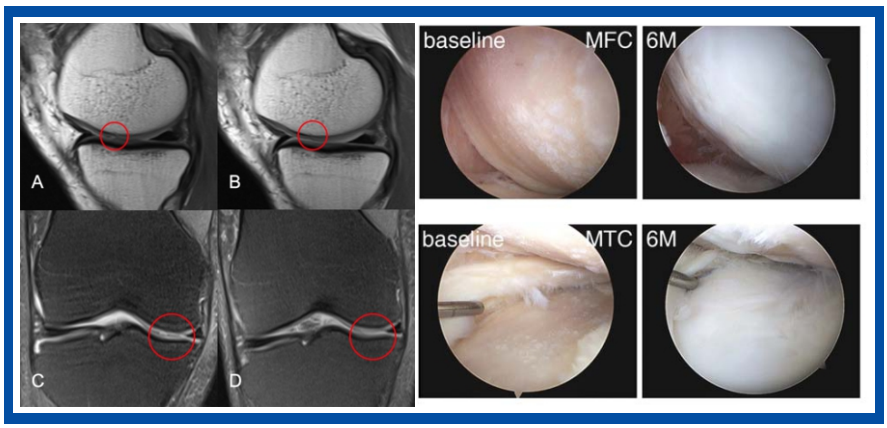
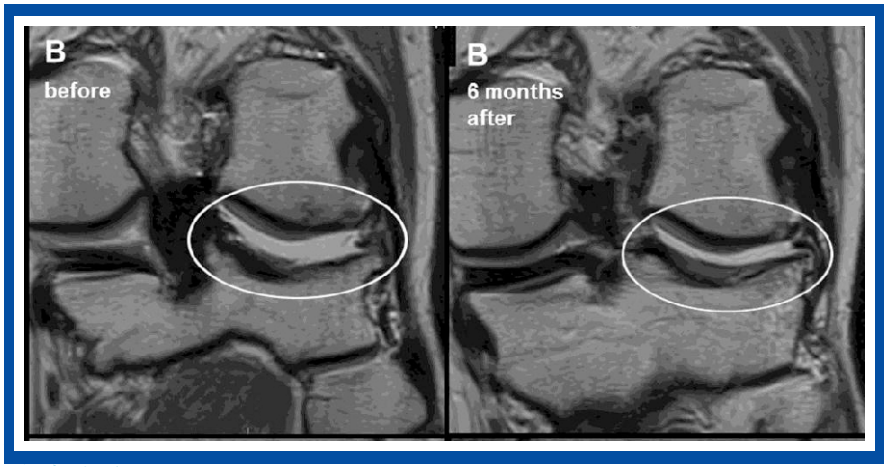
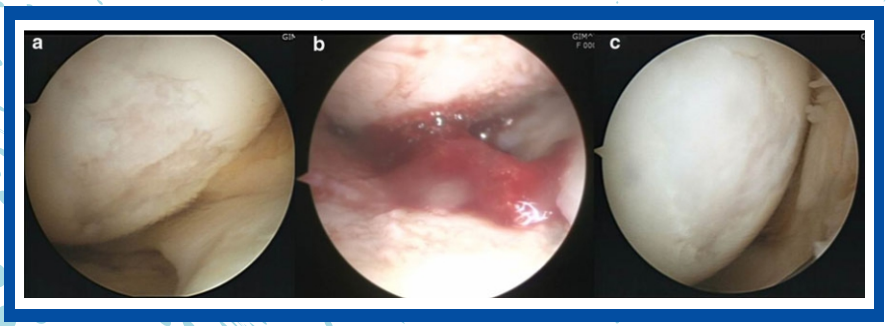
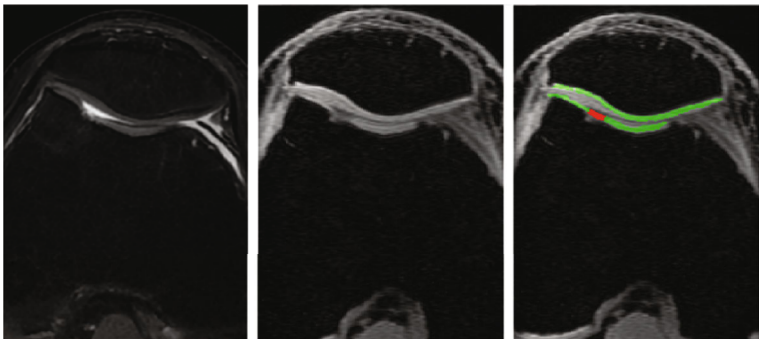
Cartilage Regeneration Evidence on MRI / Arthroscopy









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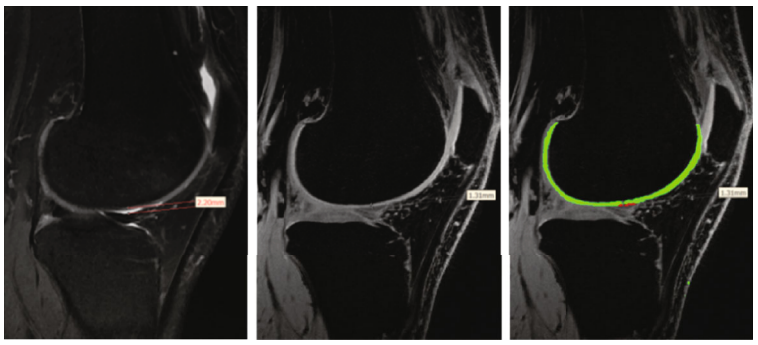

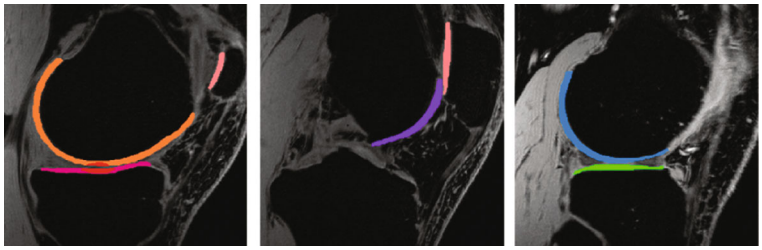

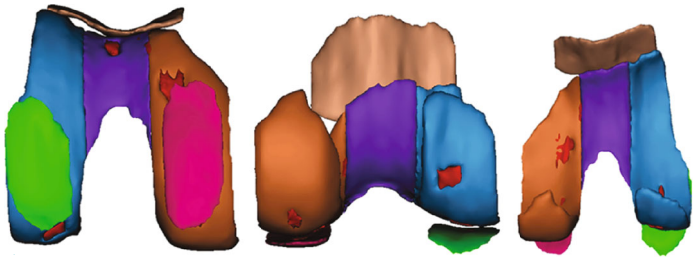

CONTENT

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ORIGINAL PAPER

Intra-articular injection of autologous adipose-derived stromal vascular fractions for knee osteoarthritis: a double-blind randomized self-controlled trial

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Abstract

Objective The purpose of this study was to compare the clinical and radiological efficacy of autologous adipose-derived stromal vascular fraction (SVF) versus hyaluronic acid in patients with bilateral knee osteoarthritis.

Methods Sixteen patients with bilateral symptomatic knee osteoarthritis (K-L grade II to III; initial pain evaluated at four or greater on a ten-point VAS score) were enrolled in this study, which were randomized into two groups. Each patient received 4-ml autologous adipose-derived SVF treatment (group test, $n = 16$) in one side of knee joints and a single dose of 4-ml hyaluronic acid treatment (group control, $n = 16$) in the other side. The clinical evaluations were performed pre-operatively and post-operatively at one month, three months, six months, and 12-months follow-up visit, using the ten-point visual analog scale (VAS), the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and the knee range of motion (ROM). The whole-organ assessment of the knees was performed with whole-organ magnetic resonance imaging score (WORMS) based on MRI at baseline, six months and 12-months follow-up. The articular repair tissue was assessed quantitatively and qualitatively by magnetic resonance observation of cartilage repair tissue (MOCART) score based on follow-up MRI at six months and 12 months.

Results No significant baseline differences were found between two groups. Safety was confirmed with no severe adverse events observed during 12-months follow-up. The SVF-treated knees showed significantly improvement in the mean VAS, WOMAC scores, and ROM at 12-months follow-up visit compared with the baseline. In contrast, the mean VAS, WOMAC scores, and ROM of the control group became even worse but not significant from baseline to the last follow-up visit. WORMS and MOCART measurements revealed a significant improvement of articular cartilage repair in SVF-treated knees compared with hyaluronic acid-treated knees.

Conclusion The results of this study suggest that autologous adipose-derived SVF treatment is safe and can effectively relief pain, improve function, and repair cartilage defects in patients with knee osteoarthritis.

Keywords Osteoarthritis · Adipose-derived stromal vascular fractions · Intra-articular injection · Articular cartilage

Introduction

Osteoarthritis (OA) results from degeneration of joint cartilage and subchondral bone and is one of the leading causes of joint pain and disability [1, 2]. The knee is the most frequently involved weight-bearing joint [3]. As a "wear to tear" disease, OA is associated with significant morbidity and healthcare expenditure [4, 5]. Many treatment modalities for knee OA such as lifestyle modification, pharmaceutical, and surgery have been advocated [6]. Intra-articular injection of hyaluronic acid (HA) is effective in improving symptoms and slowing down the progression of OA [7, 8], but fail to reverse or repair the degenerative cartilage or bone [9].

Regenerative cell therapies for knee OA such as adipose-derived stromal vascular fraction (SVF) have been recently investigated [10–14]. Adipose-derived stromal cells (ADSC) included in SVF have the potential of differentiating into adipogenic, osteogenic, chondrogenic, and other mesenchymal lineages, and have been widely applied to knee OA

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research for their immunomodulatory, anti-inflammatory and paracrine effects [15, 16]. Several recent studies showed the feasibility and safety of ADSC treatments, and it should be an ideal therapeutic option for knee OA [17–21]. However, cell expansion greatly increases the hospitalization costs. Unlike ADSC, SVF can be readily obtained from the lipoaspirate samples without the need for any cell separation or culturing conditions, which make it more cost efficient and convenient. There is a dearth of literature in the area of SVF treatments for knee OA, few clinical trials have been performed except several case reports. In addition, most of these published clinical trials failed to blind for both the participants and the outcome assessor because of the liposuction and other additional intervention procedures [10, 13, 18, 22, 23], which would lead to a high risk of performance bias. Finally, we designed a double-blind, randomized, self-controlled trial to compare the clinical and radiological efficacy of autologous adipose-derived SVF versus hyaluronic acid treatment among patients with grade II/III knee osteoarthritis of bilateral knee.

Materials and methods

Patients and study design

This trial's protocol was approved by Ethics Committees of Zhejiang Provincial People's Hospital before first patient's enrollment; all patients were provided a written informed consent voluntarily. Eligible patients were 18–70 years of age with bilateral symptomatic knee osteoarthritis of grade II to III according to Kellgren-Lawrence criteria [24] and had an initial pain evaluated at four or greater on a ten-point visual analog scale (VAS) in bilateral knee joints. More details of inclusion and exclusion criteria were listed in Table 1.

Before the study, the sample size was estimated on the basis of the results from our pilot study to obtain a power of 80% with α risk = 0.05. From January 2015 to June 2016, 16 patients (32 knees) were enrolled in this study. Three of them were male, and 13 of them were female. The completely randomization process was finished by an assistant accountant who was blinded to the patients' data using SPSS 20.0 software (IBM Corporation, NY, US). First, we listed 1–16 serial numbers (patient serial number) in accordance with the outpatient order. Second, 16 random numbers were generated by RV.UNIFORM (0, 1) in the computer that matched number-by-number with 16 patients' serial numbers. Third, the 16 random numbers were arrayed in ascending order; the corresponding patients of first eight random numbers were injected with 4-ml SVF in the left knee and 4-ml hyaluronic acid (SOFAST, Freda, china) in the right knee. The last eight patients were intervened with 4-ml hyaluronic acid (SOFAST, Freda, china) in the left knee and 4-ml SVF in the opposite. All SVF-treated knees formed the test group. By contrast, another 16 knees exposed with hyaluronic acid formed the control group. More details were shown in Fig. 1. All injections were done under the guidance of knee arthroscopy.

Table 1 Inclusion and exclusion criteria

Inclusion criteria
- Age 18–70 years old
- Bilateral knees with Grade II-III osteoarthritis, identified by two different observers, according to the Kellgren-Lawrence grading scale
- Bilateral knees with initial pain evaluated at four or greater on a ten-point visual analog scale (VAS)
- Patient is able to understand the instructions given by the doctors
- Signing informed consent form

Exclusion criteria
- Had secondary arthritis (related to rheumatoid arthritis, gouty arthritis, post-infectious arthritis, and previous articular fractures)
- Severe heart, lung, liver, and kidney disease that cannot tolerate general anesthesia
- Psychiatric disorders
- History of liposarcoma and other cancer
- Pregnancy
- Immunosuppression
- Coagulopathy
- Abdominal hernia
- Any knee joint operation or intra-articular injection of any drug within 6 months before the screening
- Sign of infection or serological positive of HIV, syphilis
- A low level of body fat content that may make liposuction difficult

Five investigators were included in the protocol for clinical evaluation, corresponding to pre-operation (1 week before operation; baseline), and one, three, six and 12-months post-operation respectively. At each visit, patients were carefully evaluated using the visual analog scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), as well as range of motion (ROM) measurement, and magnetic resonance imaging (MRI) examination (1 week pre-operation, baseline; 6 months and 12-months post-operation).

Except for the orthopedic surgeon, all patients, radiologists, and investigators were blind to treatment allocation of the participants. The orthopaedic surgeon who delivered the intervention did not take outcome measurements.

Preparation of SVF and cell counting

All patients were fasted of at least six hours and water deprivation of at least two hours before operation, general anaesthesia was performed in supine position after checking the patients' information by operator, anaesthetist, and circulating nurse. Liposuction was performed by one regular skilled plastic surgeon, who was blind to patients' information. After sterilizing on abdominal and both lower extremities skin, two small incisions about 5 mm were made around umbilicus,

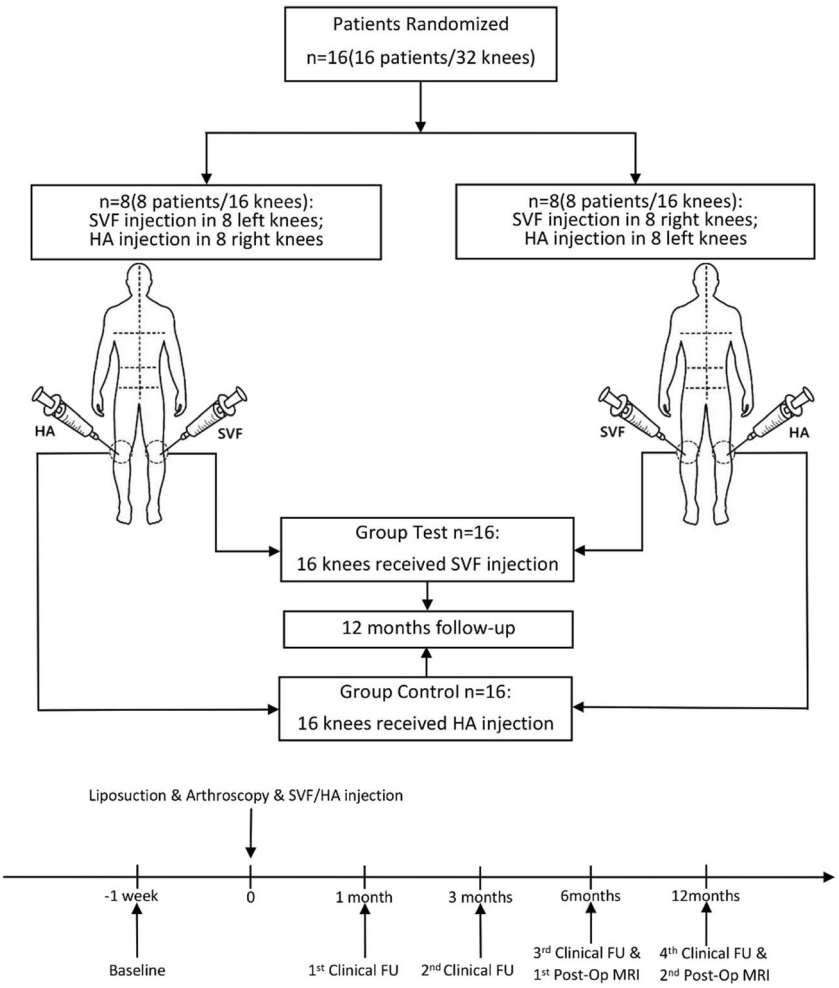

Fig. 1 Study flowchart

and a target volume of approximately 100 to 150 cc of lipoaspirate was harvested through superwet technique from the subcutaneous layer around umbilicus. The incisions were closed with sutures but not tightened to allow more drainage of the blood-tinged tumescent fluid. Abdominal binder was used after operation to prevent bruising in the surgical area.

The harvest adipose tissue was immediately put into a sterile container which was packaged in a portable cryopreservation box on the way to the laboratory. The lipoaspirate was washed twice with 37 °C phosphate buffered saline (PBS), and the residual blood cells and tissue fragments were removed by the mesh filter. Equal volume of type I collagenase (Worthington, Lakewood, NJ, USA) was added into the washed adipose tissue for digestion. The mixture was then placed in a shaking incubator at 37 °C for 30 minutes. After enzymolysis, the tube was centrifuged at 1000 g for 10 min (Eppendorf 5810R, Germany).The supernatant was discarded, and the remnant SVF pellet at the bottom was resuspended in PBS reaching a volume of 4.5-ml SVF. A 0.5-mL sample of the final product was collected for cell counting, and the cell quantity and viability was measured through an automatic cell counter (Countstar IC1000, China).

Surgical procedures and injection

While the adipose processing was going on, arthroscopic debridement was performed in bilateral knee joint by a single orthopaedic surgeon. After a standard arthroscopic examination, all unstable cartilage around the lesion was debrided to form a stabile circumstance of the cartilage. Once the SVF processing was accomplished, SVF and HA were injected under arthroscopic guidance, after the arthroscopic fluid was drained. In the test group, about 4 ml of SVF suspension was injected into the cartilage lesion surface. The contralateral knee received 4 ml of HA injection. Incisions subcuticular suture and pressure dressing after injection were confirmed. All the procedures were done under general anesthesia that the patients themselves were blind about the injection allocation.

Post-operative protocol

All patients were instructed to be non-weight bearing for one day after operation and were discharged two days post-operation with the same health propaganda. Regular daily activities were allowed during follow-up period, and all participants should contact the doctor in charge immediately once there was any sign of adverse event, including fever; cutaneous pruritus, and erythra; swelling, pyorrhea, or fissuration of the incisions. Additionally, a dosage of 200-mg Celebrex twice daily for 2 days was applied as a discharge medication, when patients complained about incision pain with an evaluation over five on a VAS scale on the discharged day. These patients were followed via telephone until the incision pain was relieved.

Clinical evaluation

Pain and functional limitation were evaluated using VAS and WOMAC questionnaire. The WOMAC measures five items for pain (score range 0–20), two for stiffness (score range 0–8), and 17 for functional limitation (score range 0–68) with a total score range from 0 (slightest) to 96 (worst). While functional limitation cannot be scored per joint, pain and stiffness were measured per joint separately by two copies of the questionnaires. In addition, ROM of bilateral knee joints was also recorded.

MRI assessment

The protocol required three MRI scan: baseline (1 week before operation), six months, and 12 months of follow-up. Each MRI was performed using SIEMENS 3.0 T Skyra MRI device, with the 15-channel knee coil. The patients lay supine 30 mintes to reduce the influence of the knee motion and weight bearing to the results of scanning. The following sequences were applied: PDWI-FS images in the sagittal, coronal, and transverse planes; T1 W1 images in the sagittal planes. All data were transmitted to Siemens post-processing workstation, two trained radiologists blinded to each other completed the measurement and recording, and finally obtained a consensus conclusion. The whole-organ assessment of the knees was performed by whole-organ magnetic resonance imaging score (WORMS) [25]. The cartilage repair tissue was assessed by magnetic resonance observation of cartilage repair tissue (MOCART) score (include 9 variables) [26].

Statistical analysis

All data are presented as means ± SD. We used SPSS software (version 20.0, IBM Corporation, NY, US) for all data calculation. Within group analysis of follow-up statistics (VAS, WOMAC score, ROM, and WORMS) were compared with baseline using the paired *t* test, and the independent *t* test was used to compare data at same follow-up time point between groups. The discrete data were analyzed by chi-square test. Differences with $P < 0.05$ were considered statistically significant.

Results

Patient characteristics

A total of 32 knees from 16 patients with bilateral knee OA were randomly allocated to the group test (knee received SVF treatment) and group control (knee received HA treatment) (Fig. 1). The patients characteristics showed no significant difference in age, gender distribution, and BMI, and preferred leg distribution between patients received SVF therapy in the left knee and patients received SVF therapy in the right knee (Table 2). No relevant baseline differences in symptom duration time, Kellgren-Lawrence OA grade, VAS score, WOMAC pain and stiffness, knee ROM, and WORMS between two groups were observed (Tables 3 and 5). In addition, there was no significant difference in preferred leg proportion between the group test, and group control showed ($P > .05$), which diminished the influence of preferred leg in the treatment and follow-up.

Safety

Four patients (25%) complained about pain of the abdomen, like muscle soreness after strenuous exercise, sustained about one week after liposuction. Six patients (37.5%) reported pain and swelling in bilateral knee joints that continued for a few days after knee surgery and all resolved within two weeks. The pain reported above all responded well to Celebrex. There were no other adverse events related to the knee surgery (including infection, allergy, and poor wound healing) and adipose harvest (including deformity and severe ecchymosis).

Clinical outcome

Mean changes of clinical scores from baseline to one month, three months, six months, and 12 months were summarized in Fig. 2 and Table 4. In the test group, all scores including VAS, WOMAC pain, WOMAC stiffness, and knee ROM significantly improved at one month, three months, six months, and 12-months follow-up visits as compared with baseline (Fig. 2). The mean VAS, WOMAC pain, WOMAC stiffness, and ROM in the test group improved by 3.19 ± 0.98, 8.00 ± 4.77, 2.25 ± 2.11, and 19.06 ± 7.76, respectively, between baseline and last follow-up (Table 4). In the control group, pain (VAS score) was significantly relieved by one month and three months after HA injection, but was amplified again

Table 2 Baseline characteristics of patients with different treatment of bilateral knees

Patient characteristics	Patients with SVF therapy in the left knee $N = 8$	Patients with SVF therapy in the right knee $N = 8$	*P* value
Age, year	53 ± 10.97	51 ± 5.95	0.561
Sex, *n*			0.522
Female	7	6	
Male	1	2	
BMI, kg/m^2	25.98 ± 1.95	26.63 ± 1.62	0.480
Preferred leg, *n*			
Left lower extremity	2	3	
Right lower extremity	6	5	
History of trauma, n	3	2	

Values are expressed as mean ± SD unless otherwise indicated. *BMI* body mass index

at six and 12-months visits, from 5.75 ± 1.24 to 5.81 ± 1.33 ($P = 0.791$) and 5.81 ± 1.83 ($P = 0.835$) (Fig. 2a). Functional improvement of ROM was significant at one month after HA therapy ($P < 0.001$). However, this trend even took a turn for the worse after three months post-operation in the control group (decreased by 1.88 ± 6.40 from baseline to last follow-up, not significantly) (Fig. 2b). Unlike the SVF treated group, the general tendency of WOMAC pain and stiffness subscores towards worsening in the control group showed significant differences compared with the test group, as showed in Fig. 2c and Fig. 2d.

Radiologic evaluation

The whole-organ assessment of the knees was performed with WORMS based on MRI at baseline, six months and 12-months follow-up (Tables 5 and 6). In the test group, WORMS showed an important improvement that the mean WORMS decreased by 11.38 ± 24.89 ($P = 0.088$) and 15.44 ± 21.95 ($P < 0.05$) from baseline to six and 12 months, respectively. By contrast the consequence in the control group was poor, WORMS deteriorated by 12.81 ± 12.66 ($P < 0.01$) and 15.50 ± 14.65 ($P < 0.01$) from baseline to six and 12 months, respectively. The repair of the articular cartilage defects was measured by MOCART system based on the MRI results at six and 12-months follow-up, details were shown in Table 7. In the test group, the mean MOCART score was 54.06 ± 11.58 at six months visit and was 62.81 ± 8.16 at 12-months follow-up, showing a significant improvement ($P < 0.01$). However, the mean MOCART, in the control group was poor in both six months (19.38 ± 9.64) and 12 months (19.06 ± 7.79), showed no improvement from six months to 12 months in the HA treated group ($P = 0.924$). It is remarkable that the MOCART in the test group was significantly better than that

Table 3 Baseline characteristics of the group test and group control

	Group test ($N = 16$) knee treated with SVF	Group control ($N = 16$) Knee treated with HA	*P value*
SVF cell density, (× 10^6/ml)	7.45 ± 3.73	–	
SVF cell viability, (%)	70.25 ± 5.04	–	
Preferred leg, *n* (%)	7 (43.75)	9 (56.25)	
Symptom duration, mo	6.88 ± 3.56	6.38 ± 2.68	0.230
Kellgren-Lawrence Grade, *n*			0.288
Grade II	10	7	
Grade III	6	9	
Baseline VAS score	5.38 ± 1.20	5.75 ± 1.24	0.392
Baseline WOMAC pain	9.44 ± 3.90	9.50 ± 3.92	0.964
Baseline WOMAC stiffness	3.00 ± 1.55	3.31 ± 1.82	0.604
Baseline knee ROM	120.13 ± 13.27	116.31 ± 14.65	0.446
Baseline WORMS	71.31 ± 24.2	69.81 ± 18.05	0.844

Values are expressed as mean ± SD unless otherwise indicated. *SVF*, stromal vascular fraction; *HA*, hyaluronic acid; *VAS*, visual analog scale; *WOMAC*, Western Ontario and McMaster Universities Osteoarthritis Index; *ROM*, range of motion; *WORMS*, whole-organ magnetic resonance imaging score

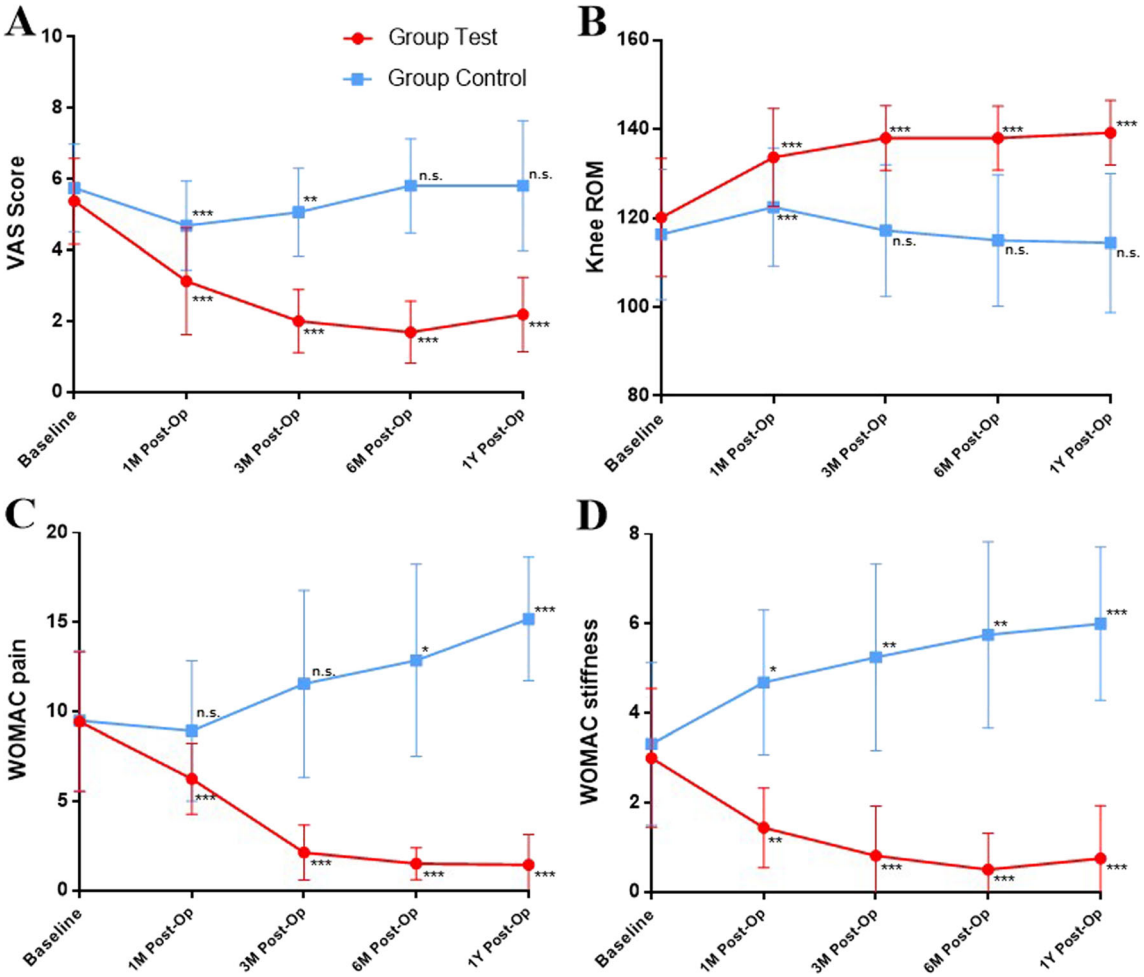

Fig. 2 Changes of VAS, WOMAC score, and knee ROM in two groups during 12-months follow-up. Values in graphs are expressed as mean ± SD in vertical bars, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, non-significant ($P > 0.05$). All values were compared with baseline. **a** VAS score. **b** Knee ROM. **c** WOMAC pain. **d** WOMAC stiffness

Table 4 Clinical and WORMS changes during 12 months follow-up

	Δ.1 month	*p* value	Δ.3 month	*p* value	Δ.6 month	*p* value	Δ.12 month	*p* value
Group test								
WOMAC pain	− 3.19 ± 3.02	< 0.001	− 7.31 ± 3.52	< 0.001	− 7.94 ± 3.84	< 0.001	− 8.00 ± 4.77	< 0.001
WOMAC stiffness	− 1.56 ± 1.59	< 0.01	− 2.19 ± 1.80	< 0.001	− 2.50 ± 1.59	< 0.001	− 2.25 ± 2.11	< 0.001
VAS score	− 2.25 ± 1.39	< 0.001	− 3.38 ± 1.09	< 0.001	− 3.69 ± 1.01	< 0.001	− 3.19 ± 0.98	< 0.001
ROM	13.56 ± 8.52	< 0.001	17.88 ± 7.82	< 0.001	17.88 ± 7.82	< 0.001	19.06 ± 7.76	< 0.001
WORMS					− 11.38 ± 24.89	0.088	− 15.44 ± 21.95	< 0.05
Group control								
WOMAC pain	− 0.56 ± 4.98	.658	2.06 ± 6.84	.246	3.38 ± 5.73	< 0.05	5.69 ± 4.29	< 0.001
WOMAC stiffness	1.38 ± 2.22	< 0.05	1.94 ± 2.49	< 0.01	2.44 ± 2.56	< 0.01	2.69 ± 2.57	< 0.001
VAS score	− 1.06 ± 0.68	< 0.001	− 0.69 ± 0.70	< 0.01	0.06 ± 0.93	.791	0.06 ± 1.18	0.835
ROM	6.13 ± 4.21	< 0.001	0.88 ± 5.80	0.556	− 1.31 ± 4.76	.287	− 1.88 ± 6.40	0.259
WORMS					12.81 ± 12.66	< 0.01	15.50 ± 14.65	< 0.01

Values are expressed as mean ± SD. *VAS*, visual analog scale; *WOMAC*, Western Ontario and McMaster Universities Osteoarthritis Index; *ROM*, range of motion; *WORMS*, whole-organ magnetic resonance imaging score

Table 5 Baseline characteristics of two groups with WORMS

Variables	Group test	Group control	*P* value
Cartilage	32.94 ± 14.24	34.44 ± 11.61	0.746
Marrow abnormality	4.44 ± 1.71	3.5 ± 1.51	0.11
Bone cysts	3.94 ± 1.95	4.81 ± 2.71	0.30
Bone attrition	1.25 ± 1.13	1.31 ± 1.2	0.88
Osteophytes	24.38 ± 16.25	22.19 ± 12.02	0.668
Menisci	3.25 ± 2.41	2.81 ± 2.43	0.613
Ligaments	0.13 ± 0.34	0.06 ± 0.25	0.559
Synovitis	1 ± 0.97	0.69 ± 0.79	0.325
WORMS total	71.31 ± 24.2	69.81 ± 18.05	0.844

Values are expressed as mean ± SD. *WORMS*, whole-organ magnetic resonance imaging score

in the control group, both at six and 12-months MRI follow-up ($P < 0.001$). In addition, in the test group, there were 11(69%) knees that showed complete or hypertrophic repair tissue filling of the defect compared with only one (6%) knee in the control group, seven (44%) knees in the test group showed complete integration with adjacent cartilage, and the value in the control group is only one (6%) (Fig. 3).

