 12-month follow-up,

Table 2. Comparison of Baseline Demographics Between the Study Groups

	Conventional (n = 54)	SVF (n = 43)	*P* Value
Age, y	63.4 ± 5.6	63.4 ± 4.1	.584
Sex, male/female, n	23/31	14/29	.315
Body mass index	26.4 ± 2.7	26.0 ± 2.8	.410
Side of involvement, right/left, n	26/28	21/22	.946
Outerbridge grade, n (%)			.946
3	26 (48.1)	21 (48.8)	
4	28 (51.9)	22 (51.2)	
Lesion size, cm^2	5.5 ± 1.2	5.6 ± 1.3	.722

SVF, stromal vascular fraction.

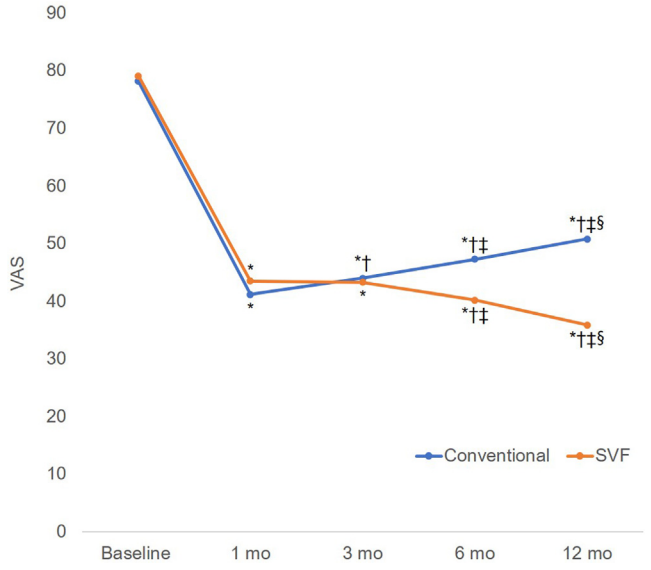

Fig 3. Changes in visual analog scale (VAS) values. Significantly different from *baseline, †1-month follow-up, ‡3-month follow-up, and §6-month follow-up. (SVF, stromal vascular fraction.)

cartilage regeneration was observed in some patients in the SVF group (Fig 4). The mean MOCART scores (conventional group: 39.7 ± 8.2; SVF group: 70.5 ± 11.1; Table 1) were significantly different between groups ($P < .001$). Variables such as degree of defect repair and filling of the defect, integration to the border zone, and signal intensity of the repair tissue were significantly different between groups (all $P < .001$; Table 1).

Correlation Between Pain Scores and MRI Outcomes

There were no significant correlations between pain scores and MOCART scores in the conventional group during the follow-up period (Table 5). Significant correlations between the pain scores and MOCART scores were not observed in the SVF group until at the 6-month follow-up. There was significant correlation between pain scores and MOCART scores at the 12-month follow-ups in the SVF group ($P = .002$; Table 5).

Table 3. Comparison of Preoperative and Postoperative Pain, as Assessed Using the Visual Analog Scale (VAS)

	Conventional (n = 54)	SVF (n = 43)	*P* Value
VAS			
Baseline	78.2 ± 6.0	79.1 ± 6.9	.532
1 month	41.2 ± 6.0	43.5 ± 8.6	.197
3 months	44.0 ± 7.1	43.3 ± 9.3	.583
6 months	47.3 ± 5.9	40.2 ± 8.8	<.001
12 months	50.8 ± 6.3	35.9 ± 7.1	<.001

NOTE. Values are expressed as mean ± standard deviation.

Discussion

The principal finding of this study is that pain improvement and MRI outcomes were more favorable in the SVF group than in the conventional group at 12-months follow-up. The mean VAS scores decreased to a similar extent in both groups until 1 month after surgery, but increased significantly from 3 to 12 months postsurgery in the conventional group. In contrast, mean VAS scores in the SVF group decreased gradually until 12 months post-treatment (Fig 3). Pain relief was significantly greater in the SVF group than in the conventional group at 6 and 12 months post-treatment (all $P < .05$; Table 3). In addition, the Outerbridge grades and MOCART scores indicated more favourable cartilage repair in the SVF group.