Discussion

In this paper, we reported our findings comparing SVF versus HA treatment for 16 pairs of knees with K-L grade II-III osteoarthritis, with 12-months follow-up. Our data demonstrated that SVF could provide effective improvements in both radiological (WORMS and MOCART), and clinical (include VAS, WOMAC pain and stiffness, knee ROM) outcomes which was significantly superior to HA treatment (single dose of 40 mg) for bilateral knee joints with osteoarthritis at II-III stage (K-L grade). In a multi-centre analysis among 2372 patients underwent MSC treatment, the major adverse event was pain post-procedure [27]. Except pain and swelling after liposuction and operation, there was no severe adverse event in the whole process of our study.

In the test group treated with SVF, the knee joints showed statistically significant improvements in the mean VAS, ROM, WOMAC pain, and stiffness compared with baseline after 12-months follow-up, but the mean VAS score of 12-months visit increased significantly ($p = 0.015$) compared with that of six months. We found these patients with increased VAS score of 12 months in the test group; all had a gradually aggravating the VAS score of the knee in the control group. When checking the history, we found that these patients were used to load more weight on the milder knee rather than the most severe knee, which may explain the worsening trend of the VAS score from six months to 12 months in the test group. From the previous literature, we knew that HA treatment was effective in ameliorating pain and symptoms for OA studied and often served as a control [28, 29]. In our study, we used a single dose of 40-mg hyaluronic acid (SOFAST, Freda) injection in the control group for a better blind and variable control, but the outcome indicated that the therapeutic effect of one-single dose of 40-mg HA injection (SOFAST, Freda) was not obvious in the intermediate and long-term follow-up. This result was different from the study of Vega et al. [28]. They used a single dose of hyaluronic acid (60 mg in 3 mL; Durolane) as control, and the VAS score was significantly improved at 12-months follow-up in the control group. More research comparing SVF and adequate course of HA treatment for knee OA is needed in the future.

The MRI follow-up showed a significant improvement of the WORMS in knees treated with SVF. Particularly notable was the reduction in the cartilage and marrow abnormality subscores, which decreased by 12 ± 21.55 ($P < 0.05$) and 2.50 ± 2.00 ($P < 0.001$) from baseline to 12-months MRI. The radiological outcome of MOCART proved that the test

Table 6 WORMS changes during 12-months follow-up

	Group test				Group control			
Variables	Δ.6 month	*P* value	Δ.12 month	*P* value	Δ.6 month	*P* value	Δ.12 month	*P* value
Cartilage	− 7.81 ± 23.42	0.20	− 12.00 ± 21.55	< 0.05	2.56 ± 5.93	0.105	4.13 ± 7.12	< 0.05
Marrow abnormality	− 2.13 ± 2.13	< 0.01	− 2.50 ± 2	< 0.001	5.38 ± 6.79	< 0.01	5.50 ± 7.17	< 0.01
Bone cysts	− 0.44 ± 2.45	0.486	− 0.56 ± 2.28	0.339	0.25 ± 1.00	0.333	0.31 ± 1.01	0.237
Bone attrition	− 0.19 ± 0.40	0.083	− 0.19 ± 0.75	0.333	3.63 ± 4.87	< 0.01	3.81 ± 5.22	< 0.05
Osteophytes	− 0.44 ± 0.73	< 0.05	0 ± 1.63	1	0.38 ± 0.89	0.111	0.69 ± 1.66	0.119
Menisci	− 0.19 ± 1.17	0.53	− 0.13 ± 1.36	0.718	0.13 ± 0.72	0.497	0.25 ± 0.93	0.3
Ligaments	− 0.06 ± 0.25	0.333	0.13 ± 0.89	0.58	0.06 ± 0.25	0.333	0.25 ± 0.68	0.164
Synovitis	− 0.13 ± 0.81	0.544	− 0.19 ± 0.75	0.333	0.44 ± 1.15	0.15	0.56 ± 1.15	0.07
WORMS Total	− 11.38 ± 24.89	0.088	− 15.44 ± 21.95	< 0.05	12.81 ± 12.66	< 0.01	15.50 ± 14.65	< 0.01

Values are expressed as mean ± SD. *WORMS*, whole-organ magnetic resonance imaging score

Table 7 MOCART results during 12-months follow-up

Variables	Maximum score	Group test, *n* (%)		Group control, *n* (%)	
		6 months	12 months	6 months	12 months
1. Degree of defect repair and filling of the defect					
Complete	20	2 (12.50)	5 (31.25)	0 (0)	0 (0)
Hypertrophy	15	5 (31.25)	6 (37.50)	1 (6.25)	1 (6.25)
Incomplete					
> 50% of the adjacent cartilage	10	4 (25.00)	2 (12.50)	2 (12.50)	2 (12.50)
< 50% of the adjacent cartilage	5	3 (18.75)	2 (12.50)	4 (25.00)	3 (18.75)
Subchondral bone exposed	0	2 (12.50)	1 (6.25)	9 (56.25)	10 (62.50)
2. Integration to border zone					
Complete	15	5 (31.25)	7 (43.75)	1 (6.25)	1 (6.25)
Incomplete					
Demarcating border visible (split-like)	10	6 (37.50)	4 (25.00)	1 (6.25)	2 (12.50)
Defect visible					
<50% of length of the repair tissue	5	3 (18.75)	4 (25.00)	5 (31.25)	4 (25.00)
> 50% of length of the repair tissue	0	2 (12.50)	1 (6.25)	9 (56.25)	9 (56.25)
3. Surface of the repair tissue					
Surface intact	10	9 (56.25)	10 (62.50)	2 (12.50)	1 (6.25)
Surface damaged					
< 50% of repair tissue depth	5	6 (37.50)	5 (31.25)	2 (12.50)	2 (12.50)
> 50% of repair tissue depth or total degeneration	0	1 (6.25)	1 (6.25)	12 (75.00)	13 (81.25)
4. Structure of the repair tissue					
Homogeneous	5	9 (56.25)	10 (62.50)	3 (18.75)	2 (12.50)
Inhomogeneous or cleft formation	0	7 (43.75)	6 (37.50)	13 (81.25)	14 (87.50)
5. Signal intensity of repair tissue					
Normal (identical to adjacent cartilage)	30	3 (18.75)	5 (31.25)	1 (6.25)	1 (6.25)
Nearly normal (slight areas of signal alteration)	15	8 (50.00)	8 (50.00)	2 (12.50)	3 (18.75)
Abnormal (large areas of signal alteration)	0	5 (31.25)	3 (18.75)	13 (81.25)	12 (75.00)
6. Subchondral lamina					
Intact	5	10 (62.50)	9 (56.25)	7 (43.75)	5 (31.25)
Not intact	0	6 (37.50)	7 (43.75)	9 (56.25)	11 (68.75)
7. Subchondral bone					
Intact	5	4 (25.00)	6 (37.50)	5 (31.25)	3 (18.75)
Not intact (edema, granulation tissue, cysts, sclerosis)	0	12 (75.00)	10 (62.50)	11 (68.75)	13 (81.25)
8. Adhesions					
No	5	11 (68.75)	10 (62.50)	3 (18.75)	4 (25.00)
Yes	0	5 (31.25)	6 (37.50)	13 (81.25)	12 (75.00)
9. Synovitis					
No synovitis	5	9 (56.25)	10 (62.50)	5 (31.25)	7 (43.75)
Synovitis	0	7 (43.75)	6 (37.50)	11 (68.75)	9 (56.25)
Mean ± SD		54.06 ± 11.58	62.81 ± 8.16	19.38 ± 9.64	19.06 ± 7.79

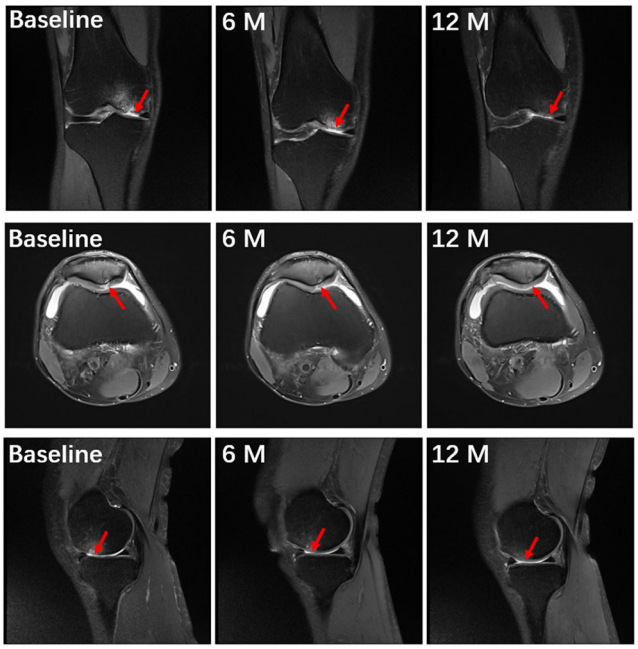

Fig. 3 Magnetic resonance imaging scans of three SVF-treated knees from baseline to 6 and 12-months follow-up showed complete repair and filling of the defects, as well as good integration with the adjacent cartilage and underlying bone in the coronal, transverse and sagittal planes (red arrows)

group had a statistically significant superior articular cartilage repair both at six months (mean MOCART 54.06 ± 11.58 in the test group and 19.38 ± 9.64 in the control group, $P < 0.001$) and 12-months (mean MOCART 62.81 ± 8.16 in the test group and 19.06 ± 7.79 in the control group, $P < 0.001$) MRI follow-up, compared with the control group (Table 7). In the group treated with SVF, four knees had a MOCART score of less than 60 at last follow-up; all accompanied with a poor subchondral lamina and bone as well as a large area of cartilage defect on baseline MRI, suggesting that SVF injection provided a less satisfactory outcome in relatively large cartilage defects. Different from the test group, the MRI outcome in the control group was poor, as the previous literature indicated that hyaluronic acid played a limited role in the repair of damaged cartilage. Furthermore, several other researches studied the relationship between cell dose and therapeutic efficacy of ADSC [18–21], but came to contradictory results. In the two year follow-up study of Jo CH et al.[18, 19], significant improvement was found mainly in the high-dose group (1×10^8), and the outcomes in the low and medium dose groups tended to deteriorate after one year; whereas, those in the high-dose group plateaued until two years. Interestingly, in another clinical trial of ADIPOA [21], significant improvement was detected only in the low-dose (2×10^6) ASCs-treated patients. In another pilot study treated with repeated injections of ADMSCs, the dosage of 5×10^7 showed the highest improvement [20]. In our study, we failed to find an actual association between SVF cell density, cell viability, and outcomes that we need more studies to explore the cell dose effect in the future. There are multiple sources of stem cells for orthopedic conditions [30–32]. Since adipose tissue-derived stem cells (ADSCs) were first characterized by Zuk et al. in 2001 [16], ADSCs have been widely studied for their regenerative and therapeutic potential. Recently, several researches indicated that the regenerative potential was also found in the SVF [33–35], a mixture of ADSCs, endothelial precursor cells (EPCs), endothelial cells (ECs), macrophages, smooth muscle cells, lymphocytes, pericytes, and pre-adipocytes [36, 37]. Traditionally, SVF is isolated by enzymatic processing from lipoaspirate. The advantages of SVF over ADSCs consist of the following parts. Firstly, unlike ADSCs, SVF is readily accessible from the lipoaspirate without the requirement for any cell separation or cell culture. Secondly, SVF therapy is much cheaper and faster than ADSCs because of the absence of culturing procedures. Thirdly, besides the similarities in immunomodulation, anti-inflammatory, and angiogenesis, the characteristic, heterogeneous cellular components of SVF may explain the better therapeutic effect observed in some animal studies [36, 38].

As far as we know, this was the first prospective, randomized, double-blind, and self-controlled clinical trial studying autologous adipose-derived stromal vascular fractions injection for bilateral human knee osteoarthritis. The study was designed according to the principle of completely random, minimizing the distinctions between two groups and reducing the interference of the preferred leg. The setting of self-control between bilateral knees ensured the consistency of sample size between groups during the follow-up process. All procedures were performed under general anaesthesia, minimizing the pain of the patients. Furthermore, adequate blinding was guaranteed in our study, all patients, radiologists, and investigators were blind of treatment allocation, and the orthopedic surgeon who delivered the intervention did not take outcome measurements, reducing the performance bias of the study.

In conclusion, our results indicates that autologous adipose-derived SVF treatment is safe and can effectively relief pain, improve function, and repair cartilage defects in patients with K-L grade II-III knee osteoarthritis. It is therefore believed that adipose tissue may be a good cell source for cartilage regenerative engineering.

Limitations of the study

We must acknowledge that there were several limitations in this study. First, the follow-up period seemed short (12 months); we need more follow-up time to determine the long-term effects of SVF. Second, the sample size was small because the incidence of bilateral knee osteoarthritis was lower than unilateral knee OA. Third, second-look arthroscopy and pathological biopsy of newborn cartilage tissue is the gold standard for evaluating cartilage repair; however, arthroscopy

and biopsy are invasive and inconvenient for dynamic follow-up, and therefore difficult to carry out in China. Fourth, we could not find a clinical rating index aiming at unilateral knee joint that patients should complete two same questionnaires focusing on the individual characteristics with different sides of knees. Fifth, it is unknown, whether SVF injection in one knee could influence the contralateral knee. Sixth, we did not find an actual association between SVF cell density, cell viability, and outcomes, more studies are needed to explore the cell dose effect of SVF treatment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in the studies involving human participants were in accordance with the ethical standards of Ethics Committee of the Zhejiang Provincial People's Hospital and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was registered at Chinses Clinical Trial Registry with identifier ChiCTR1800015125.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Autologous adipose tissue-derived stromal vascular fraction cells application in patients with osteoarthritis

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Running head of the title: SVF cells in osteoarthritis

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Abstract

Stromal vascular fraction (SVF), containing large amount of stem cells and other regenerative cells, can be easily obtained from loose connective tissue that is associated with adipose tissue. Here we evaluated safety and clinical efficacy of freshly isolated autologous SVF cells in a case control study in patients with grade 2-4 degenerative osteoarthritis (OA). A total of 1128 patients underwent standard liposuction under local anesthesia and SVF cells were isolated and prepared for application into 1-4 large joints. A total of 1856 joints, mainly knee and hip joints, were treated with a single dose of SVF cells. 1114 patients were followed for 12.1-54.3 months (median 17.2 months) for safety and efficacy. Modified KOOS/HOOS Clinical Score was used to evaluate clinical effect and was based on pain, non-steroid analgesic usage, limping, extent of joint movement, and stiffness evaluation before and at 3, 6, and 12 months after the treatment. No serious side effects, systemic infection or cancer was associated with SVF cell therapy. Most patients gradually improved 3-12 months after the treatment. At least 75% Score improvement was noticed in 63% of patients and at least 50% Score improvement was documented in 91% of patients 12 months after SVF cell therapy. Obesity and higher grade of OA were associated with slower healing. In conclusion, here we report a novel and promising treatment approach for patients with degenerative OA that is safe, cost-effective, and relying only on autologous cells.

Keywords: stromal vascular fraction, cells, adipose tissue, connective tissue, osteoarthritis, therapy

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Introduction

Degenerative osteoarthritis (OA) of large joints, especially hip and knee, is characterized by degeneration of articular cartilage, sclerosis of the subchondral bone, and marginal osteophyte formation. In the United States of America, symptomatic OA is present in 13.9% of adults 25 years and older and in 33.6% of adults 65 years and older, but it is estimated that radiographic OA is much more frequent (18). OA of weight-bearing joints is associated with chronic devastating pain, stiffness, decreasing range of motion and joint deformity, being one of the leading causes of decreased quality of life and work limitations in elderly.

Although early stages of OA can be alleviated by physical therapy, weight loss, non-steroid analgesic drugs, and chondroprotectives, the advanced disease relies on total joint replacement. Total joint arthroplasty (TJA) is the mainstay of treatment for end-stage OA of the hip or knee. Unfortunately, TJA is relatively frequently associated with serious and life-threatening complications including increased risk of infection, thromboembolism, myocardial infarction, stroke, increased risk of death at 30 and 90 days after surgery, and the life-span of the prosthesis is limited (17,24,27,29).

Recently, it was shown that mesenchymal stromal/stem cells (MSCs) hold a great promise for their healing potential in regenerative medicine (12). Preclinical animal studies that utilize MSCs demonstrated safety and efficacy in treatment of OA, cartilage defects or other orthopedic conditions (3,14,26,28). In humans, the largest collection of culture-expanded bone marrow-derived MSCs used for treatment of 339 patients with OA was recently documented and more than 75% improvement was reported in 41.4% and more than 50% improvement was reported in

63.2% of patients (6). No severe side effects and no neoplastic complications were detected at any stem cell re-implantation site in a mean follow-up 435 days (6).

MSCs can be obtained from bone marrow as well as from adipose tissue. Although bone marrow MSCs and adipose tissue-derived MSCs share many biological features, there are also some differences. Adipose tissue-derived MSCs are more genetically stable in a long term culture, display a lower senescence ratio and higher proliferative capacity (28). Bone marrow MSCs constitute only about 0.001%-0.01% of all nucleated cells in bone marrow, whereas the amount of adipose tissue-derived MSCs is approximately 1000-fold greater when isolated from equivalent volume of tissue (20,28,32). Adipose tissue can be easily obtained by standard liposuction under local anesthesia and isolated stromal vascular fraction (SVF) cells contain 1-4% MSCs as well as other cell types involved in tissue regeneration such as vascular endothelial cells, pericytes, fibroblasts, macrophages and regulatory T lymphocytes (4,10,16,28). SVF cells demonstrated anti-inflammatory and immunomodulatory effects and MSCs have the capacity to differentiate into connective tissue cells including cartilage, tendon and ligament (28,30). SVF cells can be clinically used as freshly isolated from the lipoaspirate without further in vitro expansion or manipulation. These various SVF cell components may act synergistically with MSCs and therefore may be superior to MSCs alone (32). It may be also presumed that freshly isolated cells would be safer and more efficacious compared with the cells expanded by culture, as ex vivo manipulations may lead to genetic and epigenetic alterations that may affect the functional and biological properties of the cells (2).

Autologous adipose-derived SVF cell therapy has been used since 2003 in dogs. In a randomized double-blinded multicenter controlled trial, dogs with large joint OA treated with SVF cells had

significantly improved scores for pain, lameness and range of motion compared with control dogs (3). At least 80,000 SVF cells per kilogram of animal body weight were used. Similar effects were documented for OA, cartilage, tendon and ligament injuries treated with autologous SVF cells in other species as well (3,11,21).

Based on previously published results from animal and human studies, we hypothesize that non-manipulated SVF cells freshly isolated from adipose tissue and administered to the close proximity or into the arthritic joint can demonstrate healing potential in patients with degenerative OA. Here we present data from a multicenter, case control study that demonstrate how practicing medicine with patient´s own regenerative cells freshly isolated from a stromal vascular fraction surrounding small blood vessels of the adipose tissue can significantly improve outcome of degenerative OA leading to a better quality of life.

Materials and Methods

Patients

Multicenter case control study of International Consortium for Cell Therapy and Immunotherapy (ICCTI) was performed in the United States of America, the Czech Republic, Slovakia, and Lithuania after approval by the local Ethics Committees and Investigational Review Board of American Naturopathic Research Institute/Naturopathic Oncology Research Institute. Informed consent for patients was in accordance with the Declaration of Helsinki. Certified orthopedic surgeons and/or traumatology surgeons recruited patients with OA in seven clinical centers from 2010 to 2013. Inclusion criteria consisted of: 1) 18 years of age and older; 2) chronic or degenerative joint OA

grade 2-4 (Kellgren-Lawrence) of 1-4 large weight bearing joints (including hip and knee) and additionally 0-8 other joints (including shoulder, elbow, wrist, hand, ankle, foot) causing significant functional disability verified by clinical examination and X-ray and/or magnetic resonance imaging (MRI); 3) failure of conservative management; 4) signed informed consent form. Exclusion criteria consisted of: 1) active inflammatory disease; 2) severe cardiac, pulmonary or other systemic disease; 3) history of active neoplasm and its treatment with immunosuppressive agents (including chemotherapy, radiotherapy, steroids or other immunosuppressive drugs) within the past 12 months; 4) steroids or platelet-rich plasma within the past 4 weeks; 5) health condition (including known allergy to local anesthetic drug) that does not allow to perform liposuction in local anesthesia; 6) pregnancy or lactation; 7) TJA.

Patients who were referred as candidates for TJA were allowed to participate in SVF cell therapy and this information was recorded by referring physician. All patients underwent local anesthesia of subcutaneous fat in an extent that enables collection of 20-200ml of adipose tissue by a standard tumescent liposuction.

X-ray and Magnetic Resonance Imaging

X-ray: standard weight-bearing X-ray images were performed in antero-posterior (AP) and lateral projections. The images were taken at collaborating institutions using digital X-ray machines, all of them were quality-controlled and certified. Most images were made on direct radiography system Sedecal CXDI 55G (Spain) with read-out detector Canon CXDI (Japan).

MRI: 1.5 T standard protocols pertaining each individual joint using proton density-weighted images (PD) and PD with fat saturation (FS) in coronal plane, T1 and PD FS in sagittal plane, 3D

water-excitation technique in transversal and coronal planes were applied. Examinations were performed on 1.5 T machine Toshiba Excelart Vantage (Toshiba Medical Systems, Japan).

Tissue and SVF Cell Processing

Lipoaspirate was processed using Cellthera Kit I (patent pending; in 2010-2012) or Cellthera Kit II (in 2013), Cellthera, Ltd., Brno, Czech Republic. At least 20ml of adipose tissue per each large joint (or 2 medium joints - elbow, wrist; or 5-8 small joints - hand, foot) treated was processed according to manufacturer's instructions with Cellthera Kit I containing GMP-grade collagenase mix (Cellthera, Brno, Czech Republic). Finally, to block any residual collagenase activity, SVF cells isolated by Kit I were resuspended in 1-5ml autologous plasma that was obtained from anticoagulated blood after centrifugation. When using Kit II, at least 50ml of adipose tissue per each large joint (or 2 medium joints - elbow, wrist; or 5-8 small joints - hand, foot) treated was processed with Cellthera Kit II that does not contain collagenase. Briefly, lipoaspirate was initially washed with normal saline (Ardeapharma, Sevetin, Czech Republic) to remove most red blood cells and tissue debris by sedimentation for 5 minutes. Lipoaspirate supranatant was incubated at 37°C for 20-30 minutes with the same volume of normal saline while shaking. SVF cells were collected after incubated lipoaspirate centrifugation for 5 minutes at 400g at room temperature from fluid infranatant portion. Supranatant portion of lipoaspirate was washed again with the same volume of normal saline, shaken for 1 minute and centrifuged. This step was repeated 3 times to reach maximal cell SVF cell yield. SVF pellet was finally filtered through a sterile 100µm filter (BD Biosciences, Franklin Lakes, NJ, USA). SVF cells isolated by Kit II were resuspended in 1-5ml normal saline. All isolated SVF cells were used for treatment. In both cases (isolation using Kit I or Kit II), all nucleated SVF cells were counted on Burker chamber (Glaswarenfabrik Karl Hecht

GmbH & Co KG, Sondheim/Rhön, Germany) after trypan blue (Sigma-Aldrich, St Louis, MO, USA) staining.

In Vitro SVF Cells Preclinical Testing

Freshly isolated SVF cells as well as third passage adipose tissue-derived stromal cells (ASCs) were examined for their immunophenotype. In order to obtain the third passage of ASC, isolated SVF cells were seeded at a density 20 x 10^3 cells / cm^2 in 24-well plastic plate (Costar, USA) , and then cultured in DMEM/F12 (Sigma-Aldrich) containing 2% penicillin and 5% platelet lysate at 37°C with 5% CO_2. After 24 hours of culture, non-adherent cells were removed and fresh complete medium was added to adherent cells - ASCs. The medium was changed twice per week. When 80% confluence was reached, the cells were counted and subcultured using 0.25% trypsin (Sigma-Aldrich).

The immunophenotype of SVF freshly isolated cells as well as third passage ASCs was characterized by BD FACS Canto II flow cytometer (BD Biosciences). Briefly, cells were washed twice in Dulbeco´s phosphate buffered saline (DPBS; Sigma-Aldrich) containing 1% bovine serum albumin (Sigma-Aldrich), resuspended in 100 µl DPBS (Sigma-Aldrich) and stained for 30 minutes at 4°C with 5 µl fluorescence-conjugated specific monoclonal antibodies anti-CD90 - FITC, anti-CD73 - PE, anti-CD105 - APC, anti-CD19 - APC-Cy7, anti-CD45- PECy7 and anti-CD34 - PerCP-Cy5 (BD Biosciences). Cells were then washed with PBS (Sigma-Aldrich) and characterized by flow cytometry. Doubling time (DT) was measured as followed: DT = (log2 x culture time) : (log N – log N_0) where N is cell count after the third passage and N_0 is cell count of adherent cells after removal of non-adherent cells at the beginning of cell culture.

Treatments

SVF cells were administered in 1-5ml aliquot per joint treated according to joint size. Up to 4 large joints or up to 8 other joints were treated. Single injection of SVF cells was administered intraarticularly or periarticularly to the synovial stromal tissue in the close proximity of such joint. If needed, ultrasound or C-arm X-ray navigation of the needle was employed.

Evaluations

Clinical status of all patients was closely monitored by the attending physician who indicated patients for cell therapy at least 1 week before, at the time of SVF treatment, 1 week, 1, 3, 6, and 12 months after the SVF treatment. SVF cell therapy was recorded and evaluated by the same physician. Clinical evaluation incorporated medical history, physical examination including evaluation of joint pain, number of analgesic drugs taken, joint stiffness and extent of joint movement, lameness status on a semiquantitative scale, recommendation for TJA, as well as any side effects possibly associated with SVF cell therapy. If possible, joint X-ray and/or MRI follow-up of the involved joint was performed after at least 6 months from SVF cell therapy.

All patients and their physicians were instructed to fill in the modified Knee/Hip Osteoarthritis Outcome Score (KOOS/HOOS; www.koos.nu) questionnaire that evaluated semiquantitatively the following measures:

A) Pain – patient evaluation (0 = no pain; 1 = minor not frequent pain; 2 = minor frequent pain; 3 = moderate pain; 4 = severe pain; 5 = unbearable pain requiring analgesics);

B) Painkillers per week – physician evaluation (0 = no painkillers; 1 = 1-7 pills/topical analgesic cream (TAC); 2 = 8-14 pills/TAC; 3 = 15-21 pills/TAC; 4 = 22-28 pills/TAC; 5 = 29 or more pills/TAC);

C) Limping at walk – physician evaluation (0 = no limping; 1 = less frequent minor limping; 2 = frequent minor limping; 3 = moderate limping; 4 = severe limping; 5 = impossible to walk);

D) Extent of joint movement– physician evaluation (0 = no limitation; 1 = limitation up to 20%; 2 = limitation 21-40%; 3 = limitation 41-60%; 4 = limitation 61-80%; 5 = limitation more than 80%, impossible to move);

E) Joint stiffness – patient evaluation (0 = no stiffness; 1 = minor; 2 = moderate; 3 = serious; 4 = severe; 5 = impossible to walk).

OA Score was then constructed as the mean value of variables A) – E) for each patient.

Statistical Evaluation

The nonparametric statistical analysis of changes in Scores over time (before, 3months, 6months and 12 months) in each treatment group was tested by one-way repeated measures analysis of variance ANOVA. The Kruskal–Wallis test (nonparametric one-way ANOVA) was used for comparing Score in independent treatment group (according to OA grade, and body mass index (BMI) category) and post hoc comparisons were made. Wilcoxon rank test was used for comparisons of independent pairs of groups and the Bonferroni correction was used for the test modification to multiple comparisons. Correlation analysis (Spearman correlation coefficient and also modified Spearman correlation coefficient for categorized data) was used for description of statistical association between studied variables (Score and BMI, Score and OA grade, etc.). The significance level 0.05 was used throughout. The 50% and 75% effect of Score improvement in time was calculated as a percentage of patients where the difference between Score before and

Score in a particular time was greater than half and ¾ of Score before, respectively. The data were analyzed using statistical software STATISTICA v.10 StatSoft, Inc.

Results

Patient Characteristics

A total of 1856 joints of 1128 unique patients were treated with single injection of SVF cells isolated from autologous adipose tissue. From this large group 14 patients (1.2%) were lost to follow-up and 1114 (98.8%) patients were evaluated at their follow-up visits. Median follow-up time from the procedure was 17.2 months (range 12.1-54.3 months). The median age was 62 years (range 19-94 years), 596 (52.8%) patients were males and 532 (47.2%) were females, all patients were Caucasians and all underwent single procedure of SVF cell administration to 1-8 joints. There were 557 (49.4%) patients with one joint treated, 481 (42.6%) patients with two joints treated, 51 (4.5%) patients with three joints treated, and 39 (3.5%) patients with four to eight joints treated. Patients underwent 1132 (61.0%) knee procedures, 625 (33.7%) hip procedures, and 99 (5.3%) other joint (ankle, foot, shoulder, hand, wrist or elbow) procedures, see Fig. 1. Based on clinical and X-ray examination, 226 (20.0%) patients were diagnosed with grade 2, 788 (69.9%) with grade 3, and 114 (10.1%) with grade 4 of degenerative OA (highest grade of OA in each patient is reported). There was 1 (0.1%) underweight patient (BMI bellow 18), 169 (15.0%) patients with normal weight (BMI 18-24.9), 639 (56.6%) overweight patients (BMI 25-29.9), and 319 (28.3%) obese patients (BMI 30 or over), see Fig. 1. Among all patients treated, there was always at least one large joint (hip or knee) treated and 503 patients (45.2%) of 1114 patients followed-up were candidates for TJA.

SVF Cell Characteristics

Initially, we compared isolation of SVF cells from autologous adipose tissue using Cellthera Kit I and Kit II. In the cohort of 12 patient samples of isolated SVF cells (6 isolated with Kit I and 6 isolated with Kit II) we were able to demonstrate typical ASC characteristics including 0.9-4.7% of plastic adherent cells and growing in vitro up to passage 6 and expressing CD73, CD90, CD105, losing expression of CD34 and negative for CD45 (data not shown). No significant difference in doubling time was noticed between cells isolated with Kit I and Kit II. In this preliminary optimization cohort, the yield of isolated viable SVF cells per ml of adipose tissue was 3.4-fold higher when using Kit I compared to Kit II.

Thus, for the clinical protocol of individualized cell therapy with autologous SVF cells we decided to use 20-30ml of adipose tissue per each large joint treated when Kit I was used, and 50-90ml of adipose tissue per each large joint treated when Kit II was used. Kit I was used in 478 patients and led to nucleated SVF mean cell yield of 1.63 (±0.41) x 10^6/ml of adipose tissue and viability of 87.4% (± 6.7%). Kit II was used in 650 patients and led to nucleated SVF mean cell yield of 0.39 (±0.12) x 10^6/ml of adipose tissue and viability of 95.8% (± 3.9%). Absolute number of viable SVF cells obtained from adipose tissue isolated with Kit I reached 28.4 (± 11.7) x 10^6 while absolute number of viable SVF cells obtained from adipose tissue isolated with Kit II reached 22.5 (± 8.1) x 10^6. These absolute numbers of viable SVF cells were not significantly different (p=0.19). No statistically significant differences in age, sex, BMI and degree of OA were noticed between patients treated with cells isolated with Kit I or Kit II.

SVF Cell Therapy and Patient Follow-up

All patients underwent treatment with SVF cells as scheduled and no complications related to adipose tissue processing and SVF cells preparation were noticed. There were no serious side effects associated with SVF cell therapy. Other side effects related to the procedure consisted of local pain and swelling at the site of injection, fever, reactive synovitis, headache, deep venous thrombosis, see Table 1. Pain and swelling at the site of injection were observed in patients injected with higher cell number but without significant difference between those treated with Kit I or Kit II isolated cells. Both cases of deep venous thrombosis occurred in women with unsatisfactory hydration and refusal to walk while remaining at sitting position for several hours after the procedure. There was one case of infectious synovitis reported that is unlikely to be SVF cell therapy-related but it is not possible to exclude it. Six days after SVF cell therapy a woman was complaining of localized pain and swelling at the site of SVF cell application and was admitted to another hospital where a puncture of right knee was performed and revealed to be sterile. Four days later, synovectomy of the right knee was performed and *S. epidermidis* was cultured.

Approximately 95% of joints treated were knees and/or hips (Fig. 1). Clinical effect of SVF cell therapy was evaluated with modified KOOS/HOOS Score since, based on Inclusion criteria, all patients has to be treated for at least one hip or knee joint. SVF cell application revealed at least 50% improvement of hip or knee joint after treatment in 80.6% of patients at 3 months. The Score further improved in time to 12 months of the follow-up to 91.0% as documented in Fig. 2. When 75% Score improvement was evaluated of the hip or knee joint, similar trend for improvement was noticed from 3 months to 12 months post-treatment in 39.7% to 63.0% of patients. Only up to 0.9% of patients were non-responders. Remaining patients improved for less than 50%. There was a difference in clinical responses among patients treated with SVF cells isolated with Kit I and Kit II by multiple comparison analysis, see Fig. 2D. This difference was significant at 3 months after SVF

cell therapy (p = 0.0001), but not before, at 6 and 12 months after SVF cell therapy (p = 0.2430; p = 0.0512; p = 0.4593, respectively).

Women had higher Score than men before and at 3 months after SVF cell therapy (p = 0.0089; p = 0.0020), but not at 6 and 12 months after the procedure (p = 0.0771; p = 0.5799, respectively) as demonstrated in Fig. 2E. Score evaluation before, at 3, 6 and 12 months after the SVF cell therapy was significantly increased in older patients in comparison to younger ones (p<0.0001 in all checkpoints, respectively) as shown in Fig. 2F. Higher OA grade was associated with significantly increased OA Score before, at 3 and 6 months (p = 0.0156; p = 0.0318; p = 0.0030, respectively), but not at 12 months (p = 0.5315) after SVF therapy. Patients with higher BMI had significantly higher OA Score at 3 months after the procedure (p = 0.0281), but not before and at 6 and 12 months after SVF therapy (p = 0.3002; p = 0.1004; p = 0.4022, respectively).

Patient's responses were also monitored by X-ray and MRI. Typically subtle but significant widening of joint spaces was observed on X-ray 6 - 12 months after SVF cell therapy in most patients. In some cases no change in X-ray imaging was noticed. MRI studies revealed slight chondral thickening or stable cartilage thickness 6 - 12 months after SVF cell therapy. Smoothing of surface irregularities and defects, regression of reactive subchondral bone edema, sealing of chondral fissures, healing of subchondral cortical lesions or integration of chondral flaps were frequently seen after the treatment. To illustrate the X-ray and MRI of the joint treated with SVF cells of a typical good-responders, see Fig. 3.

From 503 TJA candidates before the SVF cell therapy, only 4 (0.80 %) patients required total hip replacement during the follow-up period. These findings demonstrate that patients with lower degree of OA and non-obese patients recover from OA faster, typically within 3-6 months after

SVF cell therapy. In patients with higher degree of OA and in obese patients the regeneration of arthritic joint may take longer, but at 12 months they experience the same degree of clinical improvement as patients with lower degree of OA and non-obese patients, respectively.

Discussion

Adipose-derived cells have potential applications to a wide range of clinical disorders including myocardial infarction, stroke, Crohn's disease, multiple sclerosis, rheumatoid arthritis, limb ischemia, breast augmentation and reconstruction, decubiti ulcers, postirradiation fibrosis, and craniofacial reconstruction (8,12,13).The greatest number of patients reported have been for breast reconstruction, myocardial infarction, and fistula repair in Crohn's disease as previously reviewed (10,28).

There is also a growing body of research regarding stem cells for the treatment of degenerative OA. Recently, the largest group of patients with OA treated with bone marrow-derived cultured MSCs was reported by Centeno et al. and involved 339 patients. It demonstrated safety and clinical efficacy in most patients treated. In a subgroup of 133 patients with knee OA 50% score improvement was noticed in 63.2% cases at an average reporting time of 11.3 months from the first cell application (6). Vangsness et al. reported results of a randomized, double-blind, controlled study in 55 patients with knee OA and partial medial meniscectomy treated with allogeneic bone marrow-derived MSCs. The study demonstrated safety and no ectopic tissue formation after cell therapy. Reduction of pain as well as meniscal volume increase was noticed in MSC-treated patients but not in placebo control group (30). Despite using different cell sources, both studies

(6,30) are in accordance with our findings regarding safety and clinical effect of cell therapies in similar orthopedic indications.

Recently, there are several reports regarding adipose-derived cell therapy of degenerative OA, but all of them with relatively small number of patients (4,9,15,16,23). In our study, we are in agreement with these studies using adipose-derived cells which are safe and clinically effective in most patients with degenerative OA. The use of adipose tissue have many advantages in comparison to bone marrow: it can be easily obtained by standard liposuction under local anesthesia; adipose stem cells are plentiful and adipose tissue contains approximately 500-2500 times higher amount of mesenchymal stem cells compared to the same volume of bone marrow (1,8,20,28). While MSCs are dramatically decreasing with age in bone marrow (5), their pool in adipose tissue is quite stable during life (1,5). In addition, the adipose tissue contains unique populations of cells that suppress the inflammatory responses, and thus further contribute to regeneration and create optimal environment for adaptation of stem cells that support regeneration and repair of damaged cells and tissues (28,31). Adipose-derived stem and stromal cells do not require in vitro cultivation and are ready for use immediately after isolation from the adipose tissue. Recently, in an animal model of guinea pigs with spontaneous OA, Sato et al. demonstrated migration, differentiation, proliferation, and persistence of MSCs into the damaged cartilage and adjacent synovial tissue. There was a strong immunostaining for type II collagen around both residual chondrocytes and transplanted MSCs in the OA cartilage demonstrating direct contribution of MSCs to hyaline cartilage healing and regeneration (26).

These data are in accordance with our clinical observation in a large cohort of adult patients with grade 2-4 degenerative OA. In our SVF cell therapy case control study, 1856 joints were treated in

1128 patients and we were able to demonstrate safety with no serious side effects reported in 1-4.5 years of follow-up and clinical improvement in a vast majority of patients. Some patients experienced local pain and swelling at the injection site, but those symptoms were lasting shortly and were well controlled with common analgesics. Not surprisingly, most patients were treated for knee and/or hip OA and our treatment strategy allowed multiple joint treatments during one surgical procedure. Based on previous studies demonstrating migratory capability of MSCs (6,14,20,26,28) we allowed intraarticular or periarticular (synovial stroma or loose connective tissue immediately adjacent to the joint cartilage) application of SVF cells. We hypothesized that stem cells as well as other regenerative stromal cells may contribute to the cartilage healing process via two mechanisms: 1) paracrine effect and 2) cell migration, differentiation and proliferation. Our clinical observations are in agreement with this hypothesis and with the animal study (26), which brought direct evidence for such mechanism of cartilage regeneration using cell labeling techniques that clearly demonstrated long-term persistence of transplanted stem cells in the cartilage and adjacent synovial and other loose connective tissue. We did not observe significant difference in clinical response or side effects frequency or magnitude associated with intraarticular versus periarticular application of SVF cells. This is probably due to their anti-inflammatory capacity and the capacity to migrate to the site of injury where they are able to execute the healing effect.

At this point, we should also clarify the terminology regarding the source of SVF cells. In the vast majority of scientific publications only the term *adipose tissue* is used, but the true source of SVF cells is not the adipose part but only the stromal (ie. loose connective tissue) part of the fat obtained typically by liposuction. Histologically, the fat lobules are surrounded by a loose connective tissue and the SVF cells reside in the loose connective tissue that also home capillaries

and small vessels. *Stroma* is a broadly used term for the loose connective tissue that contains mesenchymal stem cells and other cells like fibroblasts, macrophages, adipocytes, mast cells and leukocytes. Synovia of articulated joints is also intimately associated with the loose connective tissue which is homologous to the loose connective tissue of the adipose tissue (25). Thus, in our clinical study we were aiming to enrich the population of stem and other regenerative cells in a close proximity to damaged cartilage. In a human study we are limited in direct cell imaging in comparison to animal studies (26). On the other hand, we can demonstrate indirectly the healing potential of SVF cell therapy in OA using clinical examinations and symptom scoring as well as objective visualization of damaged joints by MRI and X-ray imaging. Since imaging was not the primary aim of this case control study, the follow-up X-ray and/or MRI examination was not performed in all patients. Thus, we are not able to draw any strong conclusion on the correlation between clinical improvement and imaging studies despite, in most cases, clinical improvement corresponded well with improvement on X-ray and/or MRI imaging.

Despite several techniques for SVF cells isolation exist (8,10,20), maximal cell yields are obtained after collagenase digestion of adipose tissue (20). Here we demonstrate that collagenase digestion may lead to better short term results in a clinical outcome at 3 months, but it is not clear if such short-term effect can be caused by the autologous plasma or larger cell number contained in that cell suspension. On the other hand, later on at 6 and 12 months after SVF cell administration, we did not observe any significant difference between usage of SVF cells that were processed with or without enzymatic digestion of the adipose tissue. In addition, there are similar results with comparable numbers of viable SVF cells that can be obtained without collagenase digestion when larger amounts of adipose tissue are processed. Almost 90% of patients were diagnosed with grade 2-3 OA and almost 85% patients were overweight or obese. We were able to demonstrate

that clinical improvement is slower during the first 3 to 6 months in patients with higher BMI and in patients with higher OA grade. But later on, at 6 and 12 months after SVF cell therapy, there is no difference in clinical outcome based on BMI and OA grade status. In obese people, the mechanical pressure on cartilage of the weight-bearing joints is extremely high leading to more degenerative changes of the weight-bearing joints. We are demonstrating that despite there are still differences in a short term response (evaluated at 3 months after cell therapy), there is no significant difference in a clinical response after 6 or 12 months. The regenerative potential of SVF cells probably takes longer in obese patients to regenerate the cartilage.

Not surprisingly, higher age is associated with higher Score before and stays higher throughout the follow-up after cell therapy in comparison to younger patients. Yet, dramatic Score decrease was significant after SVF cell therapy at any age. Even patients in their seventies, eighties or even nineties, who are typically not qualified for TJA because of their age and a general health status, can undergo SVF cell therapy without any serious side effects. Also, most patients benefited from SVF cell therapy despite the fact that more than 45% of them were TJA candidates. During the median follow-up 17.2 months there were only 4 patients who required TJA. All of them underwent hip joint replacement and all of them had grade 4 OA of that hip joint. None of patients with other than hip OA required TJA. We cannot draw strong conclusions out of this finding, but we can suggest to undergo the SVF cell therapy in earlier stages, especially in case of hip OA, when clinical symptoms of OA are present and leading to decreased quality of life. In addition, our data clearly demonstrate a durable effect of single injection of adipose-derived SVF cells. Similar finding was documented previously with bone marrow-derived cultured MSCs therapy in patients with osteoarthritis (6). In this study, there were 67.8% of patients with knee OA candidates for total

knee arthroplasty and 6.9% reported that they opted for knee replacement in a median follow-up of 435 days (6).

Other treatment options are used in a clinical practice to alleviate symptoms such as pain and stiffness in OA patients, although none of them led to regeneration of joint connective tissue including cartilage: administration of analgesic, non-steroidal anti-inflammatory drugs and/or corticosteroids. However, these medications may have broad spectrum of adverse effects, namely in gastrointestinal tract, liver, kidneys, and other organs, especially during long-term use (22). In case of advanced stage large-joint OA, standard treatment consists in surgical removal of the affected joint and its replacement with an artificial joint. Total joint arthroplasty represents rather extensive surgery which is associated with considerable risk of serious side effects and post-operative complications including myocardial infarction, stroke, systemic infection or increased risk of death after TJA (17,24,27,29).

Typically, patients in our case control study were administered large amounts of painkillers, ie. mainly analgesics or non-steroidal anti-inflammatory drugs, before the SVF administration. In order to compare the amount of painkillers taken before and after the SVF cell application, the quantity of these drugs in a form of pills or topical analgesic creams used by patients was assessed, evaluated and used as one of the parameters in calculation of the Modified KOOS/HOOS Clinical Score to evaluate clinical effect of the therapy. We have observed that the quantity of painkillers (data not shown), as well as the Score (shown at Fig. 2A) were significantly decreased after the SVF therapy. Despite the limitation of our study that did not contain the control group of patients with OA, we can assume that, due to a long term use (at least 6 months, but typically several years) of painkillers prior to SVF administration, those patients would continue in painkillers consumption at

the same or even larger amounts of painkillers as the diseases progresses, without SVF cell application. Altogether, given that the amount of painkillers used was significantly decreased in patients after the SVF cell administration, we can assume that this therapeutic strategy is both safe and effective in most of the patients treated.

In addition, there are also other treatment approaches to OA, such as intraarticular administration of platelet rich plasma or hyaluronic acid. Although these methods are also available for patients with symptomatic OA, they typically involve a series of intraarticular injections. In contrast, the results of our case control study are based on single, intra- or periarticular administration of SVF cells with documented safety and a relatively long term clinical effect with a median follow-up time 17.2 months (range 12.1-54.3 months). Therefore, it would be difficult to compare the effect of a single dose of autologous SVF cells with a series of platelet rich plasma or hyaluronic acid injections.

Despite safety and efficacy of SVF cell therapy, there are some limitations in our case control study. There is no guarantee that such cell therapy can lead to a definite cure of degenerative OA. The patients are further monitored and longer follow-up data will help to answer question about durability and long-term safety of SVF cell therapy. Another limitation of our study is no randomization and no placebo control. There were two reasons for designing that case control study: 1) ethical aspect and 2) economical aspect. We believe it would be rather unethical to ask placebo group of patients to undergo liposuction and placebo administration to the joint with OA. Since this study was designed as autologous cell therapy, there is strong previously documented clinical evidence of safety of autologous non-manipulated or minimally manipulated cell therapies (19). In the first decade of the 21st century, more than 17,000 scientific articles involving 2,724 cell

therapy clinical trials were published (7). These results include 323,000 patients treated with more than 675,000 cell therapy units. The treatments were very safe and often very effective in the treatment of various diseases with the potential to significantly improve health worldwide (7,19,28). Second economical aspect of our study preparation was based on estimation of extreme costs associated with a new drug development. The costs of phase I to phase III clinical trials leading to the new drug registration are estimated to be 300 million to 1 billion USD. Furthermore, once such budget is raised, new drug is tested in a double-blind, randomized, placebo-controlled clinical trial and finally registered based on safety and efficacy profile. The health care providers worldwide are exposed to extreme costs associated with eligible patient treatments after such registration. On the other hand, a case control study, if well designed and if strong evidence for minimal risks based on previous studies exists, can lead to a cost-effective, safe, ethical and objective evaluation of a novel treatment. One of such examples can be a case control study using autologous non-manipulated or minimally manipulated cells.

Conclusion

Autologous stromal vascular fraction cell therapy of degenerative osteoarthritis is safe and clinically effective strategy leading to improved quality of life. This conclusion is based on the present case control study of 1128 adult patients.

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Disclosure of potential conflicts of interest

JM is CEO of Cellthera, Ltd., the other authors do not declare any conflicts of interest.

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Table 1: Side effects observed in patients treated with SVF cell therapy

A total number of 1,114 patients were treated and followed-up for side effects related to SVF cell therapy.

Serious side effects	Number	[%]
Myocardial infarction	0	0
Stroke	0	0
Thromboembolism	0	0
Systemic infection	0	0
Cancer	0	0
Death	0	0
Other serious side effects	0	0

Other side effects	Number	[%]
Local pain < 24 hours	47	4.22
Local pain > 24 hours	38	3.41
Local swelling < 72 hours	58	5.21
Local swelling > 72 hours	12	1.08
Fever > 38°C < 24 hours	9	0.81
Fever > 38°C > 24 hours	4	0.36
Reactive synovitis	5	0.45

Headache	3	0.27
Deep venous thrombosis	2	0.18
Infectious synovitis*	1	0.09

*One patient experienced infectious synovitis that was unlikely related to SVF therapy, but it is impossible to completely exclude it. For details, see text.

Figure legends

Figure 1. Characteristics of patients and joints treated by SVF cell therapy.

(A): 1,856 joints in 1,128 patients were treated. The columns represent the numbers of knee, hip, ankle/foot, shoulder and hand/elbow joints treated. (B): The number of patients diagnosed with grade 2, 3 and 4 (according to Kellgren-Lawrence classification) of osteoarthritis is shown. (C): The body mass index (BMI) of patients undergoing SVF cell therapy.

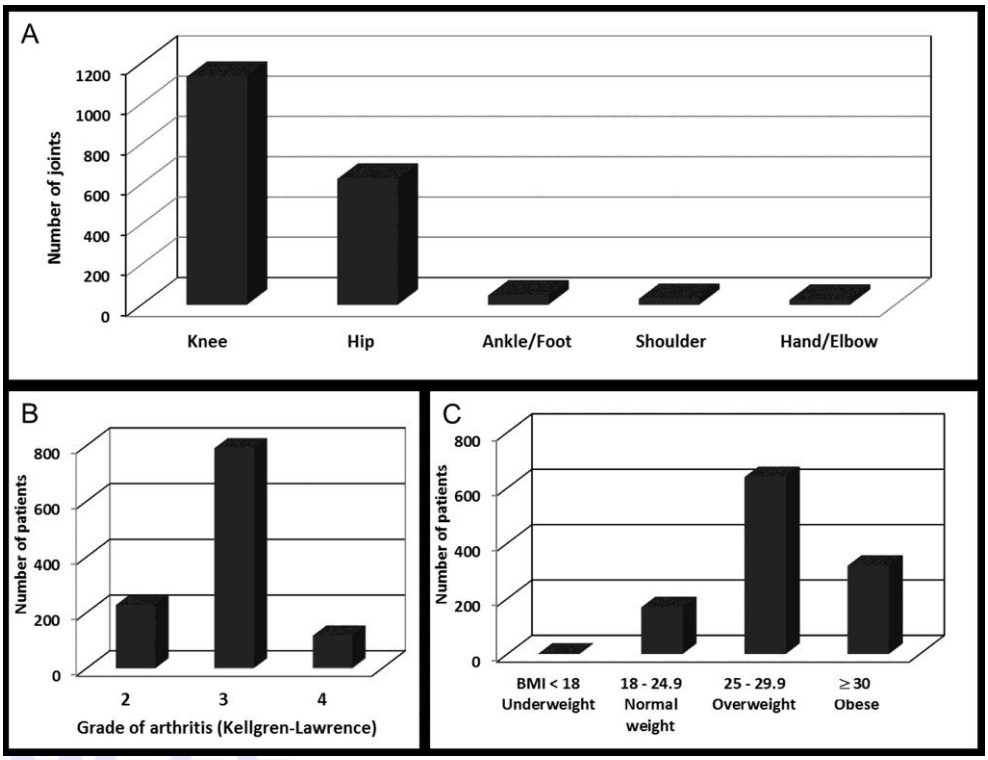
Figure 2. Evaluation of clinical outcome based on Modified KOOS/HOOS Score.

A calculation of the Score is based on 5 parameters: pain, number of painkillers per week, limping at walk, extent of joint movement and stiffness. (A): Significant improvement in Score (*) was observed 3, 6 and 12 months after SVF cell therapy compared to the status before SVF cell therapy ($p < 0.0001$). Means ± SD [black box], and ± 1.96 SD [black bars] are shown. (B): Percentage of patients with at least 50% Score improvement. (C): Percentage of patients with at least 75% Score improvement. (D): Comparison of Scores in patients treated with SVF cells isolated with Kit I or Kit II. Significant difference was noticed between Kits at 3 months after SVF cell therapy (*). Means - SD are shown for Kit I [full line] and means + SD are shown for Kit II [interrupted line]. (E): Comparison of Scores between the group of men and women. Significant difference was noticed between men and women before SVF therapy and at 3 months after the therapy (*), but not at 6 and 12 months after the procedure. Means - SD are shown for men [full line] and means + SD are shown for women [interrupted line]. (F): Comparison of Scores in patients younger than median age (< 62 years) and older than median age (≥ 62 years). Significant difference was noticed between younger and older patients before SVF therapy and at 3, 6 and 12 months after the

therapy (*). Means - SD are shown for patients younger than 62 years [full line] and means + SD are shown for patients 62 years old and older [interrupted line].

Figure 3. X-ray and magnetic resonance imaging (MRI) results of the joints before and after SVF cell therapy.

(A): X-ray results of 56 year old man with right knee grade 3 osteoarthritis and kissing bone phenomena in a medial compartment. X-ray 12 months after SVF cell therapy shows widening of the joint space [arrows], most likely due to greater cartilage volume. X-ray was performed by direct radiography system Sedecal CXDI 55G (Spain) with read-out detector Canon CXDI (Japan). (B): MRI results (proton density-weighted images in coronal plane) of 45 year old man with grade 2 osteoarthritis with chondral defects, loose chondral flap and irregularities of subchondral cortical bone of lateral compartment of the left knee joint [encircled in white]. MRI performed at 6 months after SVF cell therapy at the same level shows reintegration of the flap, reconstitution of chondral defects with a thin layer of chondral overgrowth and improved outlining of the subchondral cortex. MRI was performed by 1.5 T MRI Toshiba Excelart Vantage XGV Atlas (Japan). (C): MRI results (proton density-weighted images in coronal plane) of 49 year old man with grade 3 osteoarthritis and subchondral bone lesion with control at the same level 18 months after SVF cell therapy. The cartilage defect leading to the defect of subchondral cortical bone disappeared on the control MR image and is covered by regenerated smooth chondral layer. MRI was performed by 1.5 T MRI Toshiba Excelart Vantage XGV Atlas (Japan).



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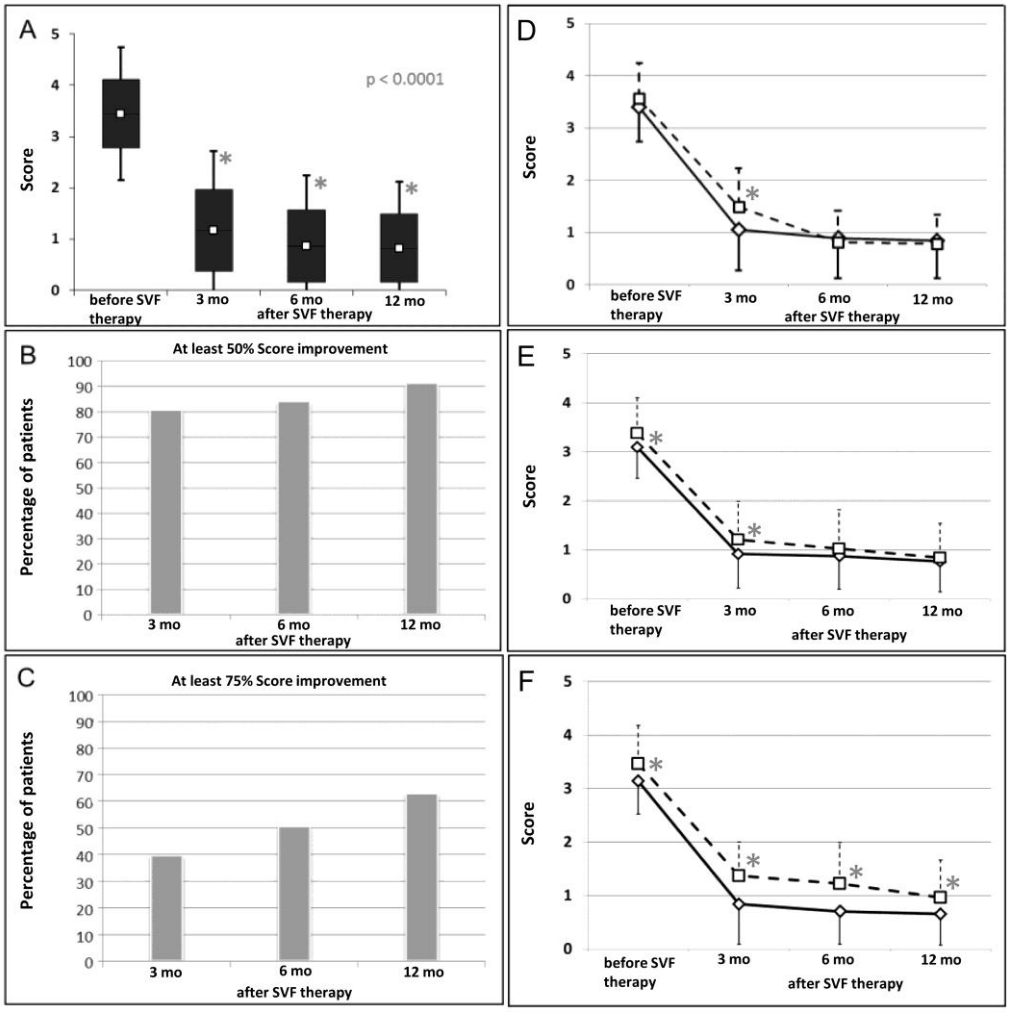

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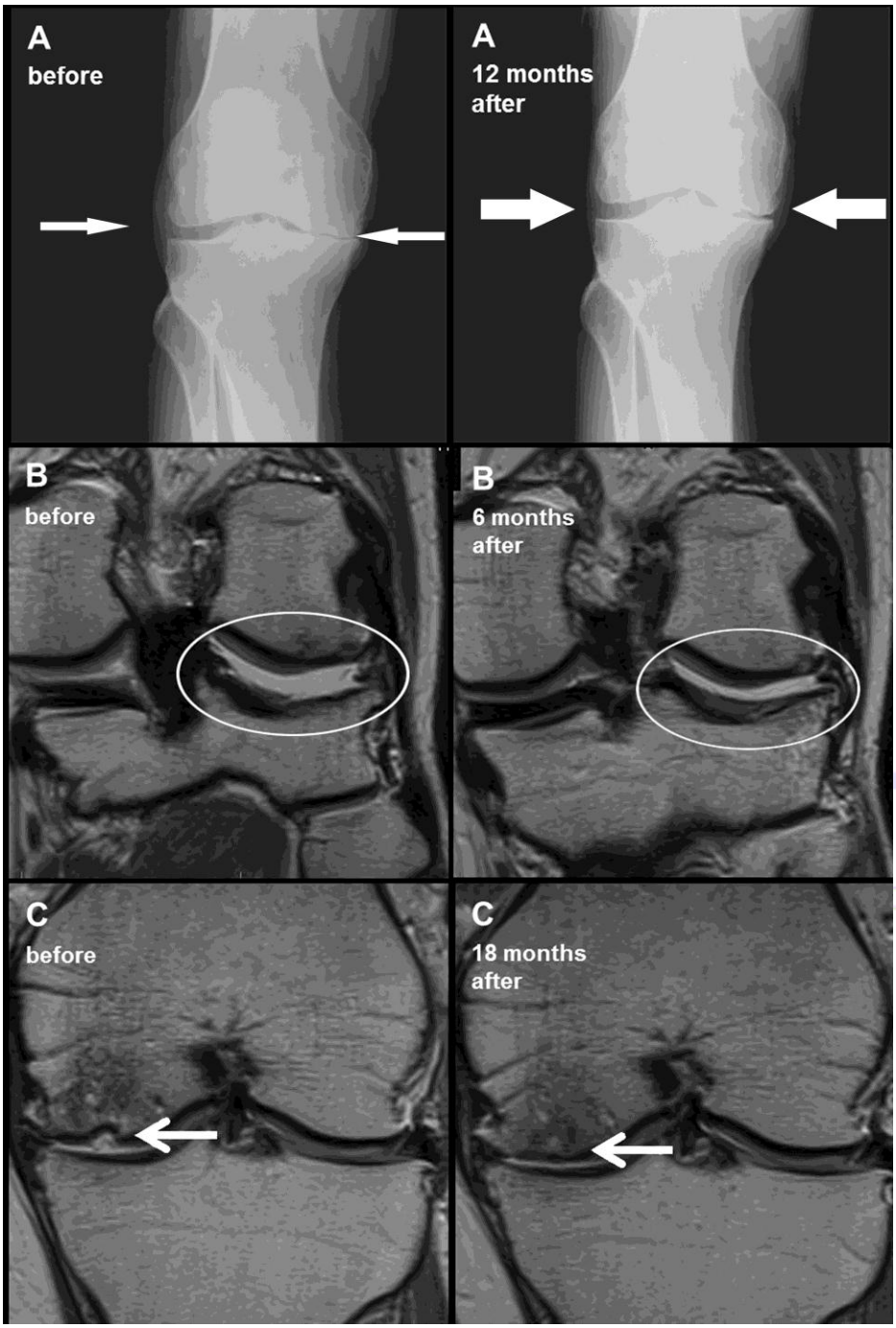

Figure 3:

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Article

Time- and Kellgren–Lawrence Grade-Dependent Changes in Intra-Articularly Transplanted Stromal Vascular Fraction in Osteoarthritic Patients

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Abstract: Knee osteoarthritis (OA) is one of the most prevalent disorders in elderly population. Among various therapeutic alternatives, we employed stromal vascular fraction (SVF), a heterogeneous cell population, to regenerate damaged knee cartilage. OA patients were classified on the basis of age, gender, body mass index (BMI), and x-ray-derived Kellgren–Lawrence (KL) grade. They were treated with SVF and followed-up for 24 months. Visual analogue scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis (WOMAC) Index were used to determine treatment efficacy. Cartilage healing was assessed using the MRI-based Outerbridge score (OS) and evaluation of bone marrow edema (BME) lesions, while a placebo group was used as a control. Time- and KL-dependent changes were also monitored. We observed a decreasing trend in VAS score and WOMAC index in the SVF-treated group up to 24 months, as compared with the placebo group. Besides, a significant increase and decrease in Lysholm and OS, respectively, were observed in the treatment group. Compared with the values before treatment, the greatly reduced WOMAC scores of KL3 than KL2 groups at 24 months, indicate more improvement in the KL3 group. Highly decreased BME in the treated group was also noted. In conclusion, the SVF therapy is effective in the recovery of OA patients of KL3 grade in 24 months.

Keywords: knee osteoarthritis (OA); KL grade; stromal vascular fraction (SVF); MRI; WOMAC; VAS; OS; BME

1. Introduction

Knee osteoarthritis (OA) is one of the most common progressive joint disorders, especially among elderly population in the United States and other developed countries [1–3]. Cartilage devolution, stiffness, loss of joint function, bone loss/rearrangement, and pain are primary

characteristics of OA [4,5]. In the clinics, OA patients are categorized on the basis of their Kellgren–Lawrence (KL) grades (1 to 4), whose range of symptomatic characteristics includes the narrowing of the joint space to definite deformity of bone ends [6]. Multiple risk factors for OA include age, gender, inflammation, genetics, mechanical wear and tear during exercise, sports, or any other stressful activity [7–10]. There is wide perception that obesity and increase in life expectancy are major causes of the increase in OA in the last decades; however, a recent study carried out by Wallace et al. suggests that life longevity and body mass index (BMI) are not the only factors for the increase in OA, and extensive research is needed to determine other factors associated with OA increase [11]. The self-renewal ability of chondrocytes is significantly lost in aged persons (>60 years), and this severely affects cartilage structure and maintainance [12]. Moreover, it has also been established that the secretion of proteolytic enzymes such as aggrecanases and metalloproteinases further degrades the damaged cartilage [13,14]. OA-related pain is treated by non-pharmacological approaches such as physical therapy, yoga, land- and water-based exercise, tai chi, and weight loss [15–20], as well as with pharmacological agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) [21,22], chondroprotective compounds, calcium, opioids [23,24], and hormones [25]. Hyaluronic acid (HA) is intra-articularly administered to restore the viscoelastic properties of injured cartilage [26,27]. Surgical treatments including arthroscopy, microfracture, subchondral drilling, and abrasion arthroplasty are used to treat late-stage OA; however, the limitations of these procedures include the formation of fibrocartilage, which has less ability to absorb shock, thereby compromising the functional characteristics of the native cartilage tissues [25].

An alternative surgical technique, the autologous chondrocyte implantation (ACI), has been recently used to overcome the limitations associated with the previously mentioned surgical techniques. ACI is a common surgical intervention to promote healing of cartilage injuries in OA [28,29]. However, the effectiveness of ACI is restricted because of the limited availability of chondrocytes and the compatibility between implanted chondrocytes and host site [30]. Cell-based regenerative therapies along with biomaterials, especially stem cells and hydrogels, are emerging and promising procedures to counter OA. Bone marrow-derived stem cells (BM-MSCs), peripheral blood-derived stem cells, adipose-derived stem cells (ADSCs), and synovial fluid-derived stem cells have been studied in the presence or absence of biomaterials [31]. The paracrine effects of stem cells have been widely associated with regeneration and repair activities [32]. The adipose tissue is considered a rich and preferable source of stem cells due to the feasibility of harvesting tissue and isolating stem cells.

Stromal vascular fraction (SVF) is a heterogeneous population of various immune, precursor, progenitor, and stem cells. SVF is considered to be as equal as or sometimes more effective than ADSCs; therefore, it provides other functional advantages, such as structural support, over ADSCs [33–36]. However, SVF is immunologically restricted because of the presence of various cells and only fit for autologous treatment [37], whereas, ADSCs are multipotent cells that can differentiate into chondrocytes, with capability of self-renewal, high plasticity, and immunomodulatory and anti-inflammatory properties [38,39]. SVF has been widely studied as an alternative therapeutic agent to treat sclerosis, myocardial and bone-related disorders, blood vessel regeneration, and pulmonary diseases [40–42]. Recent works have also been extensively focused on evaluating SVF potential in orthopedic ailments [41,42]. Various clinical studies combining SVF with plasma-rich protein (PRP) , hyaluronic acid (HA), ceramic and fibrin glue were carried out to assess the potential of SVF in the treatment of OA [43–45]. Considering the therapeutic significance of SVF, this study was carried out to assess the therapeutic efficacy of SVF in OA treatment through the regeneration of articular cartilage. During our study, we specifically investigated time- and KL grade-dependent changes up to 24 months.

2. Materials and Methods

2.1. Study Design and Participants

This study was an open-label, single-center, non-randomized, placebo-controlled, phase I/II clinical trial to evaluate the improvement in knee pain and knee function, as well as cartilage restoration. The 33 patients enrolled in the study were deliberately allocated to two groups, which were designated arthroscopic microfracture treatment only and arthroscopic microfracture treatment combined with SVF injection. Observation and follow-up data were recorded after 12 and 24 months. The eligibility criteria included: osteoarthritic knee joint with KL grades 2–3 and age >38 years. Patients meeting the following criteria were excluded: autoimmune or inflammatory disease, infection requiring parenteral administration of antibiotics, serious internal disorders, corticosteroids or viscosupplements injection into the affected knee within the past 3 months, and stiffness due to previous severe injury. The protocol was approved by the Viet Nam Ministry of Health (No. 2288/QDBYT) and the Ethical Committee in Biomedical Research of Van Hanh General Hospital (No. 90-084/QD-BVVH). Patients participating in this research provided an informed consent, in accordance with the Declaration of Helsinki.