As osteoarthritis progresses, cartilage fibrillates release debris into the joint, which induces synovitis (triggered by the phagocytosis of detritus in the synovial fluid) and causes pain.[23] The removal of debris (such as articular cartilage fragments and crystals), synovitis, and any surface irregularities can improve pain in patients with knee osteoarthritis. Arthroscopic debridement is thought to offer symptomatic relief by reducing the inflammatory response mounted against the debris that accumulates in the synovial cavity.[24,25] Edelson et al.[26] reported that arthroscopic debridement reduced pain and other symptoms in patients with advanced knee osteoarthritis at mid- to long-term follow-ups. However, Moseley et al.[27] found that in patients with knee osteoarthritis, outcomes after arthroscopic lavage or arthroscopic debridement were not better than those after a placebo procedure. In addition, most other studies have indicated that the benefits of this treatment are evident only in the short term,[23,25,28,29] which is consistent with our results. In the conventional group, the mean VAS score decreased significantly at 1-month post-treatment compared with the baseline ($P < .05$), but gradually increased from 3 months to 12 months post-treatment (all $P < .05$; Fig 3). Notably, the mean VAS score at 12 months post-treatment was still significantly better than the baseline value ($P < .05$), although long-term results need to be further analyzed.

The SVF represents is a cell population that can be retrieved with the enzymatic dissociation filtration and centrifugation of adipose tissue.[16] The SVF is a promising candidate for the regenerative treatment of osteoarthritis because it contains a significant proportion of cells involved in immunoregulation.[30,31] It also contains a variety of regenerative cells that may act synergistically with adipose-derived MSCs.[32,33] In addition, macrophages (which constitute 20% of SVF cells) are known to be involved in anti-inflammatory activities.[34] As mentioned previously, the mean VAS score in the SVF group gradually decreased until 12 months post-treatment (Fig 3). Unlike in the conventional group,

Table 4. Outerbridge Grades of Both Study Groups, as Assessed Using Preoperative and Follow-up MRIs

	Preoperative			Follow-up		
	Conventional (n = 54)	SVF (n = 43)	*P* Value	Conventional (n = 54)	SVF (n = 43)	*P* Value
Outerbridge grade, n (%)			.946			<.001
1	0	0		0	2 (4.7)	
2	0	0		0	21 (48.8)	
3	26 (48.1)	21 (48.8)		21 (38.9)	15 (34.9)	
4	28 (51.9)	22 (51.2)		33 (61.1)	5 (11.6)	

MRI, magnetic resonance imaging; SVF, stromal vascular fraction.

patients in the SVF group reported a steady improvement in pain. We speculate that the pain improvement up to 3 months postsurgery was mainly due to the paracrine and anti-inflammatory effects of SVF cells (including MSCs). Although there was no significant improvement in pain at 3 months post-treatment compared with 1-month post-treatment, significant pain improvement was reported from 6 to 12 months post-treatment. These results may be related to the effects of cartilage regeneration. MSCs stimulate chondrocyte proliferation and promote the synthesis of the extracellular matrix in osteoarthritic joints, which helps repair the damaged articular cartilage.[7,35,36] Apart from MSCs, the SVF contains additional cell types that may contribute to cartilage regeneration via tissue-specific differentiation and by secreting the extracellular matrix or various immune-modulating factors.[37,38] These characteristics confer SVF cells the potential to differentiate into adipogenic, osteogenic, chondrogenic, and other mesenchymal lineages.[39] In these regards, several authors have reported favorable cartilage regeneration after SVF therapy in patients with knee osteoarthritis.[40-43] Considering with these results, we suggest that the short-term pain improvements were mainly due to the paracrine and anti-inflammatory effects of SVF cells, rather than cartilage regeneration itself. This is because cartilage regeneration would likely require a longer time to occur after SVF therapy, although the time required is difficult to estimate. Estimating the degree of cartilage regeneration requires second-look arthroscopy or follow-up MRIs to be performed periodically, which is difficult in practice.