2.2. Fat Tissue Harvest and SVF Isolation

Lipoaspirates were harvested from patients' lower abdomen by a standard liposuction technique. Briefly, through incision, a solution of tumescent lidocain, 250 mL of normal saline, 0.9% and 0.2 mL of 1:1000 epinephrine was injected in the subcutaneous fat. Thereafter, 50–100 mL of lipoaspirate was collected through Triport Harvester Cannula (Tulip Medical Product, CA 92117 USA), and a 60 mL Luer-lock syringe. The SVF from the lipoaspirate was isolated by means of collagenase digestion (Collagenase NB 6 GMP Grade, Nordmark Biochemicals, Ho Chi Minh City, Vietnam) and the ADSC Extraction Kit (Geneworld Co. Ltd., Ho Chi Minh City, Vietnam) approved by the Viet Nam Ministry of Health. The SVF was then washed thrice with sterile PBS to remove collagenase. Finally, the SVF was diluted with normal saline 0.9% to obtain 6 mL of solution containing 90–120 million cells to administer in each knee joint.

2.3. Arthroscopy Microfracture Procedure

Spinal anesthesia for knee arthroscopy was done by using 2 mL (5 mg/mL) bupivacaine hydrochloride. The debris, crystal, and synovitis were removed, and microfracture holes were placed 3–4 mm apart by the arthroscopy microfracture technique, as described by Steadman et al [46]. After arthroscopy, the knee joint was drained for 6 hours, and the drainage tube was withdrawn before the injection of the SVF. The rehabilitation period of the patients under the guidance of a physician included three time points. In the first 6 weeks, walking with crutches, partial weight bearing, and passive motion of the joint up to 90° were allowed. During 6–12 weeks, normal walking in combination with the use of a knee protector and quadriceps and hamstring training were performed. After 12 weeks, balance and core training with unlimited knee joint movement was administered.

2.4. Follow-Up and Evaluation

Patients were monitored in the hospital for one week post-arthroscopy. After this, patients were followed for 24 months. Clinical manifestations such as pain, stiffness, and functional mobility were substantially recorded. Western Ontario and McMaster Universities Arthritis Index (WOMAC) [47], Lysholm [48], and visual analogue scale (VAS) scores were assessed before treatment and at 12 and 24 months after surgery. Magnetic resonance imaging (MRI) was performed before treatment and at 12 and 24 months after treatment. Specifically, the MRI analysis was performed to assess the extent of cartilage damage according to the Modified Outerbridge Classification [49].

2.5. Statistical Analysis

The data are expressed as the mean ± SD. The comparisons between groups were performed by one-way analysis of variance (ANOVA) and *t*-test, using SPSS-22 (IBM, New York, NY, USA), and p values <0.05 were considered statistically significant.

3. Results

3.1. Patient Characteristics

The study was conducted from September 2014 to June 2017 at Van Hanh Hospital, Ho Chi Minh city, Vietnam. The overall schematic is illustrated in Figure 1, which shows that the OA patients were identified on the basis of their clinical and MRI scores, in addition to x-ray-dependent KL grades.

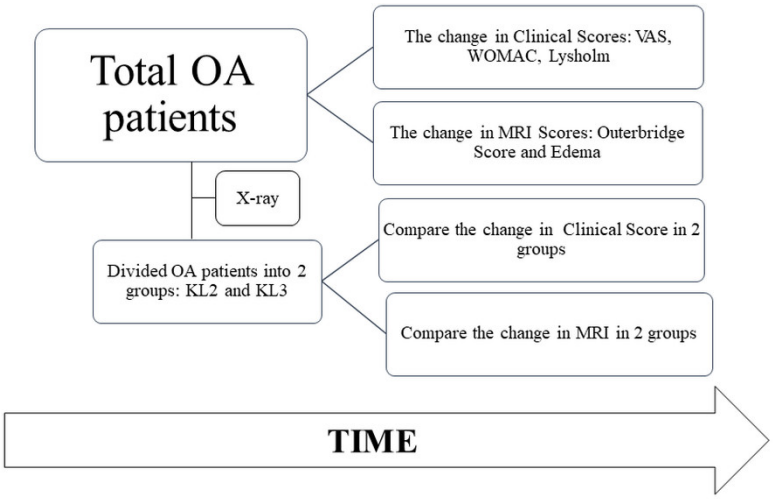

Figure 1. The schematic of the study, which shows that the osteoarthritis (OA) patients were identified on the basis of their clinical and MRI scores, in addition to x-ray-dependent Kellgren–Lawrence (KL) grades. These pateints were further treated with stromal vascular fraction (SVF), and all the outcome scores were assessed after 12 and 24 months.

Eighteen patients who satisfied the exclusive and inclusive criteria were selected to receive the treatment of SVF, a heterogeneous cell population containing mesenchymal progenitor/stem cells, preadipocytes, endothelial cells, pericytes, T cells, and M2 macrophages [50]. The demographic characteristics of the patients are shown in Table 1.

Table 1. Population characteristics of the patients. BMI: Body mass index.

Characteristics	Placebo Group	SVF-Treated Group
Age	58.2 ± 5.70	59 ± 6.04
Sex		
Male	3	5
Female	12	13
BMI		
Normal: Overweight: Obese	9:5:3	11:5:3
KL grades		
KL2	5	4
KL3	10	14

The patients were classified on the basis of their age, gender, BMI, and KL grade (Table 1). In general, the two groups (SVF treatment and placebo) shared quite similar demographic characteristics.

3.2. Changes in VAS and Western Ontario and WOMAC Index after SVF Treatment

VAS is a reliable scale for the assessment of pain in osteoarthritic condition [51], whereas WOMAC includes a questionnaire about pain, stiffness, and inability of conducting activities in daily

life [52]. In both scales, the lower score represents a better functional status of the patient. The effects of the SVF treatment on the VAS and WOMAC scores of KL2 and KL3 patients are represented in Figure 2A,B, respectively. The results revealed that after 12 months, no significant difference was found between the VAS scores of the SVF treatment and placebo groups (5.1 ± 2.5 vs. 4.9 ± 2.4). However, both scores were significantly decreased compared to that before the SVF treatment ($p < 0.05$). Further, as compared to the placebo group, a sharp decreasing trend in the VAS score of the treatment group was observed up to 24 months. The VAS score in the treated group continuously reduced after 12 and 24 months. Specifically, compared to the mean VAS score at 12 months, the score at 24 months was significantly reduced (5.1 ± 1.2 vs. 3.4 ± 1.8, $p < 0.05$). On the contrary, the score of the placebo group after 12 and 24 months increased from 4.9 ± 2 to 5.9 ± 2.47, but this difference was not significant. A similar trend was also observed for the WOMAC score in the placebo group, which was significantly decreased after 12 months of treatment (47.3 ± 17.1 vs. 28.6 ± 12.7, $p < 0.05$). However, a significant increase was observed thereafter at 24 months (36.5 ± 20.3 vs. 28.6 ± 12.7, $p < 0.05$). Meanwhile, the WOMAC score in the treated group decreased sharply after 12 months (44.7 ± 15.4 vs. 16.4 ± 12.1, $p < 0.05$) and further declined significantly to 11.1 ± 11.9 at 24 months (11.1 ± 11.9 vs. 16.4 ± 12.1, $p < 0.05$). Overall, at 24 months, both VAS and WOMAC scores in the placebo and treatment groups diminished compared with the scores before treatment. However, the decreasing trend in the treatment group was larger than in the placebo group, which is indicative of improvement after SVF therapy.

3.3. Changes in Lysholm Score after SVF Treatment

The Lysholm Knee Scale is another recommended measure of knee function [48]. As per Lysholm scale interpretation, a higher score represents better knee function. Before treatment, the Lysholm scores of the placebo and treatment groups showed a significant difference (64.1 ± 10.2, 52.8 ± 13.2; $p < 0.05$) (Figure 2C). The results showed that the score of the placebo group increased to 76.5 ± 12.4 after 12 months; thereafter, a notable decrease was recorded after 24 months (68.3 ± 15.0). However, the overall increase from the value before treatment to that at 24 months in the placebo group was found not to be significant (64.1 ± 10.2 vs. 68.3 ± 15.0). Similarly, the treatment group showed no statistically significant increase in Lysholm score after 24 months, compared to 12 months. However, compared to the value before treatment, this score was significantly increased at 24 months (52.8 ± 13.2 vs. 85.9 ± 9.9, $p < 0.05$), implying an improvement in knee function.

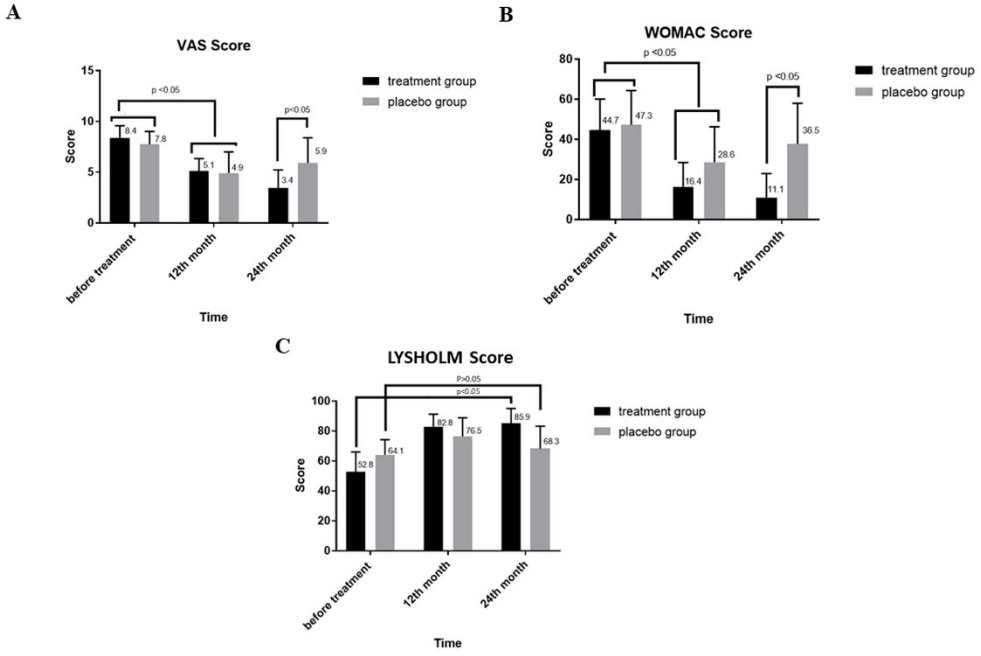

Figure 2. Assessment of clinical outcomes of OA patients treated with SVF at 12 and 24 months. (**A**) Visual analogue scale (VAS) score (**B**) Western Ontario and McMaster Universities Arthritis Index (WOMAC) index, and (**C**) Lysholm score of the SVF-treated group compared to the placebo group.

3.4. MRI-Based Evaluation of Bone Edema and Cartilage Healing

MRI results showed that after 24 months of treatment, bone marrow edema was decreased in both the placebo and the SVF treatment groups; however, the decrease in bone marrow edema in the SVF treatment group was larger (22 mm vs. 8 mm) than in the placebo group (20 mm vs. 12 mm) (Figure 3A). Similarly, the Outbridge score was decreased from 4 (at 0 months) to 3 (at 12 months) and 1 (at 24 months), implying a considerable improvement in cartilage generation in the SVF-treated group (Figure 3B).

3.5. Cartilage Injury Evaluation by MRI-based Outerbridge Score

The level of cartilage injury was measured by the Outerbridge score (OS) [53]. The OS of the study groups were recorded on the basis of MRI examination for assessment of cartilage lesions, particularly, depth of defect (Figure 3C) [54]. In the placebo group, the OS score increased slightly after 12 months (2.7 ± 1.3 vs. 2.9 ± 1.3), and this trend was maintained up to 24 months (3.2 ± 1.1). On the contrary, as compared to the values before treatment, the OS score in the treated group decreased after 12 and 24 months from 3.0 ± 0.8 to 2.7 ± 0.7 and 2.0 ± 0.7, respectively. The OS score pattern initially showed no significant difference between placebo and treatment groups (2.7 ± 1.3 vs. 3.0 ± 0.8); however, after 24 months, a significant difference between the OS scores of the two groups could be observed (3.2 ± 1.1 vs. 2.0 ± 0.7; $p < 0.05$). Taken together, the OS score of the treated group clearly decreased, while that of the placebo group displayed nearly no change.

3.6. Bone Marrow Edema (BME)

BME-like lesions are also associated with the pathogenesis of osteoarthritis and are characterized by histologic abnormalities such as bone marrow necrosis and fibrosis, in addition to trabecular abnormalities [55]. Therefore, MRI was also used to assess BME before and after 12 and 24 months of treatment (Figure 3D). Before the sham treatment, the length of BME in the placebo group was 1.9 ± 0.74 mm; an increase in BME length was observed at 12 and 24 months (2.0 ± 0.53 mm and 2.1 ± 0.64 mm, respectively $p < 0.05$). Interestingly, compared to the placebo, the BME length before SVF treatment (2.4 ± 0.34 mm) was significantly larger than after 12 and 24 months of treatment (1.5 ± 0.5 mm and 0.9 ± 0.73 mm, respectively ($p < 0.05$). On the whole, these results indicate a reduction in the formation of BME-like lesions after SVF treatment.

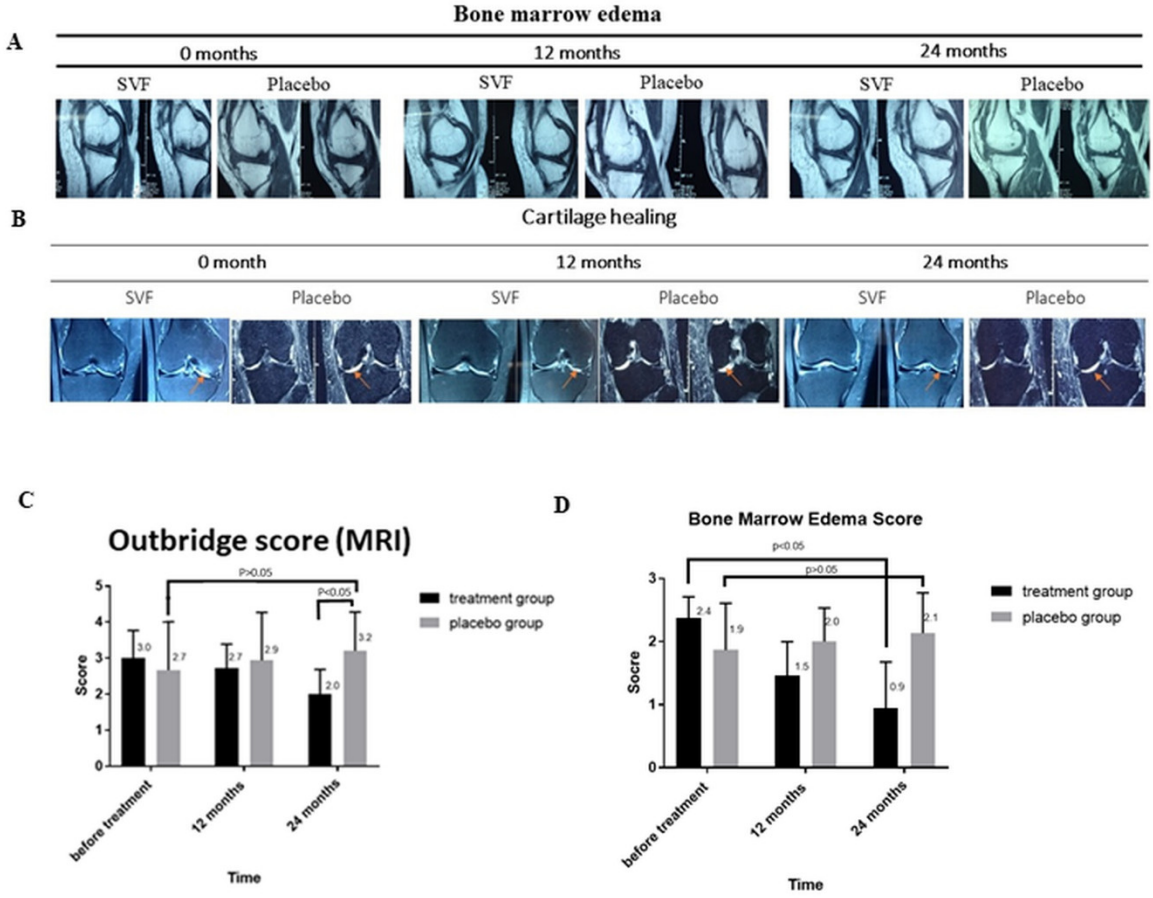

Figure 3. MRI analysis of OA knee-joints after SVF therapy. (**A**) Bone marrow edema (BME) and (**B**) (**B**) Cartilage healing and decrease in bone marrow edema (orange arrow) determined though the Outbridge score (OS) at 0, 12, and 24 month, respectively. (**C**) Cartilage injury evaluation by OS scores indicating the depth of defect in cartilage lesions before treatment and at 12 and 24 months after treatment in placebo and SVF-treated groups. (**D**) Length of BME lesions before and 12 and 24 months after treatment in placebo and treatment groups.

3.7. Comparative Assessment of the VAS Score between KL2 and KL3 Groups

The X-ray image-derived KL grading scale is a gold standard for determining the severity of OA, on the basis of which, the total OA patients were divided into KL2 and KL3 groups [6]. Further, we analyzed the relation between KL grading and VAS score in KL2 and KL3 treatment groups (Figure 4). Before treatment, the VAS score of the KL2 treatment group was 8.50 ± 1.92; it decreased to 4.50 ± 1 after 12 months. Notably, this score further decined to 3.00 ± 2 after 24 months of treatment, indicating a 57.2% decrease in the VAS score. Next, the effect of the placebo on VAS score of KL2 group was assessed. We found no considerable reduction in the VAS score of the KL2 placebo group before and after 24 months of placebo administration. Similarly, a reduction in the VAS score of the KL3 group was also observed post-treatment. Before treatment, the VAS score was 8.36 ± 1.00 and was reduced after 12 and 24 months of treatment to 5.29 ± 1.27 and 3.57 ± 1.79, respectively. This reduction in the VAS score was 64.7% after 24 months compared to the value before treatment. Taken together, the improvement in the pain status of KL3-grade patients was better than for KL2-grade patients.

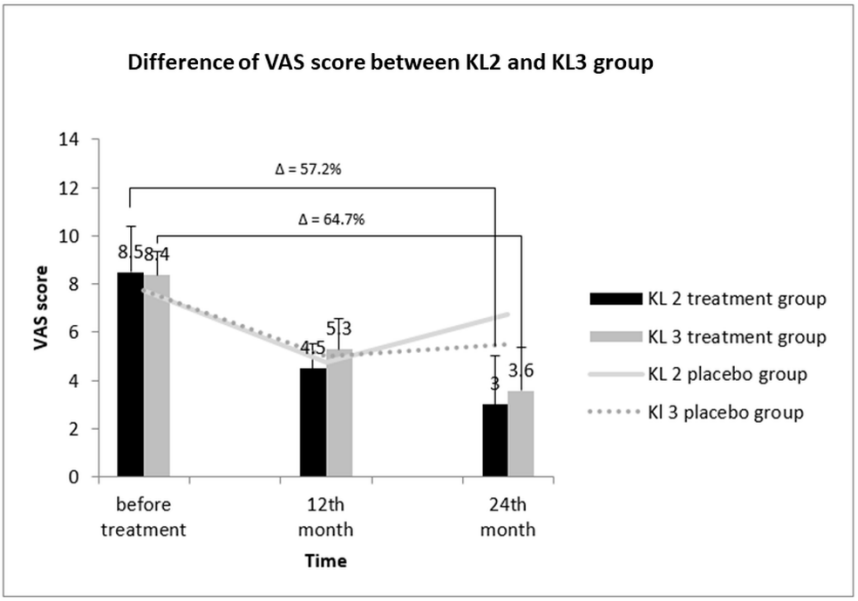

Figure 4. VAS scores of KL-grade 2 and 3 patients in SVF-treated OA groups at 12 and 24 months. After treatment, improvement was noted in patients with KL grade 2 and KL grade 3 (64.7% and 57.2%). Δ: percentage of reduction in VAS score.

3.8. Correlation between WOMAC Score and KL Grades to Determine Treatment Efficacy

Similarly, after treatment of KL2- and KL3-grade patients, differences in the WOMAC scores between the two groups were observed (Figure 5). The WOMAC scores before treatment in KL2 and KL3 patients were 52.00 ± 18.26 and 42.64 ± 14.51, respectively. After 12 and 24 months of treatment, the WOMAC score of the KL2 treatment group revealed a decreasing pattern, being 24.25 ± 19.77 and 18.25 ± 20.07, respectively. Similarly, the WOMAC score of the KL3 treatment group also dropped after 12 and 24 months of treatment to 18.21 ± 8.20 and 9.00 ± 8.46, respectively; however, this decline found to be not significant. Overall, compared with the value before treatment, at 24 months, the percentage of WOMAC score of the KL3 group was reduced with respect to that of the KL2 group (78.9% vs. 64.9%), indicating a greater extent of improvement in the KL3 group.

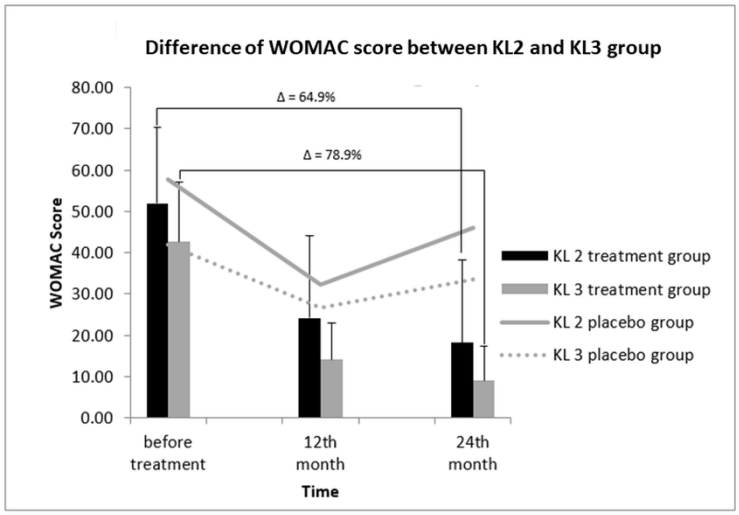

Figure 5. WOMAC scores in KL-grade 2 and 3 patients after SVF therapy at 12 and 24 months. After treatment, the reduction of the WOMAC score in KL-grade 3 patients was comparatively greater than that observed in KL-grade 2 patients (78.9% vs. 68.9%). The WOMAC scores of KL-grade 2 and 3 patients in the placebo group remained constant. Δ: percentage of reduction in WOMAC score.

3.9. Relative KL Grading and Lysholm Score between KL2 and KL3 OA Groups

The impact of KL-OA grades on the Lysholm score is represented in Figure 6. Before treatment, the Lysholm score of the KL2 treatment group was 40.25 ± 11.18; it increased rapidly to 82 ± 9.38 after 12 months of treatment. However, after 24 months, only a marginal increase in the Lysholm score in the KL 2-treated group to 86 ± 10.42 was observed, corresponding to a 33.6% increase compared to the value before treatment (40.25 ± 11.18 vs 86 ± 10.42). The Lysholm score of the KL3 treatment group followed almost a similar pattern as that of the KL2 group. The score before treatment was 56.4 ± 11.66 and increased to 83.1 ± 8.52 after 12 months of treatment, showing an increase of 53.1%. However, a slight increase to 85.0 ± 10.19 after 24 months of treatment was observed. These data showed that the improvement of the KL3 group were greater than that the KL2 group.

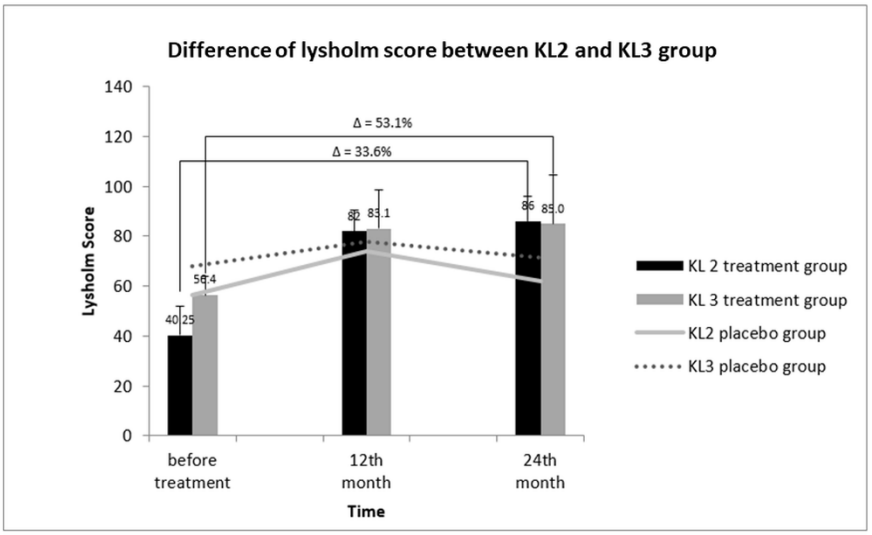

Figure 6. Lysholm scores of KL-grade 2 and 3 patients after SVF therapy at 12 and 24 months. After 24 months of treatment. The increase of the Lysholm score in KL-grade 3 patients was comparatively greater than that in KL-grade 2 patients (33.6%. vs. 53.1%). Δ: percentage of improvement in lysholm score.

3.10. Comparative Outerbridge Score (OS) between KL2 and KL3 Groups

The comparative profile of cartilage injury, as measured by the OS score in KL2 and KL3 patients after treatment, is represented in Figure 7. No significant improvement was observed in the OS of the KL 2 placebo group up to 24 months of treatment when compared to the scores before treatment. Specifically, the OS of the KL2 treatment group before treatment was 3.25 ± 0.55; however, it decreased to 2.58 ± 0.70 after 12 months of treatment and further reduced to 2.0 ± 1.19 after 24 months. The net decrease in OS score after 24 months of treatment was 38.5%. In accordance with the OS score pattern of the KL2 treatment group, the OS score of the KL3 treated group also decreased after 12 and 24 months of treatment to 2.8 ± 0.51 and 2.0 ± 0.61, respectively, compared to the value before treatment of 2.9 ± 0.51. The OS score of the KL3 placebo group showed a linear increase after 24 months of treatment. In contrast to the WOMAC and VAS scores, OS showed no difference in improvement between KL2 and KL3 groups.

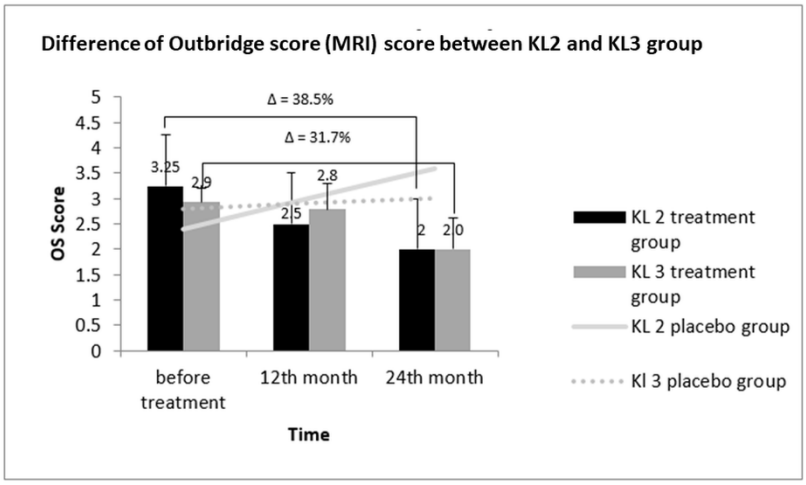

Figure 7. OS in SVF-treated and placebo groups of KL-grade 2 and 3 patients after at 12 and 24 months. After treatment, improvement was noted in KL-grade 2 and KL-grade 3 patients (38.5% and 31.7%). Δ: percentage of reduction in OS score.

4. Discussion

SVF contains a heterogeneous cell population of progenitor cells and ADSCs, which possess enhanced therapeutic potential against immune disorders, degenerative tissue pathologies, and other ischaemic conditions [37]. The complexity of knee OA related to pain, stiffness, muscle atrophy, and ligament damage has made its treatment difficult. Surgical procedures and drugs for controlling pain and inflammation have proven to be inadequate [56]. However, recent developments in regenerative therapy have provided the opportunity to address the bottlenecks associated with OA treatment. Similar to other MSCs, SVFs containing ADSCs are considered a better candidate at par with ADSCs and in some case better than pure ADSCs [35,36]. Therefore, this study assessed the efficacy of SVF treatment in OA therapy. In particular, the VAS, WOMAC, Lysholm, and MRI-based Outerbridge scores were evaluated to assess the improvement in OA status. VAS, WOMAC, and Lysholm score, closely represent the real-time status of OA; therefore, they are precise enough to evaluate the effectiveness of OA treatments [57]. The VAS score is directly measured through questionnaires [58]. The level of pain is established between two extreme points—no pain at all and worst pain imaginable [59]. This scale is simple, reliable, and valid to represent the level of pain [60]. As compared to the placebo group, a considerable reduction in the VAS score of the treatment group was observed after 24 months of treatment, reflecting an improvement of pain. On the contrary, no significant difference between the VAS scores of SVF and placebo groups after 12 months of treatment was found when an arthroscopic procedure was conducted prior to SVF administration. During this process, the inflamed tissues in both the groups were removed, which might have suppressed the pain symptom even in the placebo group, compared to the pain level before treatment. In coherence to our study, the SVF/PRP treatment has also been reported to improve the VAS score of OA patients [58]. A recent clinical study approved by the Japanese Regenerative Medicine Safety Act has documented a 40% decrease in VAS score after SVF treatment [61]. Furthermore, our study demonstrated that the WOMAC score was considerably decreased after 24 months of SVF treatment. These decreases in VAS (Figure 2A) and WOMAC scores (Figure 2B) compared to placebo groups were significant, which indicates improvement in the painful condition of OA patients. Following the pattern of VAS and WOMAC scores, the Lysholm score was also employed to assess the improvement in quality of life and status of instability post-surgery and post-treatment. The current modified Lysholm score is based on eight features, including limp, support, locking, instability, pain, swelling, stair climbing, and squatting [62]. Lysholm is mainly based on the opinion of a patient assessing function and stability of treatment; an increased score indicates improved quality of life. Our study indicates a

significant effect of SVF on the Lysholm score in OA patients 24 months post-treatment as compared to the placebo group. This increase in Lysholm score is an indication of patient relief to therapy. This result is in accordance with previous studies carried out to assess the efficacy of SVF therapy in OA treatment.

Further, the level of cartilage injury was assessed on the MRI-based OS score. An increase in OS score represents a loss of cartilage thickness. In this study, initially there was no significant difference between the OS scores of the treatment and placebo groups; however, a significant decrease in OS score was observed in the treatment group compared to the placebo group after 24 months of treatment ($p < 0.05$). These data establish the role of SVF in improving the BME score which is used as an indicator of knee OA progression and is characterized by increased accumulation of fluid [63]. A significant decrease in the BME score was observed in the SVF-treated group after 24 months of treatment with respect to the placebo which showed increased tendency. The comparison of the BME and OS scores of placebo and treatment groups at the end of 24 months of treatment indicated considerable improvements in the cartilage phenotype, particularly increased thickness.

KL classification is a five-grade scaling system in which the radiographs of eight joints are used to grade knee OA [64]. In this study, KL2- and KL3-grade patients were included to assess the effect of SVF treatment on the OA grade. On the basis of the decrease in WOMAC score and the increase in Lysholm score and considering the static response of the placebo groups during the 24 months of this study, it can be inferred that the SVF treatment was more effective in KL3-grade patients than in KL2-grade patients. The greater improvement of KL3-grade group patients might be attributed to the subjective assessment of the VAS score, WOMAC score, and Lysholm score, whereby patients with a severe condition tend to feel a greater improvement. In contrast, in the case of MRI scores (OS and BME scores) which are based on objective assessment, no differences between the two groups were witnessed. Inflammation plays a central role in pathogenesis of osteoarthritis and significantly contributes to joint pain [65]. Hence, the reduction of pain observed by us is likely to be related to the anti-inflammatory properties of SVF cells. As a corollary, it is also plausible that the better results obtained for KL3 patients, characterized by a higher level of inflammation before treatment compared to KL2 patients, depend on a better and more profitable exploitation of the anti-inflammatory activity of SVF. On the other hand, the degenerative properties of SVF will have the same effect on KL2 and KL3 patients.