Because of its noninvasive nature, reproducibility, and accuracy, MRI has been used as an effective and objective tool to evaluate the healing of cartilage lesions after surgical treatment.[40,42-44] Simunec et al.[42] reported that cartilage regeneration was visible in a 16-month follow-up MRI after SVF treatment in a patient with knee osteoarthritis. Tran et al.[40] allocated 33 patients with knee osteoarthritis into 2 groups; one group received arthroscopic microfracture treatment only, and the other received arthroscopic microfracture treatment combined with SVF injection. The authors evaluated the cartilage status based on Outerbridge scores[15] in follow-up MRIs 12 and 24 months after surgery. They reported that the Outerbridge score increased slightly after 12 months (2.7 ± 1.3 vs 2.9 ± 1.3), and that this trend was maintained for up to 24 months (3.2 ± 1.1) in the patient group treated with arthroscopic microfracture only. In contrast, in the patient group treated with arthroscopic microfracture treatment combined with SVF injection, the Outerbridge scores at 12 (2.7 ± 0.7) and 24 (2.0 ± 0.7) months after surgery were lower than the preoperative scores (3.0 ± 0.8). In addition, there was a significant intergroup difference in Outerbridge scores at 24

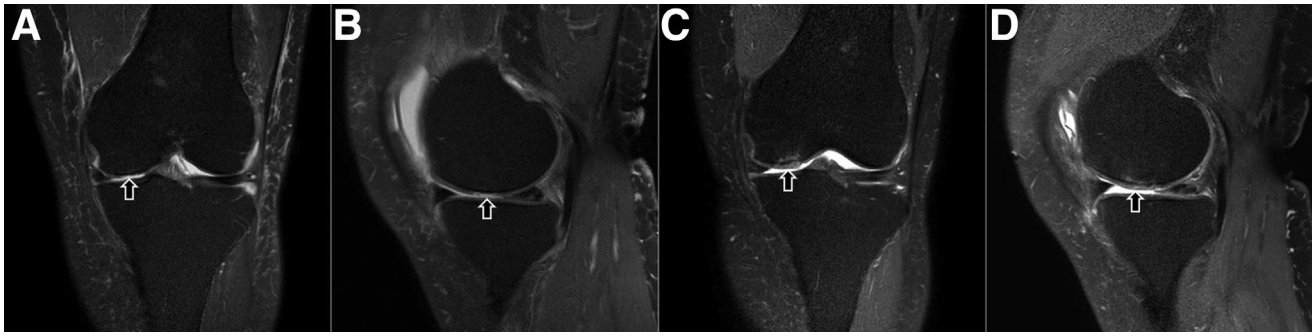

Fig 4. Preoperative (A and B) and follow-up (C and D) coronal and sagittal proton density fat-saturated images of the right knee of a 64-year-old female patient. (A and B) Cartilage loss is visible in the medial femoral condyle (arrows). (C and D) Complete filling of the defect along with complete integration with the adjacent native cartilage (arrows; MOCART score, 75 points). (MOCART, magnetic resonance observation of cartilage repair tissue.)

Table 5. Correlation Between Pain Scores and MRI Outcomes

	MOCART			
	Conventional (n = 54)		SVF (n = 43)	
	S rho	*P* value	*S* rho	*P* value
VAS				
Baseline	0.041	.767	−0.148	.344
1 mo	−0.088	.525	−0.203	.191
3 mo	−0.128	.358	−0.228	.142
6 mo	−0.110	.429	−0.201	.196
12 mo	−0.058	.674	−0.463	.002

NOTE. Calculated using the Spearman rank-order test.

MRI, magnetic resonance imaging; MOCART, magnetic resonance observation of cartilage repair tissue; SVF, stromal vascular fraction; VAS, visual analog scale.

months after surgery (3.2 ± 1.1 vs 2.0 ± 0.7; $P < .05$), indicating better cartilage regeneration in the patient group treated with arthroscopic microfracture treatment combined with SVF injection. Hong et al.[43] injected autologous adipose-derived SVF and hyaluronic acid in patients with knee osteoarthritis after arthroscopic debridement and performed 6- and 12-month follow-up MRIs to evaluate cartilage regeneration. In the patient group that received SVF treatment, the mean MOCART scores were 54.06 ± 11.58 and 62.81 ± 8.16 at 6- and 12-month follow-ups, respectively, indicating significant improvement ($P < .01$). However, in the patient group that received only hyaluronic acid treatment, mean MOCART scores were poor at 6 months (19.38 ± 9.64) and at 12 months (19.06 ± 7.79), showing no improvement between follow-up visits ($P = .924$).