The claim of SVF potential in improving clinical scores of OA patients might be attributed to SVF, which is a mixture of ADSCs, endothelial precursor cells (EPCs), endothelial cells (ECs), macrophages, smooth muscle cells, lymphocytes, pericytes, and pre-adipocytes [37,66]. The improvements in the clinical scores might be attributed to immuno-modulator and anti-inflammatory effects of SVF cells, which can lead to tissue remodeling. SVF cells secrete immunosuppressive and anti-inflammatory molecules like IL-10, IL-1, receptor antagonist (IL-1ra), indoleamine 2,3-dioxygenase, transforming growth factor (TGF)-β, and prostaglandin [67]. Further, the anti-fibrotic effect of ASDC might also play a role through the secretion of HGF or adrenomodullin, thereby reducing the fibrotic activity of overexpressed TGF-β1 and its target genes, such as collagen type I, type III, and α-SMA in OA knee [68–70].

Besides these therapeutic activities, the regenerative ability of SVF may be due to ADSCs differentiation potential into chondrocytic and osteocytic cells lineages. EPCs may also induce angiogenesis by releasing growth factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1) [71]. Macrophages and monocytes have been demonstrated to mediate the immune response through secretion of various cytokines [72]. These macrophages are modulated by T regulatory cells, which may possess immunosuppressive characteristics [73]. In a mouse model, the pericytes found in SVF were able to regenerate the muscle tissue [74], which indicates their therapeutic potential role in knee joint. Eventually, stromal cells can secrete extracellular matrix components which improve cellular adhesion, migration, cell–matrix interactions, and regeneration [75,76]. To our knowledge, this is the first study reporting time- and KL grade-dependent changes of intra-articularly transplanted SVF in osteoarthritic patients over a period of two years. The main limitation of this study is the small sample size. However, even a small

sample might have some valid scientific merit with cost effectiveness [77,78]: on the basis of it we have inferred SVF-mediated therapeutic clinical outcomes in this study. To overcome this limitation, this study will be extended to a larger population and conducted for a longer time.

5. Conclusions

On the basis of the improvements observed in treated patients during follow-up and the behavior of the placebo group, our study revealed a trend toward a better efficacy of SVF with the microfracture method for OA treatment over a period of two years. We also inferred that the SVF therapy is more effective in KL 3-grade OA patients compared with KL 2-grade OA patients.

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Research Article

The Effect of Autologous Adipose-Derived Stromal Vascular Fractions on Cartilage Regeneration Was Quantitatively Evaluated Based on the 3D-FS-SPGR Sequence: A Clinical Trial Study

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Background. Numerous reports confirmed the safety and clinical efficacy of autologous adipose-derived stromal vascular fractions (SVF), which have recently been used to treat osteoarthritis (OA). However, there is still no consensus as to whether SVF can promote cartilage regeneration. Herein, the purpose of our study was to evaluate the effectiveness of SVF versus hyaluronic acid (HA) in cartilage regeneration by establishing a cartilage model based on the three-dimensional fat-suppressed spoiled gradient recalled echo (3D-FS-SPGR) sequence. *Methods*. Patients with symptomatic OA were recruited in our research, who were randomized into two groups. Meanwhile, patients in Kellgren-Lawrence (K-L) grades 2 and 3 were distinguished in each group. In the test group, patients received SVF injections of the knee, while patients in the control group received the same dose of HA. Each patient underwent the 3D-FS-SPGR sequence to establish a cartilage model at baseline, 6 months, and 12 months, respectively. The cartilage was characterized into six regions, and relevant parameters of the cartilage model were counted. Clinical and radiographic scores were recorded in one-year follow-up. *Results*. In all regions, the thickness and volume of cartilage defect and the volume of healthy cartilage were improved to some extent in the test group, especially the medial femoral condyle (MF) and medial tibial condyle (MT). In grades 2 and 3, the thickness and volume of cartilage defect decreased by 0.92 ± 0.18 mm and 1.03 ± 0.23 mm and 84.00 ± 32.30 mm^3 and 130.30 ± 49.56 mm^3 in MF and by 0.96 ± 0.22 mm and 0.99 ± 0.14 mm and 64.18 ± 21.40 mm^3 and 95.11 ± 19.93 mm^3 in MT, respectively. No such phenomenon was observed in the control group. Meanwhile, the SVF-treated knees showed significant improvement in clinical and radiographic scores at 12 months. Nevertheless, these scores of the control group became worse at 12-month follow-up visit. *Conclusion*. Taken together, this study shows that intra-articular injection of SVF markedly improved the clinical symptoms without adverse events, thereby repairing the damaged articular cartilage through cartilage regeneration.

1. Background

Osteoarthritis (OA) is a common chronic disease of the joints, which is characterized by osteophyte formation, changes to the subchondral bone, degeneration of ligaments and menisci, pain, stiffness, and loss of joint function [1, 2]. Several studies have established that knee OA is a highly prevalent form of arthritis that contributes to arthralgia and disability, especially in elderly people [3].

To date, more than 50 therapies of pharmacological, nonpharmacological, and surgical approach have been documented by scholars. Intra-articular injection of hyaluronic acid (HA) is effective in improving symptoms and slowing the progression of OA, but they do not mention the

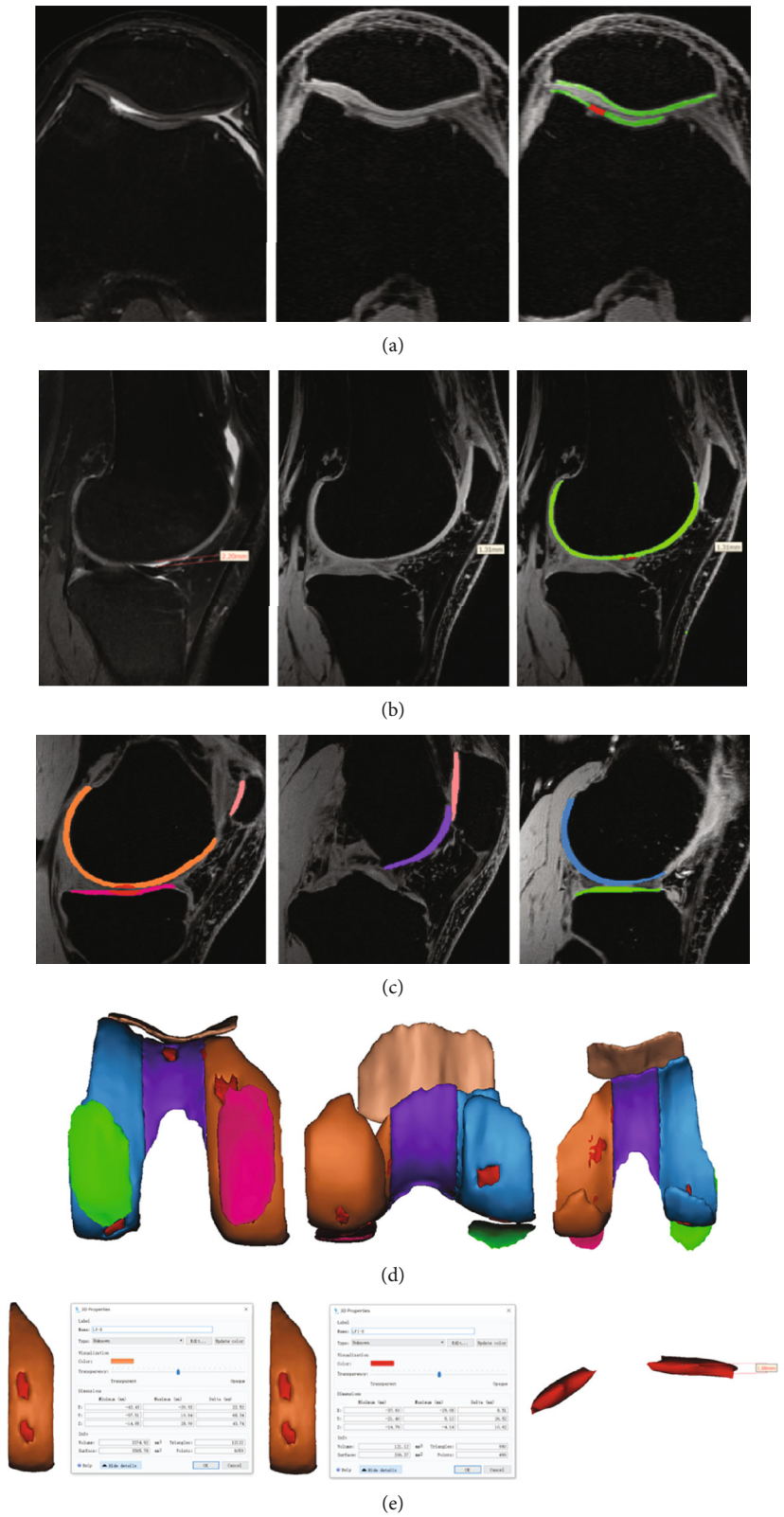

FIGURE 1: The process of establishing the cartilage model. Different color masks used to distinguish healthy cartilage from cartilage defects by setting the threshold are shown. Illustrating the injury of the whole layer of cartilage and partial cartilage defects (a, b). The cartilage of the knee joint was divided into six regions with different color masks, namely, lateral femoral condyle (LF), femoral intercondylar (T), medial femoral condyle (MF), lateral tibia condyle (LT), medial tibia condyle (MT), and patella (P), and the knee cartilage model was established (c, d). The parameters of the model were measured (e) (for example, in the lateral femoral condyle, the thickness, volume, and surface of cartilage defect were 1.88 mm, 121.12 mm^3, and 206.37 mm^2, respectively. The volume of healthy cartilage was 3374.92 mm^3).

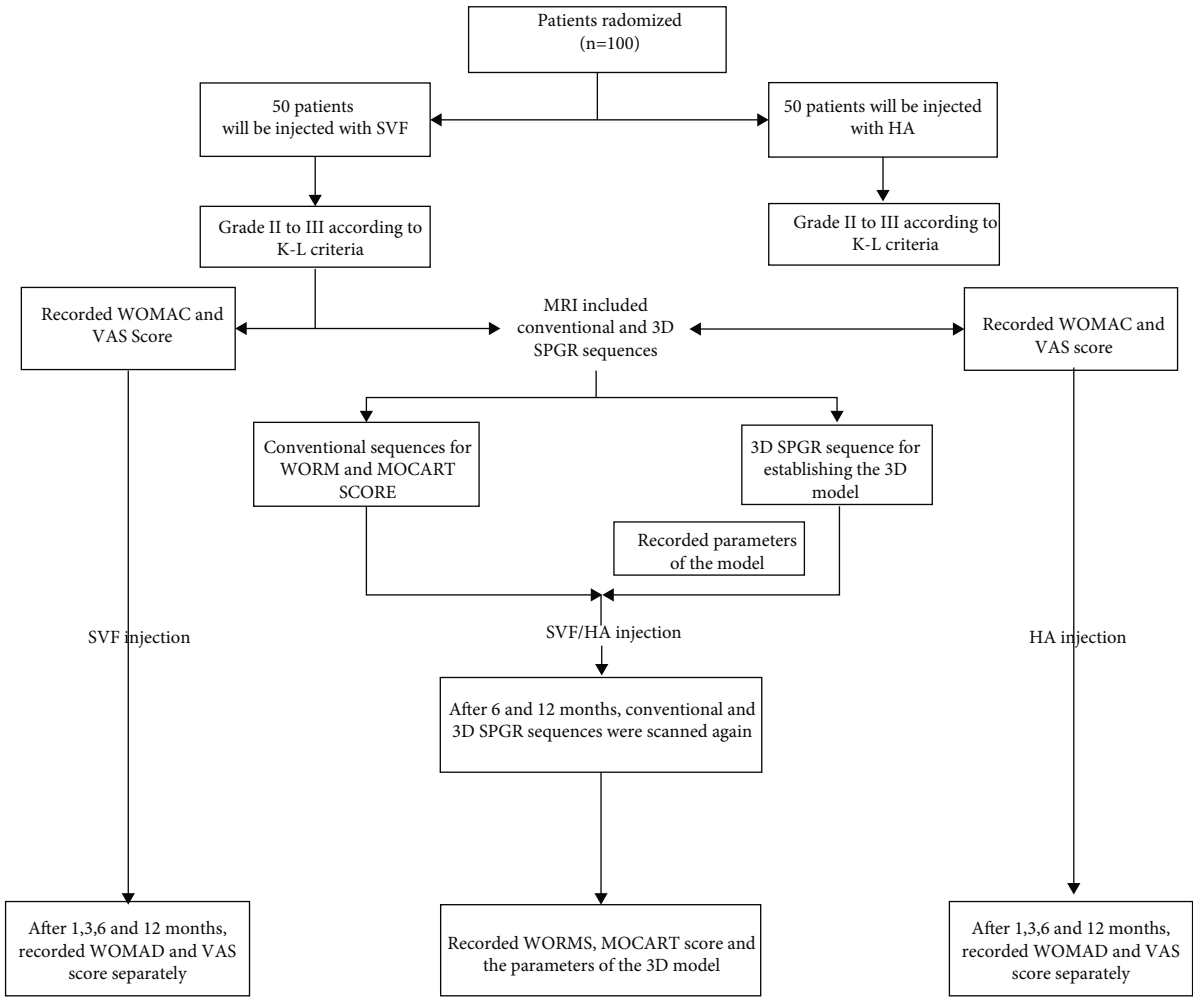

Figure 2: Flowchart of the clinical trial.

Table 1: Study participant demographic characteristics.

Characteristics	Test group (knee treated with SVF)	Control group (knee treated with HA)
Age (years)	50.83 ± 10.88	52.87 ± 9.35
Sex (M/F), *n* (%)	18/29 (38%/62%)	20/28 (42%/58%)
Knee (R/L), *n* (%)	30/23 (57%/43%)	21/30 (41%/59%)
BMI (kg/m^2)	22.67 ± 3.68	23.58 ± 4.19
K-L classification (%)		
I	0 (0%)	0 (0%)
II	29 (55%)	27 (53%)
III	24 (45%)	24 (47%)
IV	0 (0%)	0 (0%)

degeneration and regeneration of articular cartilage [4]. Therefore, most patients cannot inevitably avoid taking the road of total knee arthroplasty (TKA) in the end [5, 6]. In this respect, it is therefore of great significance to find a new and effective therapy for alleviating the clinical symptoms of OA and preventing the degeneration of articular cartilage.

Since the discovery of the multipotent stem cell population in adipose tissue by Zuk et al., cell-based regenerative therapy has gradually become a possible method for cartilage regeneration [7]. Recent related studies have also confirmed that mesenchymal stem cells (MSCs) and adipose-derived stem cells (ADSCs) possess the potential to differentiate into chondrocytes. However, MSCs and ADSCs need to take several weeks in specialized laboratory for cell isolation and expansion, which will increase the economic burden of the patients [8, 9]. Some scholars have proposed a more effective method to collect and manage ADSCs using stromal vascular fraction (SVF) [10]. Furthermore, adipose-derived SVF comprises numerous regenerative cells, such as ADSCs, blood cells, pericytes, fibroblasts, macrophages, smooth muscle cells, endothelial cells, and their precursors. It also exhibits the benefits of easy isolation and use without culturing or differentiation, consequently responding to the local environment of OA by some inflammatory factors [11–13]. Multiple recent reports have proven that the use of intra-articular SVF injections can effectively relieve the clinical symptoms of patients [14–17]. Nonetheless, despite these intriguing results, it remains unclear whether the SVF injections can promote regeneration of the articular cartilage, requiring further exploration. Inconsistent findings

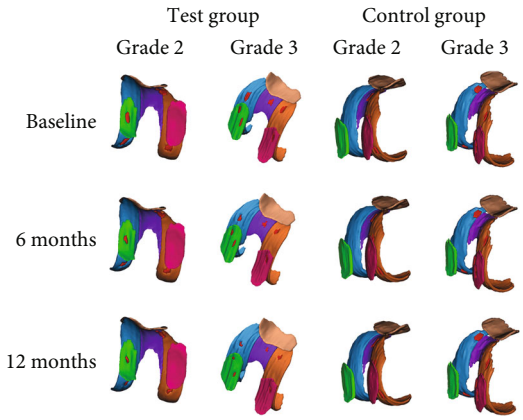

Figure 3: Cartilage model of the SVF- and HA-treated knee established at baseline and 6 and 12 months. The cartilage defect of the SVF-treated knee with K-L grade 2 and 3 OA showed good repair; the cartilage defect of the HA-treated knee with K-L grade 2 and 3 OA showed no improvement.

have been reported regarding this topic, whereby some studies reported evidence of cartilage tissue regeneration, while others claimed that no change is observed [18–21].

We thus designed a clinical trial about autologous adipose-derived SVF versus HA in the treatment of patients with knee OA Kellgren-Lawrence (K-L) grades 2 and 3 [22]. This study sought to establish a three-dimensional (3D) cartilage model by using a special sequence to quantitatively examine the effect of SVF and HA on cartilage regeneration.

2. Materials and Methods

2.1. Patients and Study Design. The trial was registered at the Chinese Clinical Trial Registry (ChiCTR2100042930). All experimental protocols used in this study were approved by the Ethics Committees of Zhejiang Provincial People's Hospital. Patients enrolled in this study provided signed written informed consent. This was a prospective double-blinded randomized study conducted at a single center. Eligible patients included were aged between 18 and 70 years, with OA K-L grades 2 and 3, exhibiting substantial pain and loss of function, failure of conservative therapy, and had an initial pain evaluated at four or greater on a ten-point visual analog scale (VAS) in the knee joint. On the other hand, exclusion criteria are comprised of secondary arthritis (for example, secondary knee OA, rheumatoid arthritis, gouty arthritis, and previous articular fractures), having problem with anesthesia (according to the American Society of Anesthesiologists score), contraindicating MRI examination, other causes of knee pain such as diffuse edema, meniscus tear, and others, a history of liposarcoma and other cancers, intra-articular injection of hyaluronic acid or other drugs in the preceding 3 months, end-stage OA, patients with recent surgery, abdominal hernia, and coagulopathy.

The complete randomization process was accomplished by an assistant accountant who was blinded to the patients' data using SPSS 20.0 software (version 20.0, IBM Corporation, NY, US). First, we listed 1–100 serial numbers (patient serial number) in accordance with the outpatient order. Second, 100 random numbers were generated by Rv.Uniform (0, 1) and matched number by number with 100 patients' serial numbers. Finally, the 100 random numbers were arrayed in ascending order; the corresponding patients of the first fifty random numbers were injected with 4 ml SVF and 4 ml hyaluronic acid (SOFAST, Freda, china) in the last 50 random numbers.

To evaluate the grade of OA, an initial X-ray image was used following the K-L criteria, and subsequently, patients belonging to grades 2 and 3 were selected. Afterward, patients who underwent MRI included conventional and three-dimensional fat-suppressed spoiled gradient recalled echo (3D-FS-SPGR) sequences; the radiologist is not informed of the patient's treatment. According to the conventional sequence, the whole-organ magnetic resonance imaging score (WORMS) was recorded to evaluate the knee, and magnetic resonance observation of cartilage repair tissue (MOCART) was recorded to assess the cartilage repair tissue. While the 3D-FS-SPGR sequence was employed to build the 3D cartilage model and measure the related parameters, the visual analog scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire were used to evaluate the pain and function of the patient. We also examined the range of motion (ROM) during the follow-up period.

2.2. Establishment of the 3D Cartilage Model. The MRI scanning was performed on a clinical 3.0 T system (GE Healthcare, Waukesha, WI, USA), including the 3D-FS-SPGR and conventional sequence (TE: 34.5 ms; TR: 2000 ms; the number of excitations: 2; FOV: 16 × 16 cm; slice thickness: 4 mm; interslice gaps: 5 mm; coil: knee coil; the total scan time: 180 s; acquired slices: 21 slices; and flip angle: 0°). Using the 3D-FS-SPGR sequence, each patient was examined before SVF injection. Acquisition parameters for the 3D-FS-SPGR sequence were as follows: TE: 3 ms; TR: 14.6 ms; acquisition matrix: 512 × 512; the number of excitations: 2; FOV: 16 × 16 cm; slice thickness: 0.6 mm; interslice gaps: 0 mm; receiver BW: ±41.7 kHz; coil: knee coil; total scan time: 1220 s; acquired slices: 276 contiguous slices; flip angle: 0°; and plane resolution: 0.60 mm × 0.60 mm [23].

To build the 3D cartilage model, the original data of the 3D-FS-SPGR sequence was converted to Digital Imaging and Communications in Medicine (DICOM) format and transferred into the Mimics 20.0 software (Materialise, Leuven, Belgium). First, all layers of cartilage defects were detected using 3D-FS-SPGR and conventional sequences. An appropriate segmentation threshold (1849-3445 GV, the segmentation threshold was determined by the cartilage to be segmented) was set for retaining the healthy cartilage of the knee joint, saving the results as the green mask. Following this, the cartilage defect was segmented by another mask, then saving it as a red mask. The healthy cartilage and cartilage defects of the knee joint are segmented by the use of different masks. After the layer-by-layer hierarchical image processing, the cartilage model was characterized into six regions, namely, medial femoral condyle (MF), lateral

Table 2: The changes of the cartilage model in the test group.

	Volume of defective cartilage (mm^3)	p value	Size of defective cartilage (mm^2)	p value	Volume of healthy cartilage (mm^3)	p value	Thickness of defective cartilage (mm)	p value
Grade 2								
MF								
Baseline	173.82 ± 63.41		353.86 ± 122.99		3102.37 ± 435.02		1.53 ± 0.23	
6 months	123.13 ± 46.87	<0.001	257.17 ± 95.64	<0.001	3231.87 ± 451.13	0.279	1.16 ± 0.20	<0.001
12 months	84.00 ± 32.30	<0.001	182.22 ± 67.00	<0.001	3317.69 ± 447.02	0.073	0.92 ± 0.18	<0.001
LF								
Baseline	146.10 ± 61.17		302.77 ± 101.75		3070.04 ± 428.12		1.46 ± 0.30	
6 months	116.49 ± 51.34	<0.05	244.22 ± 96.33	<0.05	3116.65 ± 422.88	0.557	1.25 ± 0.27	<0.05
12 months	94.73 ± 45.55	<0.001	199.93 ± 86.07	<0.001	3179.09 ± 426.00	0.343	1.17 ± 0.26	<0.001
T								
Baseline	147.91 ± 61.35		309.72 ± 99.22		2568.48 ± 406.67		1.45 ± 0.25	
6 months	127.76 ± 57.33	0.318	262.86 ± 97.90	0.172	2617.60 ± 408.53	0.645	1.34 ± 0.23	0.153
12 months	112.80 ± 56.09	0.085	222.52 ± 98.57	<0.05	2658.51 ± 410.85	0.412	1.25 ± 0.21	<0.05
MT								
Baseline	139.72 ± 46.15		281.79 ± 80.48		1647.92 ± 200.24		1.43 ± 0.26	
6 months	95.43 ± 31.56	<0.001	206.20 ± 63.30	<0.001	1720.68 ± 197.61	0.178	1.15 ± 0.23	<0.001
12 months	64.18 ± 21.40	<0.001	146.15 ± 45.47	<0.001	1783.31 ± 202.94	<0.05	0.96 ± 0.22	<0.001
LT								
Baseline	119.87 ± 32.51		256.78 ± 64.51		1613.65 ± 147.04		1.34 ± 0.19	
6 months	101.62 ± 30.18	0.055	209.44 ± 56.13	<0.05	1656.77 ± 150.76	0.284	1.22 ± 0.19	<0.05
12 months	88.66 ± 28.04	<0.05	178.79 ± 54.55	<0.001	1694.24 ± 150.56	<0.05	1.13 ± 0.18	<0.001
P								
Baseline	137.29 ± 53.30		292.45 ± 106.74		2304.81 ± 181.21		1.29 ± 0.19	
6 months	117.78 ± 46.70	0.347	247.55 ± 89.12	0.268	2354.98 ± 182.95	0.304	1.10 ± 0.16	<0.05
12 months	102.15 ± 43.47	0.095	213.88 ± 82.64	0.057	2394.72 ± 180.11	0.067	1.01 ± 0.15	<0.001
Grade 3								
MF								
Baseline	278.10 ± 110.58		525.43 ± 167.38		2382.20 ± 314.39		1.72 ± 0.32	
6 months	198.80 ± 79.19	<0.05	408.84 ± 144.89	<0.05	2540.67 ± 323.21	0.105	1.34 ± 0.25	<0.001
12 months	130.30 ± 49.56	<0.001	286.18 ± 108.47	<0.001	2712.22 ± 343.55	<0.05	1.03 ± 0.23	<0.001
LF								
Baseline	229.23 ± 94.05		459.71 ± 176.88		2379.37 ± 235.44		1.74 ± 0.28	
6 months	190.17 ± 79.75	0.111	390.81 ± 153.97	0.144	2472.52 ± 270.39	0.241	1.53 ± 0.25	<0.05
12 months	162.17 ± 70.92	<0.05	339.47 ± 144.43	<0.05	2562.15 ± 276.73	<0.05	1.36 ± 0.23	<0.001
T								
Baseline	196.75 ± 77.85		410.31 ± 152.60		2190.18 ± 198.06		1.55 ± 0.30	
6 months	166.80 ± 69.83	0.179	352.21 ± 139.03	0.189	2261.72 ± 210.30	0.256	1.34 ± 0.28	<0.05
12 months	141.78 ± 59.94	<0.05	304.62 ± 121.47	<0.05	2323.74 ± 226.45	<0.05	1.19 ± 0.27	<0.001
MT								
Baseline	200.96 ± 48.48		410.59 ± 88.53		1350.22 ± 113.84		1.62 ± 0.21	
6 months	135.99 ± 26.49	<0.001	290.12 ± 51.28	<0.001	1477.44 ± 94.51	<0.001	1.27 ± 0.19	<0.001
12 months	95.11 ± 19.93	<0.001	208.12 ± 42.70	<0.001	1596.10 ± 96.12	<0.001	0.99 ± 0.14	<0.001

Table 2: Continued.

	Volume of defective cartilage (mm^3)	p value	Size of defective cartilage (mm^2)	p value	Volume of healthy cartilage (mm^3)	p value	Thickness of defective cartilage (mm)	p value
LT								
Baseline	154.40 ± 48.17		333.83 ± 98.97		1384.14 ± 92.13		1.47 ± 0.27	
6 months	131.21 ± 44.61	0.087	283.62 ± 89.28	0.070	1438.02 ± 94.16	0.058	1.31 ± 0.24	0.030
12 months	110.57 ± 39.86	<0.05	238.78 ± 81.67	<0.001	1473.00 ± 97.45	<0.05	1.16 ± 0.23	<0.001
P								
Baseline	140.84 ± 56.97		320.57 ± 112.90		1686.92 ± 117.79		1.41 ± 0.20	
6 months	117.97 ± 49.49	0.126	250.71 ± 100.31	0.083	1771.54 ± 112.93	0.016	1.23 ± 0.19	<0.05
12 months	98.75 ± 42.84	<0.05	209.57 ± 84.85	<0.05	1847.87 ± 117.22	<0.001	1.09 ± 0.19	<0.001

femoral condyle (LF), femoral intercondylar (T), medial tibia condyle (MT), lateral tibia condyle (LT), and patella (P) [24]. Different color masks represented different areas, while cartilage defects were represented by red masks (Figures 1(a) and 1(b)). Then, the cartilage tissue for each layer was preserved, the contours of knee cartilage were calculated, and the cartilage model of each region was established (Figures 1(c) and 1(d)). The volume of healthy cartilage, as well as the volume, surface, and thickness of cartilage defects, was measured by the same professional surveyor (Figure 1(e)), and the professional surveyor was unaware of the patient's information. After one week, the cartilage model was reestablished, and the above-mentioned data were measured and averaged.

2.3. Clinical and Radiological Evaluation. The VAS and WOMAC questionnaires were used for the evaluation of pain and functional limitation. The WOMAC score includes pain (five items, score range 0-20), stiffness (two items, score range 0-8), and physical function (seventeen items, score range 0-68), with a total score ranging from 0 (best health) to 96 (worst health). The total score of VAS ranged from 0 (best) to 10 (worst). Additionally, we recorded the ROM of the knee joint during the follow-up. Finally, we assessed the safety of SVF and HA by analyzing the incidence rate of adverse events (AE) and serious adverse events (SAE).

In order to minimize the influence of knee joint loading on the results of MRI, patients were required to rest for 30 minutes before examination. We employed the MOCART score to examine the cartilage repair, while the WORMS was used for the assessment of the knee [25, 26].

2.4. SVF Isolation and Injection. For this experiment, patients were not allowed to take aspirin, thrombolytic or antiplatelet medication, corticosteroids, and nonsteroidal anti-inflammatory drugs within one week before liposuction. Also, they all fasted for liquids and solids at least six hours before the operation. The operation was performed by the same skilled plastic surgeon who was blinded to patient information. After disinfection of the abdomen, the surgeon made two small incisions around the umbilical cord and obtained 100 to 150 ml of adipose tissue from the subcutaneous tissue using the superwet technique. Briefly, lipoaspirates were washed with phosphate-buffered saline, while the mesh filter was applied to remove containing residual blood cells and tissue fragments. Next, an equal volume of digestive enzyme (type I collagenase with the concentration of 5%; Worthington, Lakewood, NJ, USA) was mixed with the washed adipose tissue and placed in a shaking incubator at 37°C for 30 minutes. The resulting mixture was then centrifuged at a rate of 1000 g for 10 minutes, and subsequently, the supernatant (Eppendorf 5810R, Germany) was discarded. After this, the remnant SVF at the bottom was resuspended in phosphate-buffered saline (PBS) up to a volume of 4.5 ml SVF, whereas an automatic cell counter (Countstar IC1000, China) was used to quantify cell quantity and viability.

In short, about 4 ml of SVF suspension was injected into the region of the cartilage defect by a trained experienced orthopedic surgeon who was blinded to patient information. The patient was supine, the knee joint was straightened, and the intersection of the upper edge of the patella and the outer edges of the patella were the location of injection. The injection was performed diagonally to the center of the patellofemoral joint at an angle of 45°. Upon the injection of SVF, subcuticular suture and pressure dressing were performed. All the operations were performed by the same experienced orthopedic surgeon.

2.5. Statistical Analysis. Changes in all follow-up data were determined using a paired t-test. The discrete data were analyzed by the chi-square test. The value of $p < 0.05$ was considered statistically significant. Data displayed in the graphs are means with standard deviation. All statistical data analyses were executed using SPSS software (version 20.0, IBM Corporation, NY, US).

3. Results

3.1. Patient Characteristics and Safety. From January 2018 to May 2021, the 95 patients who satisfied the standard were divided into two groups (Figure 2). The patients' characteristics showed no significant difference in age, gender distribution, BMI, and K-L grade between the two groups (Table 1). Finally, 47 patients (53 knees) with OA received an intra-articular injection of SVF, and 48 patients (51 knees) received HA. During the follow-up period, no serious AE (infection, allergy, and poor wound healing) happened.

Figure 4: Cartilage model of the SVF-treated knee established at baseline and 6 and 12 months. The cartilage defect of the knee joint with OA K-L grade 2 showed good repair (a). Change of cartilage defects in the LF (b), T (c), MF (d), LT (e), MT (f), and P (g) after injection.

The most common AE were pain and swelling of the knee, which occurred in 21 patients (22.11%). After treatment with anti-inflammatory and analgesic drugs, the pain and swelling of all knees were relieved in two weeks. These patients will still be enrolled in clinical trials as long as they do not develop complications such as infections and allergy.

3.2. Changes in Parameters of the 3D Cartilage Model. To establish the 3D cartilage model, all patients finished the examination of the 3D-FS-SPGR sequence at baseline and 6 and 12 months (Figure 3). In the test group, the parameters of 3D cartilage model improved in both patients with OA K-L grades 2 and 3 (Table 2). In grade 2, the thickness of cartilage defect decreased from 1.53 ± 0.23 mm to 0.92 ± 0.18 mm in MF (40% decrease; $p < 0.001$); from 1.46 ± 0.30 mm to 1.17 ± 0.26 in LF (20% decrease; $p < 0.001$); from 1.45 ± 0.25 mm to 1.25 ± 0.21 mm in T (14% decrease; $p < 0.05$); from 1.43 ± 0.26 mm to 0.96 ± 0.22 mm in MT (33% decrease; $p < 0.001$); from 1.34 ± 0.19 mm to 1.13 ± 0.18 mm in LT (16% decrease; $p < 0.001$); and from 1.29 ± 0.19 mm to 1.01 ± 0.15 mm in P (22% decrease; $p < 0.001$). The volume of cartilage defect decreased by 84.00 ± 32.30 mm^3 in MF (52% decrease; $p < 0.001$); by 94.73 ± 45.55 m m^3 in LF (35% decrease; $p < 0.001$); by 64.18 ± 21.40 mm^3 in MT (54% decrease; $p < 0.001$); and by 88.66 ± 28.04 m m^3 in LT (26% decrease; $p < 0.001$), but not in T and P, from 147.91 ± 61.35 mm^3 to 112.80 ± 56.09 mm^3 in T (24% decrease; $p = 0.085$) and from 137.29 ± 53.30 mm^3 to 102.15 ± 43.47 mm^3 in P (26% decrease; $p = 0.095$). We further found that the surface of cartilage defect decreased in MF, LF, T, MT, and LT, showing a statistically significant difference. Nevertheless, we observed no statistical difference in P (27% decrease; $p = 0.057$). As for the healthy cartilage, we generally identified no significant difference, except for MT and LT. Subsequently, we noted that the volume of healthy cartilage increased from 1647.92 ± 200.24 mm^3 to 1783.31 ± 202.94 mm^3 and from 1613.65 ± 147.04 mm^3 to 1694.24 ± 150.56 mm^3 in MT (8% increase; $p < 0.05$) and LT (5% increase; $p < 0.05$), respectively (Figure 4).