Similar MRI outcomes were observed in the present study following SVF treatment in knee osteoarthritis. The overall Outerbridge grades improved in the SVF groups, and we found significant inter-group differences in Outerbridge grades at follow-up MRIs ($P < .001$; Table 4). In addition, the mean MOCART scores were significantly higher in the SVF group (70.5 ± 11.1) than in the conventional group (39.7 ± 8.2; $P < .001$). The mean MOCART scores at the 12-month follow-up in this study (70.5 ± 11.1) were greater than those reported in a previous study by Hong et al.[43] (62.81 ± 8.16). However, the preoperative status of cartilage lesions should be considered when interpreting these differences. We speculate that this outcome was due to differences in the method of SVF application. Due to limited cell retention and survival at the target site, a simple injection of SVF cells is insufficient to repair damaged articular cartilage. Studies using cell tracking have shown that injected MSCs were mostly situated in other parts of the osteoarthritic joint (such as the synovium) rather than at the cartilage lesion site, resulting in only limited cartilage formation via chondrogenic differentiation.[45,46] We suggest that the SVF implantation technique used in this study is more effective and efficient than a simple SVF injection in delivering SVF cells to the site of cartilage lesion. This is also supported by the significant differences in variables of the MOCART score related to cartilage repair status, such as degree of defect repair and filling of the defect, integration to the border zone, and signal intensity of the repair tissue (all $P < .001$; Table 1).

Our data revealed interesting correlations between pain scores and MRI outcomes. In the SVF group, there was significant correlation between pain scores and MOCART scores only at 12 months after surgery ($P < .001$; Table 5). Although we cannot estimate the exact time required for cartilage regeneration, we anticipate that regeneration is required for at least 12 months after SVF treatment before it results in pain improvement.

Limitations

The present study has several limitations. First, the number of patients was relatively small, and the follow-up period was relatively short. A randomized comparison of arthroscopic procedures with or without SVF implantation with a larger sample size and longer follow-up period would certainly improve statistical support and allow for a more accurate evaluation of the effects of SVF in knee osteoarthritis. Second, in this study, we evaluated the pain score without any objective or subjective knee function outcomes. The evaluation of knee function outcomes is required for more precise evaluation of SVF treatment outcomes. Third, although follow-up MRIs were performed at several time points postoperatively, it is unknown how the repaired cartilage will behave over time, and the exact time period required for cartilage regeneration cannot be predicted. Fourth, it is important to examine the mechanical properties and biological functions of native cartilage and compare the properties of current cell localization approaches with those of native cartilage. Therefore, studies that use power analysis and consider histologic findings in combination with clinical and MRI outcomes are necessary to fully elucidate the effects of SVF treatment. Finally, the number of SVF implantations required to achieve the optimal outcome remains unknown.

Conclusions

The results regarding pain improvement and cartilage regeneration and the significant correlation between pain and MRI outcomes at 12-months follow-up indicate that the arthroscopic SVF implantation technique may be useful for repairing cartilage lesions in knee osteoarthritis.