Figure 5: Cartilage model of the SVF-treated knee established at baseline and 6 and 12 months. The cartilage defect of the knee joint with OA K-L grade 3 showed good repair (a). Change of cartilage defects in the LF (b), T (c), MF (d), LT (e), MT (f), and P (g) after injection.

Similar to grade 2 OA, the thickness of cartilage defect reduced in MF, LF, T, MT, LT, and P, indicating a significant difference in grade 3 (Figure 5). The volume of cartilage defect decreased from 278.10 ± 110.58 mm^3 to 130.30 ± 49.56 mm^3 in MF (53% decrease; $p < 0.001$); from 229.23 ± 94.05 mm^3 to 162.17 ± 70.92 mm^3 in LF (29% decrease; $p < 0.001$); from 196.75 ± 77.85 mm^3 to 141.78 ± 59.94 m m^3 in T (28% decrease; $p < 0.05$); from 200.96 ± 48.48 mm^3 to 95.11 ± 19.93 mm^3 in MT (53% decrease; $p < 0.001$); from 154.40 ± 48.17 mm^3 to 110.57 ± 39.8 mm^3 in LT (28% decrease; $p < 0.05$); and from 140.84 ± 56.97 mm^3 to 98.75 ± 42.84 mm^3 in P (30% decrease; $p < 0.05$). The surface of cartilage defect decreased from 525.43 ± 167.38 mm^2 to 286.18 ± 108.47 mm^2 and from 410.59 ± 88.53 mm^2 to

Table 3: The changes of the cartilage model in the control group.

	Volume of defective cartilage (mm^3)	p value	Size of defective cartilage (mm^2)	p value	Volume of healthy cartilage (mm^3)	p value	Thickness of defective cartilage (mm)	p value
Grade 2								
MF								
Baseline	183.82 ± 48.24		356.83 ± 91.08		3164.07 ± 411.84		1.63 ± 0.24	
6 months	197.64 ± 48.89	0.374	382.42 ± 92.72	0.384	3139.72 ± 412.82	0.829	1.78 ± 0.24	0.04
12 months	209.02 ± 48.30	0.114	402.29 ± 91.64	0.124	3107.26 ± 413.46	0.615	1.90 ± 0.23	0.001
LF								
Baseline	140.82 ± 43.70		275.08 ± 84.19		3077.84 ± 431.44		1.52 ± 0.29	
6 months	148.13 ± 42.87	0.545	291.72 ± 82.19	0.474	3067.12 ± 426.18	0.927	1.57 ± 0.32	0.588
12 months	154.00 ± 43.56	0.281	308.61 ± 82.01	0.152	3044.24 ± 430.33	0.776	1.60 ± 0.33	0.361
T								
Baseline	137.32 ± 59.12		279.60 ± 121.65		2607.93 ± 504.48		1.37 ± 0.21	
6 months	151.97 ± 63.42	0.318	298.40 ± 122.40	0.676	2589.15 ± 500.17	0.891	1.40 ± 0.22	0.518
12 months	165.57 ± 66.04	0.085	308.89 ± 119.60	0.512	2573.91 ± 501.18	0.805	1.44 ± 0.22	0.227
MT								
Baseline	133.01 ± 35.21		257.93 ± 59.75		1680.74 ± 196.00		1.49 ± 0.40	
6 months	142.19 ± 33.79	0.418	287.87 ± 58.95	0.129	1650.56 ± 190.03	0.568	1.58 ± 0.40	0.509
12 months	154.45 ± 37.19	0.076	318.45 ± 58.71	0.003	1618.74 ± 193.26	0.247	1.64 ± 0.41	0.272
LT								
Baseline	129.20 ± 38.74		255.47 ± 74.88		1672.37 ± 192.72		1.39 ± 0.27	
6 months	137.11 ± 39.48	0.553	270.05 ± 76.14	0.566	1651.50 ± 193.01	0.693	1.43 ± 0.27	0.603
12 months	142.37 ± 39.00	0.320	286.76 ± 74.68	0.218	1629.21 ± 188.67	0.409	1.46 ± 0.28	0.399
P								
Baseline	139.49 ± 36.09		277.21 ± 61.16		2332.80 ± 220.41		1.30 ± 0.17	
6 months	148.49 ± 36.94	0.589	293.81 ± 64.78	0.572	2307.06 ± 221.86	0.671	1.35 ± 0.16	0.463
12 months	158.32 ± 37.93	0.270	307.47 ± 62.35	0.299	2286.39 ± 219.81	0.442	1.40 ± 0.17	0.169
Grade 3								
MF								
Baseline	267.43 ± 73.34		480.77 ± 131.81		2351.03 ± 235.53		1.60 ± 0.37	
6 months	286.20 ± 77.66	0.406	512.16 ± 135.12	0.486	2317.02 ± 239.61	0.622	1.70 ± 0.36	0.401
12 months	306.14 ± 76.03	0.100	542.38 ± 136.31	0.177	2291.33 ± 241.71	0.391	1.77 ± 0.35	0.123
LF								
Baseline	240.85 ± 96.23		477.24 ± 187.46		2421.01 ± 324.67		1.73 ± 0.26	
6 months	256.56 ± 97.23	0.629	503.78 ± 187.78	0.674	2388.35 ± 318.51	0.727	1.82 ± 0.26	0.300
12 months	264.44 ± 105.07	0.788	530.49 ± 189.86	0.710	2363.33 ± 322.17	0.540	1.90 ± 0.25	0.109
T								
Baseline	214.74 ± 75.26		421.14 ± 148.53		2289.15 ± 308.65		1.51 ± 0.37	
6 months	233.95 ± 77.94	0.529	451.43 ± 145.69	0.604	2247.38 ± 310.62	0.637	1.62 ± 0.37	0.459
12 months	251.24 ± 80.86	0.245	478.47 ± 147.10	0.333	2220.16 ± 306.64	0.401	1.71 ± 0.36	0.172
MT								
Baseline	187.72 ± 31.95		368.70 ± 65.61		1368.12 ± 91.07		1.56 ± 0.33	
6 months	202.21 ± 29.26	0.108	387.01 ± 60.22	0.319	1349.76 ± 101.26	0.512	1.63 ± 0.31	0.459
12 months	216.26 ± 37.27	0.007	412.67 ± 75.35	0.036	1335.96 ± 108.87	0.273	1.68 ± 0.33	0.241

Table 3: Continued.

	Volume of defective cartilage (mm^3)	p value	Size of defective cartilage (mm^2)	p value	Volume of healthy cartilage (mm^3)	p value	Thickness of defective cartilage (mm)	p value
LT								
Baseline	152.32 ± 50.11		306.34 ± 87.99		1363.31 ± 117.82		1.54 ± 0.39	
6 months	168.41 ± 51.88	0.448	324.18 ± 92.46	0.633	1336.30 ± 121.08	0.438	1.63 ± 0.38	0.598
12 months	182.72 ± 54.90	0.171	338.17 ± 108.00	0.437	1312.25 ± 109.98	0.128	1.69 ± 0.38	0.368
P								
Baseline	160.01 ± 58.53		302.71 ± 106.39		1626.33 ± 154.17		1.55 ± 0.29	
6 months	174.43 ± 61.54	0.563	330.44 ± 107.92	0.533	1599.34 ± 149.72	0.541	1.61 ± 0.29	0.588
12 months	186.20 ± 63.81	0.306	354.39 ± 113.11	0.261	1564.71 ± 155.23	0.174	1.69 ± 0.30	0.253

$208.12 \pm 42.70\,mm^2$ in MF (46% decrease; $p < 0.001$) and MT (49% decrease; $p < 0.001$), respectively. We also noted that the volume of healthy cartilage increased from $2382.20 \pm 314.39\,mm^3$ to $2712.22 \pm 343.55\,mm^3$ and from $1350.22 \pm 113.84\,mm^3$ to $1596.10 \pm 96.12\,mm^3$ in MF (14% increase; $p < 0.05$) and MT (18% increase; $p < 0.05$), respectively. In general, we believe that the effect of cartilage repair on medial cartilage was better than that on lateral cartilage.

In the control group, no evidence of cartilage regeneration was found in patients with K-L grade 2 and 3 OA (Table 3). To make matters worse, we found that the medial cartilage was more vulnerable to damage. In patients with K-L grade 2, the thickness of cartilage defect increased from 1.63 ± 0.24 mm to 1.90 ± 0.23 mm in MF (17% increase; $p = 0.001$), more than LF and T. The volume of cartilage defect increased from $133.01 \pm 35.21\,mm^3$ to $154.45 \pm 37.19\,mm^3$ in MT (16% increase; $p = 0.076$), more than LT. Similar to the patients with K-L grade 2, the most severely damaged cartilage in K-L grade 3 remains in the medial cartilage. The volume of cartilage defect increased from $267.43 \pm 73.34\,mm^3$ to $306.14 \pm 76.03\,mm^3$ in MF (14% increase; $p = 0.100$) and from $187.72 \pm 31.95\,mm^3$ to $216.26 \pm 37.27\,mm^3$ in MT (15% increase; $p = 0.007$). These results were similar to the view of cartilage repair in the test group.

3.3. Clinical and Radiological Outcome. After one year of follow-up, the VAS, WOMAC pain, stiffness, and physical function of the patients were evaluated at baseline and 1, 3, 6, and 12 months after injection with SVF and HA (Figure 6). In the test group, the mean WOMAC pain, stiffness, and physical function scores decreased from 9.38 ± 0.96 to 2.69 ± 1.02, from 2.83 ± 0.75 to 0.93 ± 0.74, and from 24.66 ± 3.12 to 10.14 ± 2.24 in the patients with grade 2 OA, while those scores of patients with grade 3 OA also showed a significant improvement. The mean VAS scores improved from 4.31 ± 0.46 to 1.59 ± 0.93 in grade 2 and from 6.04 ± 0.61 to 2.88 ± 0.78 in grade 3. In the control group, the mean WOMAC pain, stiffness, physical function, and VAS scores were relieved by one month after HA injection in grades 2 and 3 but were amplified again at 3-, 6-, and 12-month visits.

Functional improvement of ROM was significant at one month after HA therapy, from $120.59 \pm 5.83^\circ$ to $125.24 \pm 4.15^\circ$ in grade 2 and from $114.75 \pm 5.54^\circ$ to $120.46 \pm 4.90^\circ$ in grade 3. However, this trend took a turn for the worse after three months postoperation in the control group. Unlike the HA-treated group, the improvement of ROM showed a statistically significant difference, improving from $123.72 \pm 3.44^\circ$ to $137.82 \pm 3.44^\circ$ and from $114.21 \pm 5.97^\circ$ to $130.62 \pm 5.72^\circ$ in grade 2 and 3 OA, respectively.

The whole-organ assessment of the knees was performed by the WORMS at baseline and 6-month and 12-month follow-up (Table 4). In the test group, we subsequently found no signs of new cyst formation, neoplasms of the bone, cartilage, and synovium. The mean WORMS improved from a baseline of 54.86 ± 8.15 to 40.48 ± 7.28 at 12 months, in patients with grade 2 OA. Likewise, in grade 3 OA, the WORMS decreased from a baseline of 75.67 ± 10.44 to 57.46 ± 8.03, which revealed a significant improvement. By contrast, the consequence in the control group was poor; the WORMS deteriorated to 66.90 ± 11.15 and 84.04 ± 7.31 in patients with grade 2 and 3 OA, respectively.

The repair of the cartilage defects was measured using the MOCART system at 6 and 12 months (Table 5). As for the test group, the MOCART score improved from 52.93 ± 13.87 to 62.07 ± 12.83 at 6 and 12 months, respectively, in patients with K-L grade 2. Similarly, it increased from 46.46 ± 10.05 to 57.08 ± 11.98 at 6 and 12 months in patients with K-L grade 3, respectively. However, the MOCART score of the control group was decreased from 25.37 ± 12.40 to 17.71 ± 13.43 and from 22.41 ± 9.94 to 13.54 ± 6.34, at 6 and 12 months in grade 2 and 3 OA, respectively.

In addition, there were 12 knees (41.38%) that showed complete or hypertrophic repair tissue filling of the defect in grade 2 OA, while 13 knees (44.83%) elucidated most of the repair of cartilage defects (Figure 7). Although only one knee (4.17%) showed complete repair of the cartilage defects in grade 3 OA, there were 18 knees (75.00%) that showed substantial repair of cartilage defects. In the control group, there were 5 knees (18.52%) that showed substantial repair of cartilage defects in grade 2, and only one knee (4.17%) showed substantial repair of cartilage defects in grade 3.

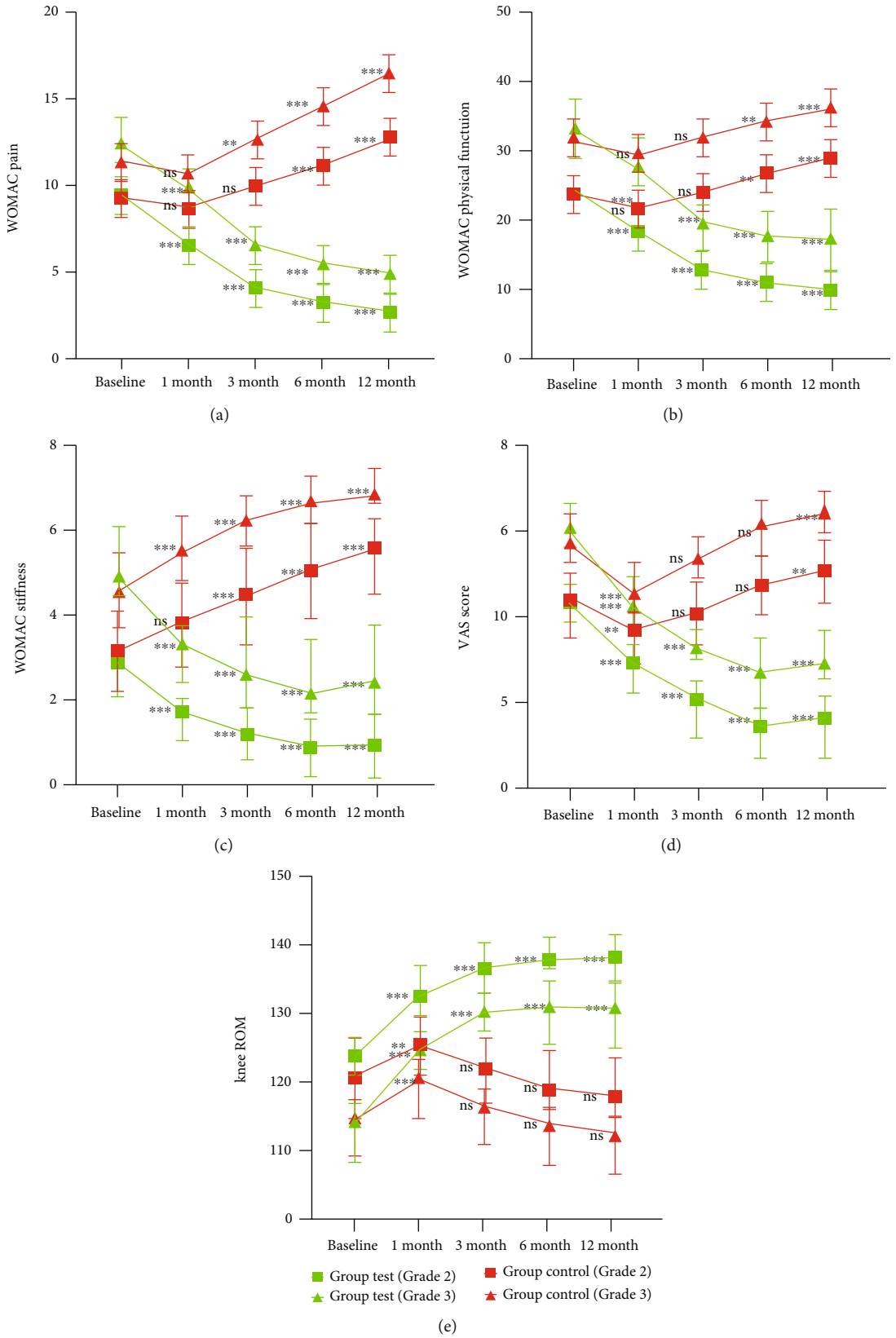

Figure 6: Changes of the VAS, ROM, WOMAC pain, stiffness, and physical function during 12-month follow-up after intra-articular injection of SVF and HA. Values in graphs are expressed as mean ± SD in vertical bars. $^{**}p < 0.01$ and $^{***}p < 0.001$. ns: nonsignificant ($p > 0.05$). All values were compared with baseline: (a) WOMAC pain; (b) WOMAC physical function; (c) WOMAC stiffness; (d) VAS score; (e) knee ROM.

Table 4: WORMS changes during 12-month follow-up.

Variables	Grade 2					Grade 3				
	Baseline	6 months	p value	12 months	p value	Baseline	6 months	p value	12 months	p value
Test group										
Cartilage	26.48 ± 3.43	19.38 ± 2.91	<0.001	15.17 ± 2.96	<0.001	34.33 ± 5.89	25.75 ± 4.39	<0.001	19.58 ± 3.83	<0.001
Marrow abnormality	3.07 ± 1.01	1.97 ± 0.96	<0.001	1.72 ± 0.91	<0.001	4.42 ± 1.11	3.25 ± 0.88	<0.001	2.88 ± 0.60	<0.001
Bone cysts	2.31 ± 1.02	1.76 ± 0.94	<0.05	1.69 ± 0.91	<0.05	3.71 ± 0.68	3.04 ± 0.68	<0.05	2.91 ± 0.70	<0.001
Bone attrition	1.03 ± 0.93	0.90 ± 0.80	0.535	0.83 ± 0.75	0.353	2.50 ± 0.65	2.38 ± 0.63	0.500	2.25 ± 0.60	0.179
Osteophytes	19.97 ± 3.99	19.69 ± 4.07	0.799	19.59 ± 4.03	0.726	27.08 ± 4.75	26.67 ± 4.76	0.766	26.63 ± 4.68	0.743
Menisci	0.83 ± 1.12	0.59 ± 0.81	0.325	0.55 ± 0.77	0.261	1.67 ± 1.28	1.50 ± 1.08	0.628	1.54 ± 1.18	0.716
Ligaments	0.07 ± 0.25	0.03 ± 0.18	0.538	0.03 ± 0.18	0.538	0.17 ± 0.37	0.13 ± 0.33	0.669	0.08 ± 0.28	0.393
Synovitis	1.10 ± 0.71	0.93 ± 0.74	0.374	0.90 ± 0.71	0.286	1.79 ± 0.64	1.63 ± 0.75	0.436	1.58 ± 0.76	0.330
WORMS total	54.86 ± 8.15	45.24 ± 7.52	<0.001	40.48 ± 7.28	<0.001	75.67 ± 10.44	64.33 ± 9.09	<0.001	57.46 ± 8.03	<0.001
Control group										
Cartilage	26.41 ± 4.48	28.59 ± 4.73	0.078	30.48 ± 4.82	0.002	34.08 ± 5.12	35.96 ± 4.39	0.18	37.17 ± 3.18	0.017
Marrow abnormality	3.48 ± 1.35	6.31 ± 2.16	<0.001	6.76 ± 1.57	<0.001	4.46 ± 1.25	7.54 ± 0.83	<0.001	7.67 ± 0.64	<0.001
Bone cysts	2.13 ± 1.19	2.38 ± 1.01	0.409	2.89 ± 0.71	0.036	3.33 ± 0.76	3.67 ± 0.48	0.078	3.79 ± 0.41	0.014
Bone attrition	1.38 ± 0.86	3.17 ± 1.34	<0.001	3.38 ± 1.01	<0.001	2.21 ± 0.78	4.79 ± 1.14	<0.001	4.96 ± 1.08	<0.001
Osteophytes	20.21 ± 4.90	20.59 ± 5.15	0.775	20.76 ± 4.87	0.669	26.29 ± 5.86	26.58 ± 5.56	0.86	26.83 ± 5.28	0.738
Menisci	0.97 ± 0.94	1.10 ± 0.94	0.579	1.24 ± 0.87	0.253	1.42 ± 0.72	1.54 ± 0.66	0.532	1.67 ± 0.56	0.186
Ligaments	0.14 ± 0.35	0.17 ± 0.38	0.723	0.24 ± 0.44	0.324	0.29 ± 0.46	0.33 ± 0.48	0.762	0.42 ± 0.50	0.376
Synovitis	0.97 ± 0.73	1.21 ± 0.73	0.212	1.34 ± 0.61	0.037	1.25 ± 0.68	1.46 ± 0.59	0.260	1.54 ± 0.51	0.098
WORMS total	55.69 ± 10.25	63.52 ± 11.79	0.009	66.90 ± 11.15	<0.001	73.33 ± 9.92	81.88 ± 8.19	0.002	84.04 ± 7.31	<0.001

4. Discussion

Nonoperative therapy is a frequently prescribed option for knee osteoarthritis treatment. Unfortunately, conservative treatment has been found to only temporarily relieve clinical symptoms, while their long-term efficacy is not satisfactory, eventually requiring an alternative intervention, TKA. Previous studies have highlighted that TKA may be associated with life-threatening complications such as infection, thromboembolism, myocardial infarction, and even death. In addition, the life span of the prosthesis is between 10 and 15 years [27]. Therefore, it will be of great significance to find an effective treatment particularly for reversing the progression of this disease. Interestingly, numerous studies have recently confirmed that intra-articular injection of autologous adipose-derived SVF for the treatment of OA pain is safe and feasible [28–31]. However, most clinical studies on SVF had small sample sizes, so estimates from individual studies may be imprecise, and their radiological evaluation only remains at the 2D level. So far, it remains enigmatic whether SVF can promote the growth of cartilage.

Furthermore, multiple recent studies have reported inconsistent findings of the effect of SVF on cartilage regeneration. For instance, Hong et al. found that the knee joint exhibited significant defect filling and cartilage repair after receiving SVF. Similarly, WORMS and MOCART scores verified this conclusion [32]. Jo et al. used the parameters of the 3D cartilage model to verify the efficacy of cartilage repair but did not use the special MRI sequence; the appearance of the cartilage model was poor [31]. In 2017, Nguyen proposed that the cartilage regeneration of the knee joint after Arthroscopic Microfracture (AM) combined with SVF/PRP injection was probably due to the combination of SVF and platelet-rich plasma (PRP), where SVF is the primary factor of this healing reaction. Elsewhere, several studies confirmed that PRP significantly reduced short-term pain without cartilage regeneration [33, 34]. In a double-blinded prospective randomized controlled clinical trial, Garza et al. reported no significant difference in cartilage thickness between the test group injected with SVF and the control group injected with placebo. However, in this study, participants were followed for only six months [35]. In the final analysis, these studies had small sample sizes, so estimates from individual studies may be imprecise, and their radiological evaluation remains at the 2D level.

Table 5: MOCART changes during 12-month follow-up.

Variables	Maximum score	Group test (grade 2/3), *n* (%)		Group control (grade 2/3), *n* (%)	
		6 months	12 months	6 months	12 months
1. Degree of defect repair and filling of the defect					
Complete	20	0 (0)/0 (0)	2 (6.90)/1 (4.17)	0 (0)/0 (0)	0 (0)/0 (0)
Hypertrophy	15	9 (31.03)/5 (20.83)	10 (34.48)/8 (33.33)	2 (7.41)/0 (0)	2 (7.41)/0 (0)
Incomplete					
>50% of the adjacent cartilage	10	12 (41.38)/9 (37.50)	13 (44.83)/10 (41.66)	4 (14.81)/2 (8.33)	3 (11.11)/1 (4.17)
<50% of the adjacent cartilage	5	7 (21.14)/7 (29.17)	4 (13.79)/4 (16.67)	11 (40.74)/7 (29.17)	11 (40.74)/6 (25.00)
Subchondral bone exposed	0	1 (3.45)/3 (12.50)	0 (0)/1 (4.17)	10 (37.04)/15 (62.50)	11 (40.74)/17 (70.83)
2. Integration to the border zone					
Complete	15	7 (21.14)/4 (16.67)	14 (48.28)/7 (29.16)	0 (0)/0 (0)	0 (0)/0 (0)
Incomplete					
Demarcating border visible (split-like)	10	17 (58.62)/10 (41.67)	12 (41.38)/9 (37.50)	5 (18.52)/3 (12.50)	4 (14.81)/3 (12.50)
Defect visible					
<50% of length of the repair tissue	5	3 (10.34)/7 (29.17)	3 (10.34)/6 (25.00)	11 (40.74)/7 (29.17)	12 (44.45)/6 (25.00)
>50% of length of the repair tissue	0	2 (6.90)/3 (12.50)	0 (0)/2 (8.33)	11 (40.74)/14 (58.33)	11 (40.74)/15 (62.50)
3. Surface of the repair tissue					
Surface intact	10	16 (55.17)/13 (54.17)	19 (65.52)/16 (66.67)	1 (3.70)/0 (0)	1 (3.70)/0 (0)
Surface damaged					
<50% of repair tissue depth	5	11 (37.93)/9 (37.50)	10 (34.48)/7 (29.16)	14 (51.85)/8 (33.33)	12 (44.45)/7 (29.17)
>50% of repair tissue depth or total degeneration	0	2 (6.90)/2 (8.33)	0 (0)/1 (4.17)	12 (44.45)/16 (66.67)	14 (51.85)/17 (70.83)
4. Structure of the repair tissue					
Homogeneous	5	20 (68.97)/16 (66.67)	23 (79.31)/19 (79.17)	9 (33.33)/5 (20.83)	8 (29.63)/7 (29.17)
Inhomogeneous or cleft formation	0	9 (31.03)/8 (33.33)	6 (20.69)/5 (20.83)	18 (66.67)/19 (79.17)	19 (70.37)/17 (70.83)
5. Signal intensity of repair tissue					
Normal (identical to adjacent cartilage)	30	2 (6.90)/1 (4.17)	7 (24.14)/3 (12.50)	2 (7.41)/1 (4.17)	2 (7.41)/0 (0)
Nearly normal (slight areas of signal alteration)	15	20 (68.96)/16 (66.67)	18 (62.07)/17 (70.83)	7 (25.92)/5 (20.83)	6 (22.22)/5 (20.83)
Abnormal (large areas of signal alteration)	0	7 (21.14)/7 (29.16)	4 (13.79)/4 (16.67)	18 (66.67)/18 (75.00)	19 (70.37)/19 (79.17)
6. Subchondral lamina					
Intact	5	21 (72.41)/17 (70.83)	21 (72.41)/20 (83.33)	12 (44.44)/7 (29.17)	7 (37.04)/4 (16.67)
Not intact	0	8 (27.59)/7 (29.17)	8 (27.59)/4 (16.67)	15 (55.56)/17 (70.83)	17 (62.96)/20 (83.33)
7. Subchondral bone					
Intact	5	8 (27.59)/8 (33.33)	8 (27.59)/11 (45.83)	6 (22.22)/6 (25.00)	4 (14.81)/4 (16.67)
Not intact (edema, granulation tissue, cysts, sclerosis)	0	21 (72.41)/16 (66.67)	21 (72.41)/13 (54.17)	21 (77.78)/18(75.00)	23 (85.19)/20 (83.33)

Table 5: Continued.

Variables	Maximum score	Group test (grade 2/3), *n* (%) 6 months	Group test (grade 2/3), *n* (%) 12 months	Group control (grade 2/3), *n* (%) 6 months	Group control (grade 2/3), *n* (%) 12 months
8. Adhesions					
No	5	18 (62.07)/12 (50.00)	18 (62.07)/16 (66.67)	8 (29.63)/7 (29.17)	6 (22.22)/6 (25.00)
Yes	0	11 (37.93)/12 (50.00)	11 (37.93)/8 (33.33)	19 (70.37)/17 (70.83)	21 (77.78)/18 (75.00)
9. Synovitis					
No synovitis	5	9 (31.03)/2 (8.33)	9 (31.03)/4 (16.67)	8 (29.63)/7 (29.17)	6 (22.22)/6 (25.00)
Synovitis	0	20 (68.97)/22 (91.67)	20 (68.97)/20 (83.33)	19 (70.37)/17 (70.83)	21 (77.78)/18 (75.00)
Mean ± SD		52.93 ± 13.87/46.46 ± 10.05	62.07 ± 12.83/57.08 ± 11.98	25.37 ± 12.40/17.71 ± 13.43	22.41 ± 9.94/13.54 ± 6.34

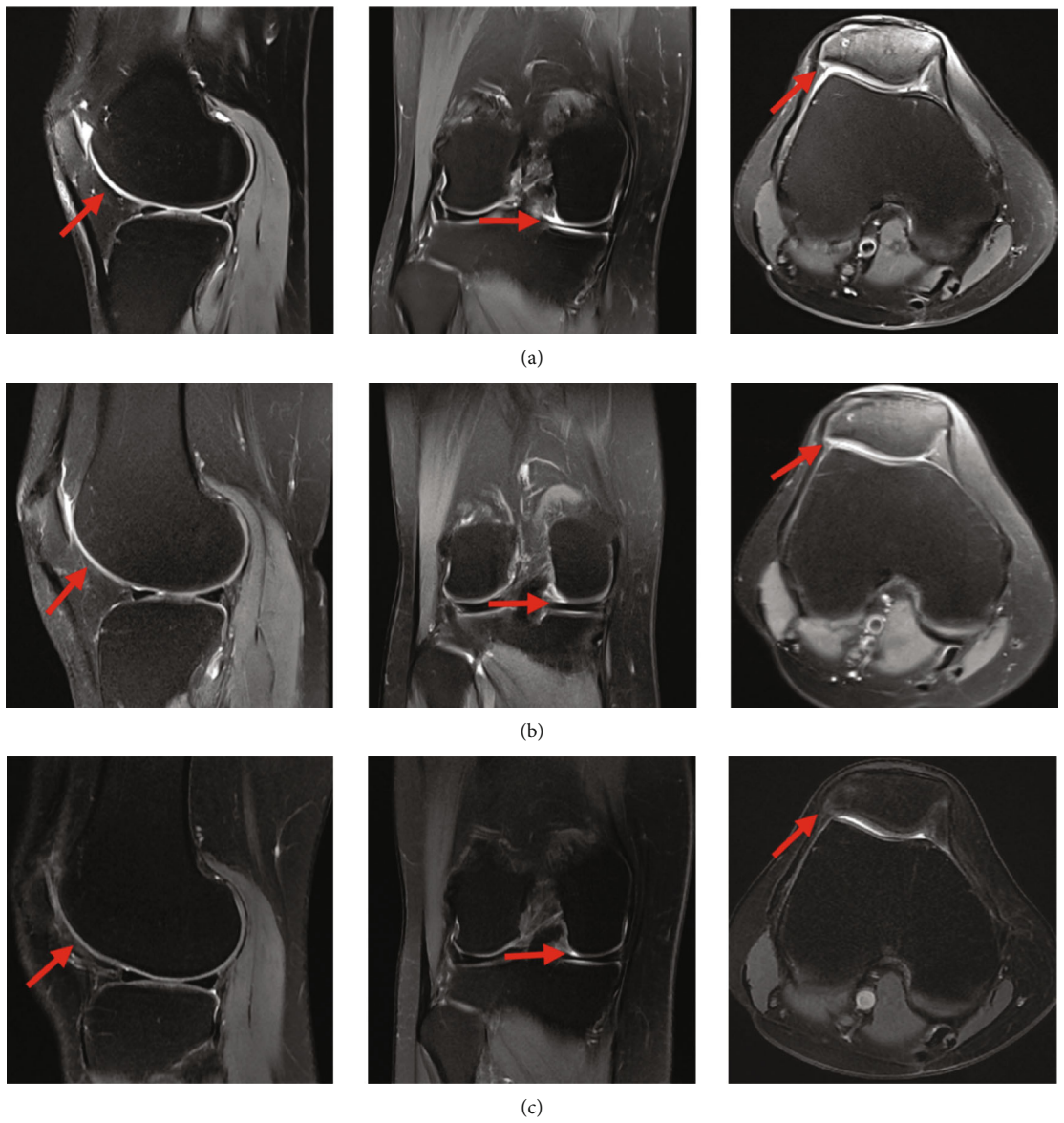

Figure 7: MRI scans of the SVF-treated knees with OA performed at baseline and 6 and 12 months, respectively. It was found that the defect was completely repaired and filled, and the cartilage fused well with adjacent cartilage and subchondral bone in the coronal, transverse, and sagittal planes (red arrow): (a) baseline; (b) 6 months; (c) 12 months.

The emergence of the 3D-FS-SPGR sequence and finite element analysis provided a new method to evaluate cartilage quantitatively. Kijowski et al. proposed that routine MRI with a 3D sequence can improve the diagnostic performance for detecting cartilage lesions in the knee [36]. Jang et al. believed that the 3D-SPGR sequence can provide better diagnostic performance for the evaluation of knee articular cartilage lesions by detecting partial-thickness cartilage lesions in patients with OA [37]. In 2014, Peterfy et al. combined the 3D-SPGR sequence with finite element analysis to establish the cartilage model for accurate prediction of normal intra-articular pressure and force under different loads [25]. By finite element simulation, Li et al. proposed that meniscectomy can relieve pain for some time, resulting in more severe biomechanical changes and increase progression of cartilage injury [38]. Taken together, these studies confirmed that the 3D-SPGR sequence can provide better diagnostic performance, and the cartilage model is reliable. However, no scholar has applied this technology to evaluate cartilage regeneration of SVF.