References

1. Kong L, Zheng LZ, Qin L, Ho KKW. Role of mesenchymal stem cells in osteoarthritis treatment. *J Orthop Translat* 2017;9:89-103.
2. Zhang L, Hu J, Athanasiou KA. The role of tissue engineering in articular cartilage repair and regeneration. *Crit Rev Biomed Eng* 2009;37:1-57.
3. Zhao D, Pan JK, Yang WY, et al. Intra-articular injections of platelet-rich plasma, adipose mesenchymal stem cells, and bone marrow mesenchymal stem cells associated with better outcomes than hyaluronic acid and saline in knee osteoarthritis: A systematic review and network meta-analysis. *Arthroscopy* 2021;37:2298-2314, e2210.
4. Barry F, Murphy M. Mesenchymal stem cells in joint disease and repair. *Nat Rev Rheumatol* 2013;9:584-594.
5. Horie M, Choi H, Lee RH, et al. Intra-articular injection of human mesenchymal stem cells (MSCs) promote rat meniscal regeneration by being activated to express Indian hedgehog that enhances expression of type II collagen. *Osteoarthritis Cartilage* 2012;20:1197-1207.
6. Vézina Audette R, Lavoie-Lamoureux A, Lavoie JP, Laverty S. Inflammatory stimuli differentially modulate the transcription of paracrine signaling molecules of equine bone marrow multipotent mesenchymal stromal cells. *Osteoarthritis Cartilage* 2013;21:1116-1124.
7. Wu L, Prins HJ, Helder MN, van Blitterswijk CA, Karperien M. Trophic effects of mesenchymal stem cells in chondrocyte co-cultures are independent of culture conditions and cell sources. *Tissue Eng Part A* 2012;18:1542-1551.
8. Pers YM, Ruiz M, Noël D, Jorgensen C. Mesenchymal stem cells for the management of inflammation in osteoarthritis: State of the art and perspectives. *Osteoarthritis Cartilage* 2015;23:2027-2035.
9. Garza JR, Campbell RE, Tjoumakaris FP, et al. Clinical efficacy of intra-articular mesenchymal stromal cells for the treatment of knee osteoarthritis: A double-blinded prospective randomized controlled clinical trial. *Am J Sports Med* 2020;48:588-598.
10. Yokota N, Hattori M, Ohtsuru T, et al. Comparative clinical outcomes after intra-articular injection with adipose-derived cultured stem cells or noncultured stromal vascular fraction for the treatment of knee osteoarthritis. *Am J Sports Med* 2019;47:2577-2583.
11. Han J, Koh YJ, Moon HR, et al. Adipose tissue is an extramedullary reservoir for functional hematopoietic stem and progenitor cells. *Blood* 2010;115:957-964.
12. Traktuev DO, Prater DN, Merfeld-Clauss S, et al. Robust functional vascular network formation in vivo by cooperation of adipose progenitor and endothelial cells. *Circ Res* 2009;104:1410-1420.
13. Bansal H, Comella K, Leon J, et al. Intra-articular injection in the knee of adipose derived stromal cells (stromal vascular fraction) and platelet rich plasma for osteoarthritis. *J Transl Med* 2017;15:141.
14. Yokota N, Yamakawa M, Shirata T, Kimura T, Kaneshima H. Clinical results following intra-articular injection of adipose-derived stromal vascular fraction cells in patients with osteoarthritis of the knee. *Regen Ther* 2017;6:108-112.
15. Posadzy M, Desimpel J, Vanhoenacker F. staging of osteochondral lesions of the talus: MRI and cone beam CT. *J Belg Soc Radiol* 2017;101:1 (suppl 2).
16. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng* 2001;7:211-228.
17. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 1997;276:71-74.
18. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation* 1974;17:331-340.
19. Smith MB, Whiteside MG, Campbell DG. The occurrence of heterozygous beta-thalassaemia as screened by quantitative haemoglobin electrophoresis in pregnancy. *Med J Aust* 1971;1:1273-1274.
20. de Girolamo L, Niada S, Arrigoni E, et al. Repair of osteochondral defects in the minipig model by OPF hydrogel loaded with adipose-derived mesenchymal stem cells. *Regen Med* 2015;10:135-151.
21. Marchal JA, Picón M, Perán M, et al. Purification and long-term expansion of multipotent endothelial-like cells with potential cardiovascular regeneration. *Stem Cells Dev* 2012;21:562-574.
22. Marlovits S, Singer P, Zeller P, Mandl I, Haller J, Trattnig S. Magnetic resonance observation of cartilage repair tissue (MOCART) for the evaluation of autologous chondrocyte transplantation: Determination of interobserver variability and correlation to clinical outcome after 2 years. *Eur J Radiol* 2006;57:16-23.
23. Felson DT. Arthroscopy as a treatment for knee osteoarthritis. *Best Pract Res Clin Rheumatol* 2010;24:47-50.
24. Cameron-Donaldson M, Holland C, Hungerford DS, Frondoza CG. Cartilage debris increases the expression of chondrodestructive tumor necrosis factor-alpha by articular chondrocytes. *Arthroscopy* 2004;20:1040-1043.
25. Rosenthal AK. Crystals, inflammation, and osteoarthritis. *Curr Opin Rheumatol* 2011;23:170-173.
26. Edelson R, Burks RT, Bloebaum RD. Short-term effects of knee washout for osteoarthritis. *Am J Sports Med* 1995;23:345-349.
27. Moseley JB, O'Malley K, Petersen NJ, et al. A controlled trial of arthroscopic surgery for osteoarthritis of the knee. *N Engl J Med* 2002;347:81-88.
28. Aaron RK, Skolnick AH, Reinert SE, Ciombor DM. Arthroscopic débridement for osteoarthritis of the knee. *J Bone Joint Surg Am* 2006;88:936-943.
29. Yang SS, Nisonson B. Arthroscopic surgery of the knee in the geriatric patient. *Clin Orthop Relat Res* 1995;316:50-58.
30. da Silva Meirelles L, Caplan AI, Nardi NB. In search of the in vivo identity of mesenchymal stem cells. *Stem Cells* 2008;26:2287-2299.
31. Leto Barone AA, Khalifian S, Lee WP, Brandacher G. Immunomodulatory effects of adipose-derived stem cells: Fact or fiction? *Biomed Res Int* 2013;2013:383685.