Herein, we enrolled 95 patients with K-L grade 2 and 3 OA in this study. Each patient underwent the examination of the 3D-FS-SPGR sequence before treatment and at 6 and 12 months. We employed the 3D-FS-SPGR sequence to develop a 3D cartilage model, thereby dividing the cartilage at the 3D level. Compared with the conventional MRI sequence, the slice thickness of the 3D-FS-SPGR sequence was 1 mm, which can reduce the volume effect on imaging, and consequently, the resulting data is more accurate. In addition, the 3D SPGR sequence was clearer and more stratified for the imaging of articular cartilage, the interslice gaps of the 3D-SPGR sequence is 0 mm, and the error of the 3D

modeling is less, which can be used for the quantitative analysis of cartilage. Following this, we recorded the changes of cartilage parameters in each region. Remarkably, the cartilage of all regions was improved to some extent in the test group, especially the MF and MT. In grade 2 OA, the thickness, volume, and size of cartilage defect in MF decreased to 0.92 ± 0.18 mm, 84.00 ± 32.30 mm^3, and 182.22 ± 67.00 m m^2, respectively. These parameters decreased to 0.96 ± 0.22 mm, 64.18 ± 21.40 mm^3, and 146.15 ± 45.47 mm^2 in MT. Similarly, these parameters of cartilage defects in MF and MT were greatly improved in grade 3, more than other regions. We identified that the efficacy of patients with medial cartilage injury was better compared with that of patients with other region injuries, whether pain improvement, functional recovery, or cartilage repair. We did not compare the MRI results with arthroscopy; the secondary surgery can cause injury to the patient, even though it is a minimally invasive procedure.

Besides, we further observed that the cartilage of patients with K-L grades 2 and 3 had different responses to SVF injection. The WOMAC score, ROM, and rehabilitation speed of patients with grade 2 were better than those of patients with grade 3. The WOMAC pain, stiffness, and physical function scores decreased from 9.38 ± 0.96 to 2.69 ± 1.02 (71% decrease), from 2.83 ± 0.75 to 0.93 ± 0.74 (67% decrease), and from 24.66 ± 3.12 to 10.14 ± 2.24 (59% decrease) in grade 2, while those scores in grade 3 improved to 4.92 ± 1.22 (60% decrease), 2.41 ± 1.35 (51% decrease), and 17.58 ± 4.35 (48% decrease), respectively. Likewise, based on the degree of cartilage repair, the increase in grade 2 OA was higher compared to that of grade 3 OA. There were 12 knees (41.38%) that showed complete or hypertrophic repair tissue filling of the defect in grade 2, and 13 knees (44.83%) elucidated most (beyond 50%) repair of cartilage defects. Only one knee (4.17%) showed complete repair of the cartilage defects in grade 3 OA. In summary, these results confirmed that SVF cell therapy can effectively improve clinical symptoms and promote cartilage regeneration before the excessive development of cartilage degeneration.

However, despite these promising results, this work has some limitations that are worth noting. First, the segmentation of the image was done through manual segmentation, which would increase some errors. Then, the follow-up period was short (12 months), whereby clinical evaluations were performed at baseline and 1, 3, 6, and 12 months after intra-articular injection of SVF cells into the knee. Third, we did not evaluate the relationship between the intra-articular injection dose of SVF cells and clinical results; hence, the effect of dose on clinical efficacy is not clear. Finally, although the MRI and parameters of the cartilage model clearly elucidated the regeneration of articular cartilage, it remains elusive whether the regenerated cartilage was either fibrocartilage or hyaline cartilage.

5. Conclusion

Collectively, our study demonstrates that autologous adipose-derived SVF can effectively relieve pain and improve function. We noted that the method of establishing the model and calculating parameters through the 3D-FS-SPGR sequence can accurately evaluate the effect of cartilage repair. Quantitative data of the cartilage model showed significant improvements in cartilage regeneration. Therefore, this research suggests that intra-articular injection of SVF is a promising minimally invasive therapy for cartilage regeneration, particularly for K-L grades 2 and 3.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the Zhejiang Provincial People's Hospital and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was registered in the Chinese Clinical Trial Registry (trial registration number ChiCTR2100042930).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

The authors designed the studies and analyzed and interpreted the data. All authors have made substantial contributions: (1) the conception and design of the study, acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, and (3) final approval of the version to be submitted. Qiong Zhang takes responsibility for the integrity of the work as a whole. Yin Zhang and Qing Bi contributed equally to this work.

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Mid-term prognosis of the stromal vascular fraction for knee osteoarthritis: a minimum 5-year follow-up study

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Abstract

Background: The short-term safety and efficacy of stromal vascular fraction (SVF) in treating knee osteoarthritis (KOA) have been extensively studied but the mid-term and long-term prognoses remain unknown.

Methods: 126 KOA patients were recruited and randomly assigned to SVF group and hyaluronic acid (HA) group (control group). The scores of visual analogue scale (VAS) and the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) were assessed and compared between the two groups 1, 2, 3, and 5 years after treatment. The endpoint was defined as surgeries related to KOA or clinical scores exceeding the patient acceptable symptom state (PASS).

Results: The VAS and WOMAC scores in the SVF group were significantly better than those in the HA group during the 5-year follow-up after treatment. The average responsive time to SVF treatment (61.52 months) was significantly longer than HA treatment (30.37 months). The adjusted Cox proportional hazards model showed that bone marrow lesion (BML) severity, body mass index (BMI) and treatment were independent risk factors and that the use of SVF reduced the risk of clinical failure by 2.602 times. The cartilage volume was reduced in both the SVF and control groups at 5 years but reduced less in the SVF group.

Conclusions: Up to 5 years after SVF treatment, acceptable clinical state was present for approximately 60% of patients. BML severity and BMI were independent predictors of the prognosis.

Trial Registry: This study was retrospectively registered at Chinses Clinical Trial Registry with identifier ChiCTR2100052818 and was approved by ethics committee of the First Affiliated Hospital of Zhejiang Chinese Medical University, number 2013-X-063.

Keywords: Knee osteoarthritis, Stromal vascular fraction, Bone marrow lesion, Full-thickness cartilage defect, Mid-term follow-up, Prognosis

Background

Knee osteoarthritis (KOA), the most common clinical degenerative disease, is characterized by cartilage destruction, subchondral bone damage, synovial inflammation and osteophyte formation and affects 10% of men and 16% of women over age 60 worldwide [1]. As opposed to medications and physical therapy used to treat early-stage KOA and total knee arthroplasty where

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KOA progresses to end-stage, emerging regenerative therapy has the potential to change this treatment paradigm [2]. The stromal vascular fraction (SVF) obtained by adipose tissue enzyme digestion contains adipose-derived stem cells (ADSCs) and progenitor cells with the ability to differentiate into a variety of cell types, such as chondrocytes, which can be a therapeutic option, and SVF is considered to be comparable to and sometimes even more effective than ADSCs due to the other functional advantages it provides over ADSCs, such as structural support [3, 4]. In recent years, several studies have addressed the short-term outcomes of SVF for KOA, demonstrating their analgesic effect and joint function improvement [5–7]. Nevertheless, owing to the high cost of SVF therapy and its hoped regenerative capacity, patients may not be content to achieve only a short-term improvement in symptoms, which can also be obtained with conservative treatment. Therefore, it is essential to clarify its mid-term efficacy, which is beneficial for the patient's choice of treatment.

Indications for such regenerative therapy are unclear. For the most part, it is highly recommended for patients with apparent cartilage damage on MRI, but the extent to which cartilage damage is prognostically meaningful is not known. Tiny cartilage defects and thinned thickness, with a prevalence of > 80% among patients with symptomatic KOA, do not appear to be disastrous [8]. However, a previous study has shown that full-thickness cartilage defects are an independent risk factor for total knee arthroplasty in asymptomatic KOA [9]; the outcome of such cartilage defects in SVF treatment and their impact on prognosis are of concern. Instead of being a pure cartilage disorder, more joint structure abnormalities contribute to the progression of KOA [10]. Upon reviewing the literature, it is also notable that bone marrow lesion (BML), characterized by bone marrow oedema, fibrosis, and necrosis, is tied to total knee arthroplasty failure [11, 12]. The relationship between BML and cartilage loss and pain is becoming increasingly recognized [13].

Therefore, we conducted a single centre, parallel group, assessor blinded, and randomized controlled clinical trial to determine the mid-term outcomes and clinical failure of SVF for KOA and whether a number of factors, including full-thickness cartilage damage and BML, are predictive of prognosis.

Methods

Study design

The study is a single centre, parallel group, assessor blinded, and randomized controlled clinical trial, that was retrospectively registered at Chinses Clinical Trial Registry with identifier ChiCTR2100052818 and was approved by ethics committee of the First Affiliated Hospital of Zhejiang Chinese Medical University, number 2013-X-063. KOA patients at the First Affiliated Hospital of Zhejiang Chinese Medicine University between May 2013 and July 2015 were recruited in the study (Fig. 1). The criteria included the following: the diagnosis met the diagnostic criteria in the American Rheumatism Association Revised Classification Criteria for Knee Osteoarthritis [14]; Kellgren–Lawrence (KL) grade 2–3 [15]; age 20–85 years; and no history of significant trauma. The exclusion criteria included the following: local infection of the knee joint; systemic diseases such as blood disorders or diabetes; rheumatoid arthritis, gout, autoimmune disease, or malignancy in the past 5 years; prior injection or use of oral steroids within 3 weeks before screening; knee surgery within 6 months before screening; or pain attributed to displaced meniscal tear and torn ligaments.

Enrollment and randomization

Patients with KOA were recruited at the First Affiliated Hospital of Zhejiang Chinese Medical University. We obtained written informed consent from each eligible patient. Patients were randomly divided into the SVF group or hyaluronic acid (HA) group (control group). Randomization assignments were generated by using a computer generated, randomized number sequence, and kept the assessor who collected and analyzed outcome data blinded.

Preparation of therapeutic SVF

Adipose tissues of patients in SVF group were obtained from the abdomen by liposuction surgery performed by a skilled orthopaedic surgeon. The patient lay supine with full exposure of the abdomen. Routine sterilization and drape operation were performed. Local anaesthesia was applied to the abdomen with 10 mg/ml lidocaine (10 ml), two small incisions of approximately 5 mm were created around the umbilicus, and approximately 40 ml of abdominal subcutaneous adipose tissue was aspirated through a sterile syringe. The incisions were closed with sutures, and the abdomen was wrapped with pressure. Harvested adipose tissue was stored in a small freezer and transported to the laboratory. The adipose tissue was washed 3–5 times with PBS containing penicillin at a 2% concentration and then centrifuged at 1000 rpm for 5 min. The upper layer of adipose tissue was removed and cut to chyme with sterilization scissors. The chylomicron adipose tissue was collected in a clean 15 ml centrifuge tube with the addition of an appropriate amount of 1% collagenase type IV at 400 rpm and 37 °C for digestion. After that, the filtrate was collected through a 100-mesh cell sieve and centrifuged at 1200 rpm for 5 min, and the supernatant was removed, the residual SVF pellet at the bottom was

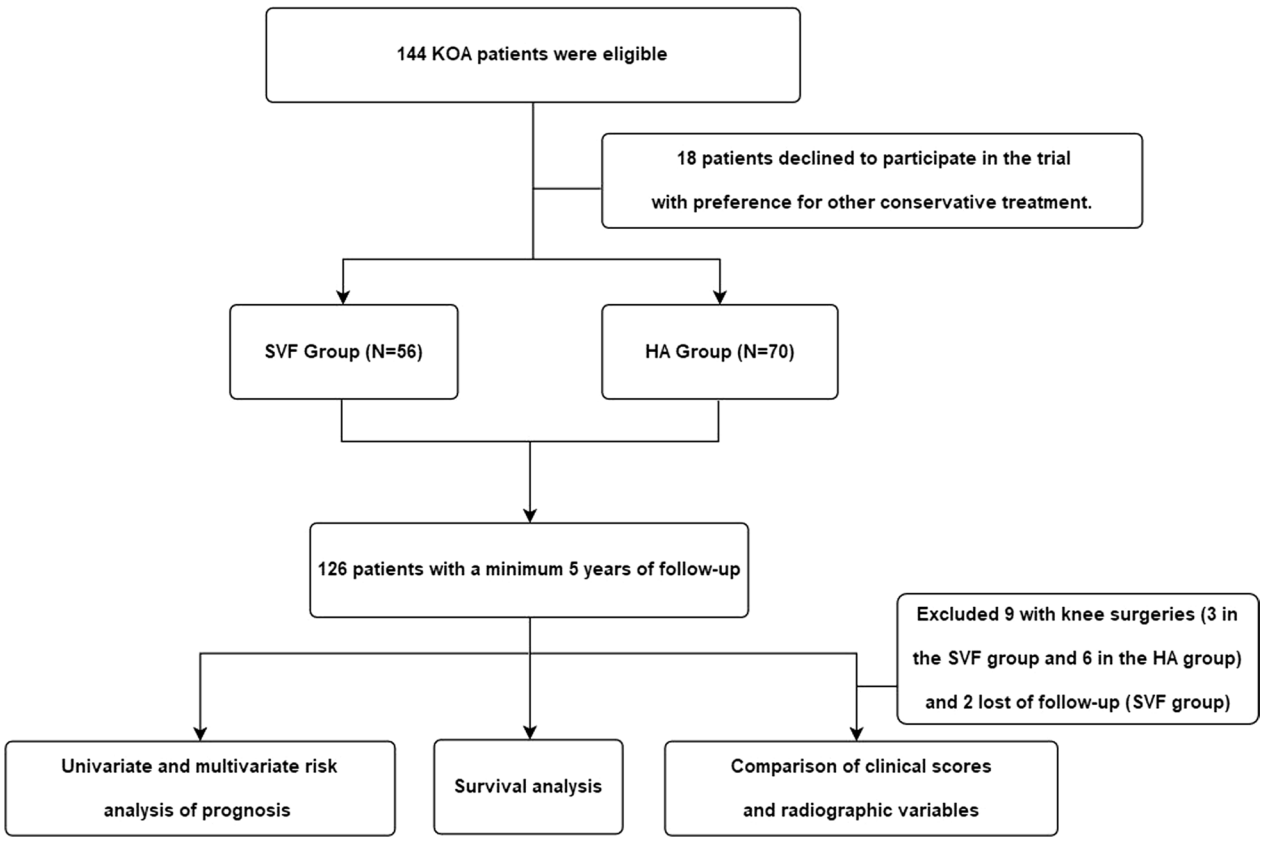

Fig. 1 Study flow diagram

Table 1 Cell characterization by flow cytometry

Subpopulation	Avg (%)	Type pf cells
CD45−/CD31−/CD34+	33.1	SVF progenitor cells
CD45−/CD31−/CD34−	45.6	SVF non-progenitor cells
CD45−/CD31+	9.3	Endothelial cells
CD45−	92.7	Stromal vascular cells
CD45+	5.5	Leukocytes

resuspend in PBS to a volume of 6 ml, in which 1 ml of the sample was retained for cell counting. The SVF was characterized by flow cytometry, and the constituent cell subpopulations of live nucleated SVF cells were shown as a percentage of the total number of live nucleated cells, without counting RBCs (Table 1). The remaining 5 ml, with an average count of 4.84 ± 1.61 million viable SVF cells according to the counting result, was used for injection. SVF was injected into the knee joint within one hour after successful preparation.

Intra-articular injection

The patient was placed in the supine position with the knee straight. Local sterilization was performed. 5 ml of SVF was injected into the joint cavity via a superior-lateral approach under sterile technique by percutaneous puncture with a disposable syringe once a month for a total of three times. After the injection, a local sterile dressing was applied, and the patient was instructed to bend and extend the knee joint several times. HA was injected in patients of control group as described above at a dose of 5 ml once a month for a total of three times.

Post-injection protocol

Patients were instructed to be non-weight bearing for two days and to undertake only light activity and avoid previously painful activities for the first 3 weeks after the injection. Patients were informed of the possibility of adverse reactions, including fever, swelling, or skin rash, after the injection and were asked to contact their physician immediately if any adverse reactions occurred during the follow-up period. Patients should inform their physician to evaluate pain and function if they suffer

from knee pain and take pain medication during the follow-up period.

Primary outcomes

Pretreatment baseline data, including sex, age, body mass index (BMI), etc. were collected from both groups of patients. Patients were followed up at 1, 2, and 3 years after treatment and every 2 years thereafter, and their pain and function were evaluated by a blinded and skilled orthopaedic surgeon using the scores of visual analogue scale (VAS) (0–10 cm) and Western Ontario and McMaster University Osteoarthritis Index (WOMAC).

Second outcomes

X-rays were used to determine the KL grade change and mechanical axis at the time of assessment, and MRI (3.0 T) was performed to evaluate cartilage structure and volume, patella-femoral pathology and BML. MRI data: a T1-weighted image, repetition time 3000 ms, echo time 33 ms, 512 × 512-pixel matrix; sagittal images were obtained at a slice thickness of 1.5 mm without an interslice gap; (2) a T2-weighted image, repetition time 4590 ms, echo time 62 ms, 320 × 320-pixel matrix; sagittal images were obtained at a slice thickness of 3 mm with an interslice gap of 3.85 mm. Cartilage structure and volume and BML were assessed over the medial tibia, medial femur, medial patella, lateral tibia, lateral femur, and lateral patella.

The KL grade and mechanical axis were assessed by a skilled orthopaedic surgeon blinded to treatment allocation and clinical data.

Patella-femoral pathology was assessed by a skilled orthopaedic surgeon blinded to treatment allocation and clinical data.

Cartilage structure and volume was assessed by a skilled orthopaedic surgeon blinded to treatment allocation and clinical data. Measurement of individual cartilage plate volumes was performed using Rhinocero 5.0 (Robert McNeel, USA) software. Contour tracing of cartilage boundaries was performed layer by layer of MRI images in isometric sections and separated from the total volume to create a cartilage 3D model [16], and the volume was calculated using the software. A full-thickness cartilage defect was defined as cartilage stripping to subchondral bone exposure, regardless of size. The full-thickness cartilage defect area of each coronal and transverse slice was measured and divided into three levels [9]: 0, no defect; 1, defect < 2 cm^2; 2, defect ≥ 2 cm^2.

Subchondral BML was assessed on T2-weighted images by a skilled orthopaedic surgeon blinded to treatment allocation and clinical data and were defined as areas of high signal in the subchondral bone marrow, including cystic changes. BML size was scored by measuring the maximum area of the lesion (mm^2) at baseline and follow-up [17]. The areas of BML in the six positions were added to determine the total size. BML severity was scored and summarized according to the number of slices covered by BML in each measurement site with reference to the previous method [18]: 0, no BML; 1, cover one slice, 2; cover two consecutive slices; 3, cover three or more consecutive slices, score 0–18. It was scored 3 if more than one lesion was present at the same site (Fig. 2).

Definition of clinical failure

Clinical failure was defined as surgeries related to KOA, such as total knee arthroplasty, unicondylar knee arthroplasty and debridement under arthroscopy, or clinical scores exceeding the patient acceptable symptom state (PASS) (VAS > 3.23 or WOMAC function score > 31) [19]. Information about the surgery was collected at each follow-up. For patients who underwent surgery, clinical scores were not included in the final comparison analysis, but only the time of surgery was recorded for Kaplan–Meier survival analysis. We use the term responsive to denote survival for Kaplan–Meier survival analysis, which represents the knee remains responsive to treatment, i.e., the lasting impact of the treatment. To avoid overestimating responsive time, for patients with very poor clinical scores after 1 year, we carefully questioned the patient's medical history prior to that time to determine the exact time beyond PASS.

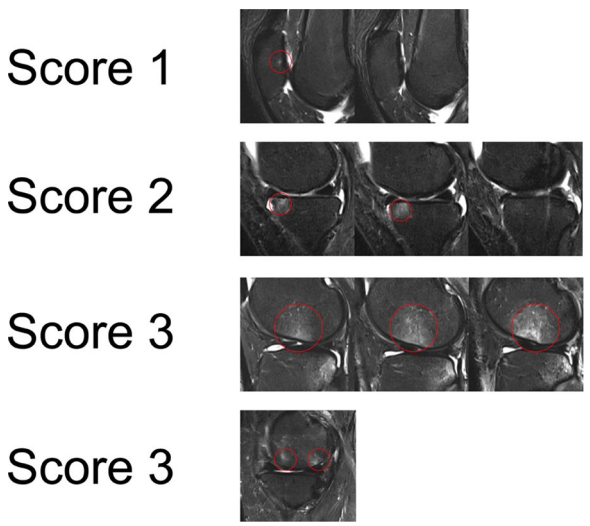

Fig. 2 Three levels of bone marrow lesions in the red circle position. 0, no BMLs; 1, cover one slice, 2; cover two consecutive slices; 3, cover three or more consecutive slices. A score of 3 was assigned if more than one lesion was present at the same site

Statistical analysis

SPSS statistics 25 software (IBM, USA) was used to perform the statistical analysis, and the data are presented as the mean ± standard deviation and percentage. Differences in baseline data were assessed by independent samples t-tests for continuous variables and chi-square tests for categorical variables. For the clinical score comparison, the main effect (within- and between-subjects) and crossover effect were analysed by two-factor repeated-measures ANOVA. The separate effects were analysed by 2-way ANOVA for grouping factors at each time point and repeated measures ANOVA for time factors. With clinical failure as the endpoint, Kaplan–Meier responsive curves were generated to compare the responsive probability of the two groups. The crude risk factors for clinical failure were obtained through univariate Cox regression using the same endpoint, and the significance level was set at $p < 0.10$. After that, with diagnosing collinearity with variance inflation factor (VIF), a multivariate Cox regression was performed to exclude confounding factors for independent risk factors and to develop independent prediction models. KL grade, mechanical axis, patella-femoral pathology, full-thickness cartilage defect, total cartilage volume and BML-related variables were mandatory to be included in the multivariate analysis. Differences were considered significant with $p < 0.05$.

Results

Study population

We enlisted 144 KOA patients between May 2013 and July 2015 who were accorded with inclusion criteria. 18 patients declined to participate in the trial with preference for alternative treatment. 126 patients were enrolled and randomly assigned: 56 patients in SVF group and 70 patients in the control group. Two patients in the SVF group were lost to follow-up due to a change in contact details, and 9 patients underwent surgery during the follow-up period (3 in the SVF group and 6 in the HA group); these patients were included in the Kaplan–Meier responsive analysis but not in the comparison analysis of clinical scores (Fig. 1). There were no significant differences in the baseline data between the two groups of patients ($p > 0.05$, Table 2). There were no adverse reactions during postoperative follow-up in either group.

Primary outcomes

A total of 115 patients at 1 year, 2 years, 3 years, and 5 years received a complete clinical score evaluation, including 51 in the SVF group and 64 in the HA group. The comparison of VAS scores and WOMAC scores between the SVF group and HA group before and after treatment is shown in Tables 3 and 4 and Fig. 3. There was a significant difference in clinical scores between time before and after treatment (VAS: $F = 64.348$,

Table 2 Baseline data of included patients

	SVF ($N = 56$)	HA ($N = 70$)	*p* value
Sex			0.692
Male	14 (25%)	16 (22.9%)	
Female	42 (75%)	54 (77.1%)	
Age, years	53.98 ± 13.69	55.63 ± 12.18	0.790
BMI	23.73 ± 2.99	23.86 ± 2.55	0.447
Mechanical axis, °	Varus 1.63 ± 2.21	Varus 1.49 ± 2.12	0.715
KL grade			0.366
2	41 (73.2%)	46 (65.7%)	
3	15 (26.8%)	24 (34.3%)	
Full-thickness defect			0.069
0	40 (71.4%)	45 (64.3%)	
1	6 (10.7%)	18 (25.7%)	
2	10 (17.9%)	7 (10%)	
Total cartilage volume (mm^3)	16,377.16 ± 2692.40	15,851.51 ± 2143.45	0.225
BML severity	3.30 ± 4.34	2.77 ± 3.42	0.455
BML size (mm^2)	127.68 ± 193.42	108.07 ± 149.89	0.522
Baseline VAS score	4.04 ± 1.46	3.64 ± 0.98	0.088
Baseline WOMAC score	34.57 ± 22.85	29.97 ± 19.87	0.229
Patella-femoral pathology present	28 (50%)	24 (34.3%)	0.075

SVF, stromal vascular fraction; HA, hyaluronic acid; BMI, body mass index; KL, Kellgren–Lawrence; VAS, visual analogue scale; WOMAC, Western Ontario and McMaster University Osteoarthritis Index; BML, bone marrow lesion

Table 3 Comparison of the VAS scores before and after treatment in the SVF and the control group

Before or after treatment								
Group	**Pre-treatment**	**1 year**	**2 years**	**3 years**	**5 years**	**Sum**	***F***	***p* value**
SVF	3.96 ± 1.46	1.69 ± 1.63***	2.04 ± 1.78***	2.43 ± 1.66***	2.86 ± 1.83**	2.60 ± 1.84	75.990	< 0.001
HA	3.55 ± 0.91	3.42 ± 0.99^{ns}	3.50 ± 1.39^{ns}	3.73 ± 1.29^{ns}	3.95 ± 1.23^{ns}	3.63 ± 1.18	9.067	< 0.001
Sum	3.73 ± 1.19	2.65 ± 1.57	2.85 ± 1.73	3.16 ± 1.60	3.47 ± 1.61	3.17 ± 1.59[a]	64.378[a]	< 0.001[a]
F	2.414	42.441	30.065	23.921	16.751	18.030[a]	(*F* = 49.319 *p* value < 0.001)[b]	
p value	0.121	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001[a]		

SVF, stromal vascular fraction; HA, hyaluronic acid

[a] *F* statistic and *p* value of main effect

[b] *F* statistic and *p* value of crossover effect

* *p* value < 0.05; ** *p* value < 0.01; *** *p* value < 0.001; ns, non-significant (*p* value > 0.05), compared with pre-treatment

Table 4 Comparison of the WOMAC total score before and after treatment in the SVF and the control group

Before or after treatment								
Group	**Pre-treatment**	**1 year**	**2 years**	**3 years**	**5 years**	**Sum**	***F***	***p* value**
SVF	33.24 ± 21.93	18.02 ± 18.87***	20.57 ± 20.13**	23.14 ± 21.03*	27.04 ± 22.47^{ns}	24.40 ± 21.43	36.195	< 0.001
HA	28.44 ± 18.23	28.27 ± 21.07^{ns}	31.28 ± 22.33^{ns}	33.36 ± 22.88^{ns}	36.05 ± 22.52^{ns}	31.48 ± 21.54	46.619	< 0.001
Sum	30.57 ± 20.00	23.72 ± 20.68	26.53 ± 21.95	28.83 ± 22.57	32.05 ± 22.85	28.34 ± 21.7[a]	45.087[a]	< 0.001[a]
F	1.449	6.609	7.015	6.378	5.108	3.335[a]	(*F* = 41.307 *p* value < 0.001)[b]	
p value	0.229	0.010	0.008	0.012	0.024	0.070[a]		

SVF, stromal vascular fraction; HA, hyaluronic acid

[a] *F* statistic and *p* value of the main effect

[b] *F* statistic and *p* value of the crossover effect

* *p* value < 0.05; ** *p* value < 0.01; *** *p* value < 0.001; ns, non-significant (*p* value > 0.05), compared with pre-treatment

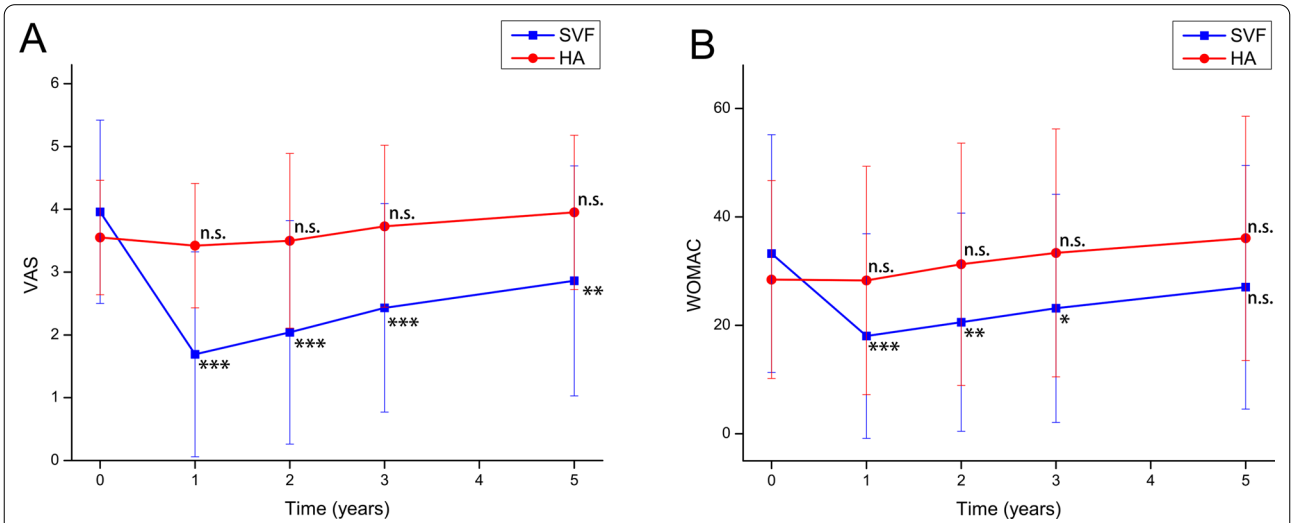

Fig. 3 Changes in the VAS score and the WOMAC score during the 5-year period before and after treatment in the SVF group and control group. **A** The mean VAS score change. **B** The mean WOMAC total score change. *p value < 0.05, **p value < 0.01, ***p value < 0.001, ns, non-significant (*p* value > 0.05), compared with pre-treatment

$p<0.001$; WOMAC: $F=45.087$, $p<0.001$), with separate effect analyses in the SVF group (F[VAS] = 75.990, F[WOMAC] = 36.195) and HA groups (F[VAS] = 9.067, F[WOMAC] = 46.619), all at $p<0.001$. The VAS and WOMAC scores in the SVF group were lowest after 1 year and then increased annually but remained lower than pretreatment scores at 5 years. The VAS and WOMAC scores in the HA group did not change much from pretreatment at 1 year, then increased annually and were significantly higher than pretreatment at 5 years. The post hoc tests were conducted for different time points in the HA and SVF groups. VAS scores in the SVF group were significantly lower than pre-treatment at all post-treatment time points ($p<0.05$), and WOMAC scores in the SVF group were significantly lower than pre-treatment at years 1, 2, and 3 post-treatment ($p<0.05$), but did not differ from pre-treatment at year 5 ($p>0.05$). VAS scores and WOMAC scores in the HA group did not differ from pre-treatment at all post-treatment time points ($p>0.05$). The VAS scores in the SVF group were significantly lower than those in the HA group overall after treatment ($F=18.030$, $p<0.001$), and the WOMAC scores were not significantly different between the two groups in the overall effect ($F=3.335$, $p>0.05$). Due to a crossover effect between treatment and time (F[VAS] = 49.319, $p<0.001$; F[WOMAC] = 41.307, $p<0.001$). We performed an analysis of the separate effects for each time point. The VAS and WOMAC scores of the SVF group were significantly lower than those of the HA group at all time points after treatment ($p<0.05$).

To observe the clinical outcome of each patient more accurately, the Kaplan–Meier responsive curves of all patients in the two groups were plotted and compared. The SVF group showed a responsive rate of 62.5% (35/56) at the 5-year follow-up, and the rate in the HA group was 20% (14/70). According to the log-rank analysis, the mean responsive time (61.52 ± 4.14 months) of the SVF group was significantly longer than that of the HA group (30.37 ± 2.69 months) ($p<0.001$, Fig. 4).

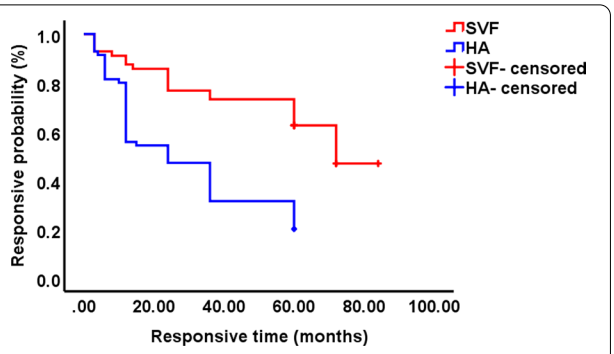

Fig. 4 Kaplan–Meier responsive curve with clinical failure as the endpoint

Secondary outcomes

The radiological changes as secondary outcomes at 5 years are documented in Table 5. At the final radiological examination, the total cartilage volume was significantly reduced in both groups from baseline to 5 years and was less in the HA group than in the SVF group at 5 years. Compared to the HA group, a higher percentage of patients in the SVF group had a reduced or unchanged grade of full-thickness cartilage defects, and a lower percentage of patients experienced progression (Fig. 5). There was no significant difference in BML size, severity, patella-femoral pathology or mechanical axis from baseline to 5 years and no difference between the two groups. There was no significant difference in the change in KL grade from baseline to 5 years between the two groups.