32. Lin K, Matsubara Y, Masuda Y, et al. Characterization of adipose tissue-derived cells isolated with the Celution system. *Cytotherapy* 2008;10:417-426.
33. Zimmerlin L, Donnenberg VS, Rubin JP, Donnenberg AD. Mesenchymal markers on human adipose stem/progenitor cells. *Cytometry A* 2013;83:134-140.
34. Morris DL, Oatmen KE, Wang T, DelProposto JL, Lumeng CN. CX3CR1 deficiency does not influence trafficking of adipose tissue macrophages in mice with diet-induced obesity. *Obesity (Silver Spring)* 2012;20:1189-1199.
35. Mokbel AN, El Tookhy OS, Shamaa AA, Rashed LA, Sabry D, El Sayed AM. Homing and reparative effect of intra-articular injection of autologus mesenchymal stem cells in osteoarthritic animal model. *BMC Musculoskelet Disord* 2011;12:259.
36. Acharya C, Adesida A, Zajac P, et al. Enhanced chondrocyte proliferation and mesenchymal stromal cells chondrogenesis in coculture pellets mediate improved cartilage formation. *J Cell Physiol* 2012;227:88-97.
37. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002;13:4279-4295.
38. Bowles AC, Wise RM, Gerstein BY, et al. Immunomodulatory effects of adipose stromal vascular fraction cells promote alternative activation macrophages to repair tissue damage. *Stem Cells* 2017;35:2198-2207.
39. Pers YM, Rackwitz L, Ferreira R, et al. Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: A phase I dose-escalation trial. *Stem Cells Transl Med* 2016;5:847-856.
40. Tran TDX, Wu CM, Dubey NK, et al. Time- and Kellgren-Lawrence grade-dependent changes in intra-articularly transplanted stromal vascular fraction in osteoarthritic patients. *Cells* 2019;8(4).
41. Nguyen PD, Tran TD, Nguyen HT, et al. Comparative clinical observation of arthroscopic microfracture in the presence and absence of a stromal vascular fraction injection for osteoarthritis. *Stem Cells Transl Med* 2017;6:187-195.
42. Simunec D, Salari H, Meyer J. Treatment of grade 3 and 4 osteoarthritis with intraoperatively separated adipose tissue-derived stromal vascular fraction: A comparative case series. *Cells* 2020;9(9).
43. Hong Z, Chen J, Zhang S, et al. Intra-articular injection of autologous adipose-derived stromal vascular fractions for knee osteoarthritis: A double-blind randomized self-controlled trial. *Int Orthop* 2019;43:1123-1134.
44. Hagen MS, Hannay WM, Saluan Q, Lynch TS, Westermann RW, Rosneck J. Magnetic resonance imaging predictors of chondral lesions in patients with femoroacetabular impingement: An analysis of 545 cases. *Arthroscopy* 2021;37:2497-2501.
45. Agung M, Ochi M, Yanada S, et al. Mobilization of bone marrow-derived mesenchymal stem cells into the injured tissues after intraarticular injection and their contribution to tissue regeneration. *Knee Surg Sports Traumatol Arthrosc* 2006;14:1307-1314.
46. Matsumoto T, Cooper GM, Gharaibeh B, et al. Cartilage repair in a rat model of osteoarthritis through intra-articular transplantation of muscle-derived stem cells expressing bone morphogenetic protein 4 and soluble Flt-1. *Arthritis Rheum* 2009;60:1390-1405.