Univariate (unadjusted) risk factors

Univariate Cox regression results indicated age (per year increase, HR 1.032; 95% CI 1.013–1.051; $p=0.001$), BMI (per point increase, HR 1.111; 95% CI 1.032–1.197; $p=0.005$), treatment (SVF vs HA, HR 3.067; 95% CI 1.849–5.089; $p<0.001$), KL grade (2 vs 3, HR 1.718; 95% CI 1.277–2.311; $p<0.001$), mechanical axis (per degree increase, HR 1.101; 95% CI 0.993–1.221; $p=0.068$), full-thickness cartilage defect (per grade increase, HR 1.581; 95% CI 1.177–2.123; $p=0.002$), total cartilage volume (per mm^3 increase, HR 1.000; 95% CI 1.000–1.000; $p=0.018$), BML size (per mm^2 increase, HR 1.002; 95% CI 1.001–1.003; $p<0.001$) and BML severity (per point increase, HR 1.154; 95% CI 1.093–1.219; $p<0.001$) as possible risk factors. Sex and patella-femoral pathology were not risk factors in the unadjusted analysis ($p>0.1$). The results are shown in Table 6.

Independent risk factors

Linear regression showed no collinearity (VIF < 3) for age, BMI, treatment, KL grade, mechanical axis, patella-femoral pathology, full-thickness cartilage defect, total cartilage volume, BML size or BML severity score. In the final multivariate Cox regression model, BML severity score (per point increase, HR 1.104; 95% CI 1.013–1.202; $p=0.024$), BMI (per point increase, HR 1.096; 95% CI 1.003–1.198; $p=0.043$) and treatment (SVF vs HA, HR 3.602; 95% CI 2.116–6.131; $p<0.001$) were independent risk factors for prognosis. The use of SVF reduces the risk of clinical failure by 2.602 times compared with HA. Each score increase in BML severity increased the risk of clinical failure by 0.104 times. Each score increase in body mass index increased the risk of clinical failure by 0.096 times. Age, KL grade, mechanical axis, patella-femoral pathology, total cartilage volume, full-thickness cartilage

Table 5 Changes in radiographic variables

		SVF ($N=51$)	HA ($N=64$)	*p* value
BML size, mm^2	Baseline	123.48 ± 197.02	105.49 ± 151.12	0.581
	5 years	90.33 ± 141.01	95.54 ± 146.76	0.848
	p value	0.149	0.516	
BML severity	Baseline	3.02 ± 4.14	2.64 ± 3.34	0.588
	5 years	2.59 ± 3.16	2.56 ± 3.30	0.966
	p value	0.125	0.773	
Total cartilage volume, mm^3	Baseline	16,467.89 ± 2739.13	15,718.20 ± 2071.90	0.109
	5 years	15,121.11 ± 3174.45	13,473.30 ± 2489.59	0.003
	p value	< 0.001	< 0.001	
Mechanical axis, °	Baseline	Varus 1.48 ± 2.16	Varus 1.24 ± 2.02	0.536
	5 years	Varus 1.75 ± 2.11	Varus 1.40 ± 2.03	0.373
	p value	0.164	0.208	
Patella-femoral pathology	Baseline	25 (49.0%)	22(34.4%)	0.112
	5 years	30 (58.8%)	29 (45.3%)	0.150
	p value	0.321	0.206	
Full-thickness defect	Decrease	3 (5.9%)	0 (0%)	0.043
	No change	44 (86.3%)	52 (81.3%)	
	Increase	4 (7.8%)	12 (18.8%)	
KL grade	Decrease	0 (0%)	0 (0%)	0.524
	No change	43 (84.3%)	51 (79.7%)	
	Increase	8 (15.7%)	13 (20.3%)	

SVF, stromal vascular fraction; HA, hyaluronic acid; KL, Kellgren–Lawrence; BML, bone marrow lesion

defects and BML size were not risk factors in the overall model ($p > 0.05$) after adjusting for confounding factors. The results are shown in Table 6.

Discussion

The most important finding of this study is that acceptable clinical state was present for approximately 60% of patients after SVF treatment. In terms of change in pain scores, the SVF group was superior to the control group, and although the WOMAC scores did not show an advantage for HA in overall effect, a crossover effect of time and grouping was present, and we turned to assess the scores at each time point alone, which were superior to the control group. Although the difference in WOMAC scores was not significant in the SVF group compared to pre-treatment after 5 years, it was still better than the control group However, what is not sufficiently convincing is that reports based on the mean and standard deviation of clinical score changes often reflect the average level of the subject and do not address the individual patient's perspective, making it difficult to determine the efficacy [20]. Therefore, we defined clinical failure based on the above evaluation and set it as KOA-related surgery and scores that did not meet the PASS, which fit the patients' own willingness to accept the symptoms. Comparing the responsive curves of the two groups, the 5-year responsive rate of SVF group was significantly better than that of the control group and exceeded 60%, indicating that patients treated with SVF were less likely to experience clinical failure in 5 years. Tran et al. [5] observed an improvement in clinical symptoms in SVF-treated patients after two years of follow-up, in which they attributed to paracrine mechanisms related to the anti-inflammatory effects of cell therapy. However, for mid-term prognosis, there is no evidence that the anti-inflammatory effect could be sustained over such a long period of time, so cartilage changes remain a significant consideration. Imaging of 5-years postoperative results revealed that cartilage volume was reduced in both groups compared to the preoperative period, but total cartilage volume was still higher in the SVF group than in the HA group, and a small proportion of SVF patients showed signs of repair of the full-thickness cartilage defect. This is consistent with a previous short-term study in which Song et al. observed an increase in cartilage volume in patients treated with ADSCs for 72 weeks, which began to decrease at 96 weeks [21]. This phenomenon may be related to an unavoidable natural consequence of ageing [22, 23]. Regarding the mechanism of cartilage volume effect, we suggest that firstly, SVF may promote cartilage regeneration through

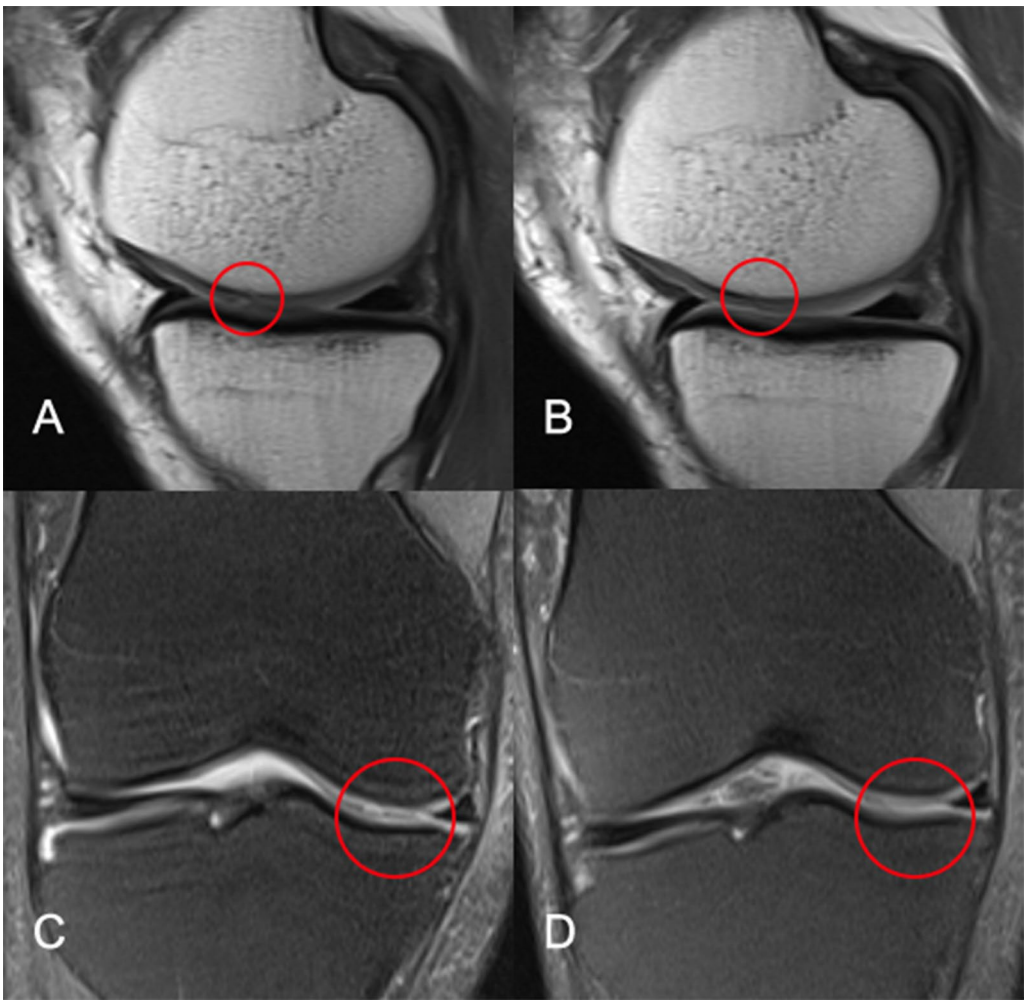

Fig. 5 MRI evaluation of full-thickness cartilage defect changes at 5 years. **A**, **C** Coronal and sagittal images of the medial femur and tibia before injection of SVF. A grade 1 full-thickness cartilage defect can be observed in the circle. **B** Coronal and sagittal images of the medial femur and tibia 5 years after SVF injection. The full-thickness cartilage defect in the circled area disappeared, and the cartilage edge was smooth

Table 6 Unadjusted and adjusted risk of clinical failure

Variable	Univariate analysis		Multivariate analysis	
	p value	Unadjusted HR (95% CI)	*p* value	Adjusted HR (95% CI)
Sex (male vs female)	0.420	1.248 (0.728–2.139)		
Age (per year increase)	0.001	1.032 (1.013–1.051)	0.140	1.014 (0.995–1.034)
BMI (per point increase)	0.005	1.111 (1.032–1.197)	0.043	1.096 (1.003–1.198)
Treatment (SVF vs HA)	< 0.001	3.067 (1.849–5.089)	< 0.001	3.602 (2.116–6.131)
KL grade (2 vs 3)	< 0.001	1.718 (1.277–2.311)	0.218	1.277 (0.865–1.885)
Mechanical axis (per degree increase)	0.068	1.101 (0.993–1.221)	0.689	1.023 (0.917–1.141)
Full-thickness cartilage defect (per grade increase)	0.002	1.581 (1.177–2.123)	0.403	1.158 (0.821–1.634)
Total cartilage volume (per mm^3 increase)	0.018	1.000 (1.000–1.000)	0.919	1.000 (1.000–1.000)
BML size (per mm^2 increase)	< 0.001	1.002 (1.001–1.003)	0.627	1.000 (0.999–1.002)
BML severity (per point increase)	< 0.001	1.154 (1.093–1.219)	0.024	1.104 (1.013–1.202)
Patella-femoral pathology	0.310	1.263 (0.805–1.981)	0.873	0.960 (0.578–1.592)

HR, hazard ratio; CI, confidence interval; BMI, Body mass index; KL, Kellgren–Lawrence; BML, bone marrow lesion

specific differentiation and paracrine signalling of different cell groups [24], but there is no evidence that SVF cells can directly differentiate into chondrocytes or tissues in human body. Similar to this study, several short-term clinical studies have observed the repair of cartilage defects and the widening of joint space by MRI, which indicates the result of cartilage regeneration but the processes involved need further study [5, 6, 25]. Secondly, inflammatory factors such as IL-1 and TNF-α play an important role in the progression of OA, which can promote the release of matrix metalloproteinases and make the catabolism of articular cartilage [26, 27]. After injection of SVF into the knee joint, the ADSCs produced IL-1 receptor antagonists and the tissue protective protein tumor necrosis factor-stimulated gene-6 (TSG-6), and exerted anti-inflammatory effects on chondrocytes and synovial cells via prostaglandin E2 [28, 29]. In addition, ADSCs promote the polarization of non-polarized macrophages and mature dendritic cells towards anti-inflammatory and phagocytic phenotypes [30]. Other substances in SVF may also play an anti-inflammatory role. Morris et al. [31] found that macrophages (CD11b) in adipose tissue accounted for 20% of the cells obtained from SVF, 70% of which were positive for CD301, a marker of M2 macrophages, which has anti-inflammatory and pro-angiogenic functions. And in the fat grafting procedure performed by Dong et al. [32], the inclusion of SVF resulted in increased expression of CD206 (another phenotypic marker of M2 macrophages) and negative regulation of the pro-inflammatory agents IL-1β and IL-6.The reduction in inflammation resulted in less cartilage damage, destruction, and cartilage regeneration occurred in the SVF group, but not in the HA group, ultimately causing less cartilage volume loss in the SVF group than in the HA group, although cartilage in both groups still inevitably degenerated. In addition to ADSCs, SVF contains heterogeneous cell types and different factors with paracrine effects, which may result in more significant benefits and cartilage healing potential [33]. Maintenance of existing MSCs and their functions through molecular and structural synergy is a possible mechanism. Traktuyev et al. [34] demonstrated that certain factors produced by MSCs in SVF, such as VEGF, enable better migration and survival of endothelial precursor cells (EPCs), while EPCs, by producing PDGF-BB, in turn enable MSCs to proliferate and migrate to the site of injuried tissue. Other differentiated cells such as progenitor cells in SVF may also promote cartilage regeneration. Zhao et al. measured the composition changes of articular cartilage in KOA patients before and after intra-articular injection of adipose-derived progenitor cells by multi-compositional MRI, and observed the improvement of articular cartilage [35]. We speculate that the cartilage volume advantage achieved by SVF treatment over controls may be more relevant to mid-term clinical acceptable state.

Adipose-derived cell therapies commonly use culture-expanded ADSCs and SVF. Agarwal et al. meta-analyzed 18 studies of ADSCs and SVF for KOA and concluded that although the dose or number of injections of ADSCs or SVF varied, patients showed improvement in pain and function from 2 to 24 months postoperatively [36], which is consistent with our short-term results, suggesting that compared to ADSCs, less preparation for SVF injections may be an advantage, as both therapies have achieved good clinical outcomes. The amount of ADSCs in SVF tends to be less than culture-expanded ADSCs, but the effect of ADSCs dose on efficacy is controversial. Jo et al. found more significant improvement in KOA pain at high cell doses (1×10^8 cells) [37], while another study showed better results at lower doses (2×10^6 cells) [38]. There are few clinical studies directly comparing the two therapies, only Yakota et al. conducted a study in this area and found that ADSCs were more clinically significant than SVF for KOA with more rapid action and fewer complications after a 6-month follow-up [39]. However, a recent animal study showed that SVF was more effective than culture-expanded ADSCs in the short-term repair of damaged cartilage and reduction of inflammatory factors such as IL-6 and TNFα in the synovial fluid [40]. Clinical studies on the differences in cartilage repair between the two therapies have not been reported. Therefore, more clinical studies are still needed to draw strong conclusions about which treatment is better. Based on the available evidence in the literature, we need to be aware that SVF is still a good treatment option.

To the best of our knowledge, our study has a mid follow-up period and exploring the factors influencing prognosis for the first time. BML severity which reflects the depth of spread of BML in bone tissue at multiple MRI slices was an independent predictor of prognosis after adjusting for confounding factors rather than cartilage-related variables. The pathology of BML is often thought to be related to bone resorption, with continued progression of BML secondary to the expansion of the area of necrosis, fibrosis subchondral tideline drift and subchondral remodelling, leading to focal, vertical shear stresses [41–43], which accelerate loss of cartilage so that BML seem to be dominant. In this study, we also focused on BML size, as the largest area shown on a single MRI slice, which was shown not to be an influencing factor, and we consider that the BML status at one level alone does not reflect the grade of BML and the impact on prognosis. Although a previous study found that SVF has the potential to reduce bone marrow lesions during

short-term treatment [5], we found, in the present study, that BML was not significantly improved at 5 years after SVF treatment compared with the preoperative results and compared with the data in the control group, suggesting that SVF injection in the joint cavity does not seem to improve BML in the subchondral bone. Intra-articular injections supplemented by subchondral injections may be an option to try. The most direct correlation between BML and clinical symptoms is pain, and intolerable pain is often the immediate cause of patients seeking medical treatment. A 6-month retrospective study [44] showed that the pretreatment presence of BML was also associated with daily activities and function in the short term, suggesting that BML is more responsive to PASS regardless of pain or function. Since preoperative cartilage factors are not influential factors in prognosis for clinical failure, we believe that sufficient attention should be given to BML severity in serial MRI slices, as this may imply a higher risk of failure with the treatment of SVF. Therefore, we continue to recommend active intervention with BML.

The KL grade is the most widely used method in clinical practice for assessing KOA severity. A 2-year follow-up of 30 patients undergoing stem cell therapy found that the KOOS score of KL grade 2 was superior to that of grade 3 [45]. Another study showed that the KL3 group improved more than the KL2 group after SVF treatment [5]. We compared the prognosis of KL grades 2 and 3, which turned out not to be an independent risk factor. The KL grade may miss meaningful changes in the bone marrow and cartilage and therefore is not recommended for the evaluation of regenerative therapies [46].

It is well known that obese patients have an increased load on weight-bearing joints and thus an increased risk of KOA [47]. A cross-sectional study showed a dose–response relation between high BMI and pain and function in patients with KOA [48]. This may also apply to prognostic analysis, where patients with high BMI are also at increased risk of clinical failure.

There are limitations in the present study. First, the efficacy of SVF in patients with KL grade 4 is unknown. Second, since we performed a simple intra-articular injection without lesion site localization, the exact destination of SVF cells in the joint is unknown, which limits our further understanding of the mechanism of action of SVF. Targeting SVF injections to specific lesion sites and tracking the localization of SVF cells under MRI is a direction for future research.

Conclusions

Up to 5 years after autologous SVF treatment, acceptable clinical state was present for approximately 60% of patients with less cartilage volume loss. In addition, the high severity of BML and high BMI increased the risk of clinical failure. Intra-articular injection of SVF does not improve subchondral BML.

Abbreviations

SVF: Stromal vascular fraction; KOA: Knee osteoarthritis; ADSCs: Adipose derived stem cells; HA: Hyaluronic hcid; VAS: Visual analogue scale; WOMAC: Western Ontario and McMaster University Osteoarthritis Index; PASS: Patient acceptable symptom state; BML: Bone marrow lesion; BMI: Body Mass Index; VIF: Variance inflation factor; KL: Kellgren–Lawrence.

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None.

Authors' contributions

SZ and HX: collection and assembly of data, manuscript writing; MF, JZ and MX: data analysis and interpretation, manuscript writing; BH: imaging assessment, manuscript writing; DC: data analysis and manuscript preparation; QM and PT: conception and design, imaging assessment, manuscript writing, final approval. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was retrospectively registered at Chinses Clinical Trial Registry with identifier ChiCTR2100052818 and was approved by ethics committee of the First Affiliated Hospital of Zhejiang Chinese Medical University, number 2013-X-063. All enrolled patients' written informed consents were obtained.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

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TRANSLATIONAL AND CLINICAL RESEARCH

Intra-Articular Injection of Mesenchymal Stem Cells for the Treatment of Osteoarthritis of the Knee: A Proof-of-Concept Clinical Trial

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Key Words. Osteoarthritis • Adipose-tissue derived mesenchymal stem cells • Intra-articular injection • Cartilage regeneration

ABSTRACT

Mesenchymal stem cells (MSCs) are known to have a potential for articular cartilage regeneration. However, most studies focused on focal cartilage defect through surgical implantation. For the treatment of generalized cartilage loss in osteoarthritis, an alternative delivery strategy would be more appropriate. The purpose of this study was to assess the safety and efficacy of intra-articular injection of autologous adipose tissue derived MSCs (AD-MSCs) for knee osteoarthritis. We enrolled 18 patients with osteoarthritis of the knee and injected AD MSCs into the knee. The phase I study consists of three dose-escalation cohorts; the low-dose (1.0×10^7 cells), mid-dose (5.0×10^7), and high-dose (1.0×10^8) group with three patients each. The phase II included nine patients receiving the high-dose. The primary outcomes were the safety and the Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) at 6 months. Secondary outcomes included clinical, radiological, arthroscopic, and histological evaluations. There was no treatment-related adverse event. The WOMAC score improved at 6 months after injection in the high-dose group. The size of cartilage defect decreased while the volume of cartilage increased in the medial femoral and tibial condyles of the high-dose group. Arthroscopy showed that the size of cartilage defect decreased in the medial femoral and medial tibial condyles of the high-dose group. Histology demonstrated thick, hyaline-like cartilage regeneration. These results showed that intra-articular injection of 1.0×10^8 AD MSCs into the osteoarthritic knee improved function and pain of the knee joint without causing adverse events, and reduced cartilage defects by regeneration of hyaline-like articular cartilage. STEM CELLS *2014;32:1254–1266*

INTRODUCTION

Osteoarthritis of the knee is the most common form of arthritis that cause pain, stiffness, and decreased function, and one of leading causes of disability among noninstitutionalized adults [1, 2]. More than 50 modalities of pharmacological, nonpharmacological, and surgical treatment are reported in the literature [3]. However, the current most common treatments for osteoarthritis except for joint replacement have at best modest albeit clinically relevant effects and can endanger substantial adverse events or costs, or both [4]. Furthermore, these treatments are generally intended to decrease pain, maintain or improve joint function, and minimize disability, not to regenerate articular cartilage, whereas osteoarthritis is characterized by the degeneration of the extracellular matrix resulting in loss of articular cartilage [5, 6].

For regeneration of articular cartilage, various efforts including cell therapy and tissue engineering have been tried. Chondrocytes are one of the most extensively investigated cells showing positive clinical outcomes [7–10]. Nevertheless, chondrocyte implantation has inherent disadvantages such as a two-stage surgical procedure that may cause further cartilage damage and degeneration [8, 10, 11] and chondrocyte dedifferentiation during culture that might result in fibrocartilage rather than hyaline cartilage [8, 12]. Moreover, its use has been limited to focal cartilage defect caused by injury while generalized cartilage loss seen in osteoarthritis has been its exclusion criterion [8, 10], suggesting the need to find a different approach for cartilage regeneration in osteoarthritis.

Mesenchymal stem cells (MSCs) have also been focused as an emerging regime for cartilage regeneration. Unlike chondrocytes implantation, the use of MSCs for regeneration of human articular cartilage is still investigational [13–15]. Recently, some authors reported results of direct intra-articular injection of

MSCs into the knee for the treatment of focal defect or more generalized cartilage loss in osteoarthritis [16–21]. Direct intra-articular injection of MSCs would offer great advantages if it could be translated into clinical practice as it would avoid surgeries and associated side effects, such as hypertrophy and ossification of periosteal coverage, immune reaction and disease transmission caused by xenograft coverage. More importantly, simplicity and ease of the injection could provide better treatment opportunities, especially for the elderly with comorbidity. Despite this potential, no clinical trials have been performed but a few case reports. Therefore, we conducted a proof-of-concept phase I/II clinical trial to assess the safety and the efficacy of intra-articular injection of autologous adipose tissue derived MSCs (AD MSCs) in patients with knee osteoarthritis. We report the clinical, radiological, arthroscopic, and histological results.

Materials and Methods

Study Design and Patients

This study is a phase I/II clinical trial with no active control conducted between March 2009 and September 2011 at SMG-SNU Boramae Medical Center, Seoul, Korea. The protocol was approved by the institutional review board of our institute. All participants provided written informed consent.

The phase I study consisted of three dose-escalation cohorts; the low-, mid-, and high-dose group with three patients each. Patients in each dose group received 1.0×10^7, 5.0×10^7, and 1.0×10^8 cells in 3 mL of saline, respectively. After three patients in each cohort were followed up for 28 days after injection, a safety review was done before moving to the next dose or phase (Supporting Information Method 1). The phase II included nine patients receiving the high-dose. Therefore, 18 patients were granted by the Korean Food and Drug Administration and were consecutively enrolled in the trial.

Eligible patients were between 18 and 75 years of age with idiopathic osteoarthritis of the knee of grade 2 or more according to Kellgren-Lawrence criteria and had an average pain intensity of grade 4 or more on a 10-point visual analog scale (VAS) for at least 4 months. Details of inclusion and exclusion criteria are listed in the Supporting Information Method 2.

Patients underwent physical examination, laboratory tests including routine blood and urine tests, serologic tests, tumor screening, and the pregnant test if indicated, and magnetic resonance imaging (MRI) of the knee at screening after providing informed consent. All pain medications except the rescue analgesic, acetaminophen, were discontinued (Supporting Information Method 3). Eligible patients returned to the hospital within 1 week for liposuction. Arthroscopy and cell injection was performed 3 weeks after liposuction. Patients were followed up at 1, 2, 3, and 6 months after injection. At each visit, the safety and efficacy assessments were performed. Furthermore, MRI of the knee was obtained at 3 and 6 months after injection. Second-look arthroscopy was performed at 6 months after injection. A 2-mm-punch biopsy specimen was obtained from the center of the cartilage defect of the medial femoral condyle at the first arthroscopy, and from the adjacent area to the first biopsy site at the second-look arthroscopy in patients who gave consent in the high-dose group. Independent safety and data monitors oversaw the overall trial process.

MSC Preparation

AD MSCs (Jointstem; K-STEM CELL, Seoul, Korea, http://www.k-stemcell.com/) were prepared from the abdominal subcutaneous fats by liposuction under good manufacturing practice conditions, as previously described (Supporting Information Method 4) [22]. Cells were tested before shipping for cell number, viability, purity (CD31, CD34, CD45), identity (CD73, CD90), sterility, endotoxin, and mycoplasma (Supporting Information Table 1).

Arthroscopy and Stem Cell Injection

All procedures were performed in the supine position under spinal anesthesia. A single orthopedic surgeon performed all procedures. Standard arthroscopic examination of the knee was performed; articular cartilage lesions were measured with a calibrated arthroscopic probe and graded according to the international cartilage repair society (ICRS) cartilage injury classification [23]. After diagnostic exploration, AD MSCs in 3 mL of saline were injected into the knee joint through the medial portal via 22G spinal needle. No debridement, synovectomy, or meniscectomy was performed during arthroscopy, and no drainage was used. Postoperative rehabilitation is described in the Supporting Information Method 5.

Outcome Measures

Primary outcomes were the safety and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) at 6 months after injection [24]. The safety was assessed with vital signs, physical examination, laboratory tests, adverse events, and serious adverse events. Adverse events were categorized using National Cancer Institute-Common Terminology Criteria for Adverse Events version 4.0 scale (NCI-CTCAE v4.0). The WOMAC is a validated, self-administered outcome measure designed to evaluate knee and hip osteoarthritis; higher scores mean increased pain, stiffness, and decreased function [24].

Secondary outcomes included four categories: clinical, radiological, arthroscopic, and histological. Clinical outcomes included a visual analog scale for knee pain on a scale from 0 to 10, and Knee Society Clinical Rating System (KSS) score [25]. Radiological outcomes were measured with Kellgren-Lawrence grade [26], joint space width of the medial compartment [27], mechanical axis with weight bearing line [28], and anatomical axis using x-ray. The size, depth of cartilage defect, and signal intensity of regenerated cartilage was also measured using MRI by a blinded musculoskeletal radiologist as previously described (Supporting Information Method 6) [29, 30]. In addition, changes of the cartilage volume of the knee joint were measured using a semiautomated segmentation method by a blinded researcher (Supporting Information Method 7) [31]. Arthroscopy was performed to evaluate any change in cartilage defect at the time of cell injection and at 6 months after injection. The size and ICRS grade of cartilage defect was measured. If cartilage was regenerated at second-look arthroscopy, ICRS grade of the defect was changed only when regenerated cartilage covered more than 50% of the original defect. For histological assessment, biopsy specimens were subject to safranin O staining and immunohistochemistry for type I and II collagen as previously described with slight

modification (Supporting Information Method 8) [32]. Thickness of regenerated cartilage was measured, and specimens were evaluated with ICRS II by a histopathologist [33].

Statistical Analysis

The sample size (18 patients) was decided in consultation with the Korean FDA. Outcome measures were analyzed based on the intention-to-treat population. Missing data were replaced with multiple imputations (10 sets) under a missing-at-random assumption [34]. Ten imputed datasets were generated, analyzed separately for each outcome measure, and then combined into a single set of estimates according to the Rubin rules [35]. For sensitivity test, single imputation using the last-observation-carried forward method and a complete-case analysis were additionally performed [36]. Because all of the three methods did not yield meaningful changes in each measurement, we presented only the imputation analyses. Changes from baseline in all the measures that were scale variables were determined with a paired t test. Kellgren-Lawrence grade, depth of the cartilage defect measured by MRI, and ICRS grade determined with arthroscopy were determined with a Wilcoxon signed rank test. The analysis was performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC).

Results

Demographics of Patients

Twenty-five patients were assessed for eligibility, and 18 patients were consecutively allocated to treatment groups and received AD MSCs (Fig. 1). Generally, all the patients enrolled in the study showed similar baseline characteristics of age, height, weight, body mass index, and radiographic grade of osteoarthritis. One patient in the mid-dose group withdrew consent after cell injection. Another patient completed follow-up except for the second-look arthroscopy. The other 16 patients completed 6 months of follow-up. Analysis was performed according to the level of cell doses (low-, mid-, and high-dose), not to the phase of the trial, and according to the intention-to-treat principle in clinical, radiological, and arthroscopic assessments. Histological assessments were performed in specimens from eight patients in the high-dose group who gave consent for biopsies at both arthroscopies.

Patients in each group had similar baseline characteristics (Table 1). Generally, females aged 60 years with an average body-mass index around 26 who suffered for more than 5 years despite conservative treatments were included in the study. All patients had osteoarthritis of the knee of Kellgren-Lawrence grade 3 or 4. Baseline cartilage defect of the medial femoral condyle measured with MRI was 407.0, 535.0, and 497.9 mm^2 in the low-, mid-, and high-dose group, respectively.

Safety

Adverse events occurred in two (67%), two (67%), and five (42%) patients in the low-, mid-, and high-dose group, respectively (Table 2). None of them was grade 3 or 4 by NCI-CTCAE scale or treatment-related. The most common adverse event was nasopharyngitis, which developed in one patient in each group (Supporting Information Table 2). There was one serious adverse event, urinary stone, which occurred in a patient in the low-dose group with a previous history. He was treated with extracorporeal shock wave lithotripsy and medicine. He was fully recovered and completed follow-up. Two patients reported arthralgia. One patient in the mid-dose group reported bilateral knee pain; the ipsilateral pain and tenderness was due to pes bursitis which has been known commonly accompanied in the osteoarthritis knee. And the contralateral pain was due to osteoarthritis of the contralateral knee. The other patient in the high-dose group also reported pain and tenderness in the pes anserinus of the ipsilateral knee. Both patients were managed with knee stretching and quadriceps setting exercise and intermittent acetaminophen. Both of them completed follow-up. No patients were discontinued from the study because of adverse events. There were no clinically important trends in the results of physical examination, vital signs, laboratory test during the study.

Clinical Outcomes

AD MSCs injection was associated with improvement of the WOMAC score at 6 months after injection as compared with baseline in the high-dose groups (Fig. 2A; Supporting Information Table 3). The mean reduction from the baseline over 6 months was 39% in the high-dose group, from 54.2 ± 5.2 to 32.8 ± 6.3 ($p = .003$). Patients in the low- and mid-dose group did not improve over 6 months. Visual analog scale for knee pain significantly decreased from 79.6 ± 2.2 to 44.2 ± 6.3 in the high-dose group only (45% decrease; $p < .001$) (Fig. 2B).

The knee score of KSS significantly increased in the low-dose group from 41.3 ± 6.8 to 79.0 ± 12.5 (91% increase; $p = .025$) and in the high-dose group from 47.2 ± 2.6 to 71.0 ± 4.4 (50% increase; $p < .001$) (Fig. 2C). Meanwhile, the function score of KSS significantly increased in the low-dose group only from 60.0 ± 5.8 to 83.3 ± 8.8 (39% increase; $p = .020$) (Fig. 2D).

Radiological Outcomes

Kellgren-Lawrence grade, joint space width, mechanical axis, and anatomical axis did not change significantly over 6 months in all dose groups (Supporting Information Table 4). Serial MRI examinations found gradual regeneration of articular cartilage in the medial femoral and tibial condyles over 6 months (Fig. 3A). At 3 months, thin cartilage was noticed in the both condyles. It thickened and became mature with isointensity at 6 months.

The size of cartilage defect measured with MRI significantly decreased both in the medial femoral and tibial condyles as well as in the lateral femoral and tibial condyles at 6 months in the high-dose group. (Fig. 3A; Supporting Information Table 5); from 497.9 ± 29.7 mm^2 to 297.9 ± 51.2 mm^2 in the medial femoral condyle (40% decrease; $p = .004$), from 333.2 ± 51.2 mm^2 to 170.6 ± 48.2 mm^2 in the medial tibial condyle (49% decrease; $p < .001$), from 103.6 ± 27.1 mm^2 to 51.1 ± 24.9 mm^2 in the lateral femoral condyle (51% decrease; $p = .011$), and from 19.4 ± 7.3 mm^2 to 10.4 ± 4.2 mm^2 in lateral tibial condyle (46% decrease; $p = .041$), but not in the patella, from 93.3 ± 33.3 mm^2 to 79.1 ± 27.6 mm^2 (15% decrease; $p = .340$). There were no significant changes in the other dose groups. The depth of the cartilage defect did not show significant changes over 6 months in all dose groups (Supporting Information Table 6). The signal intensity

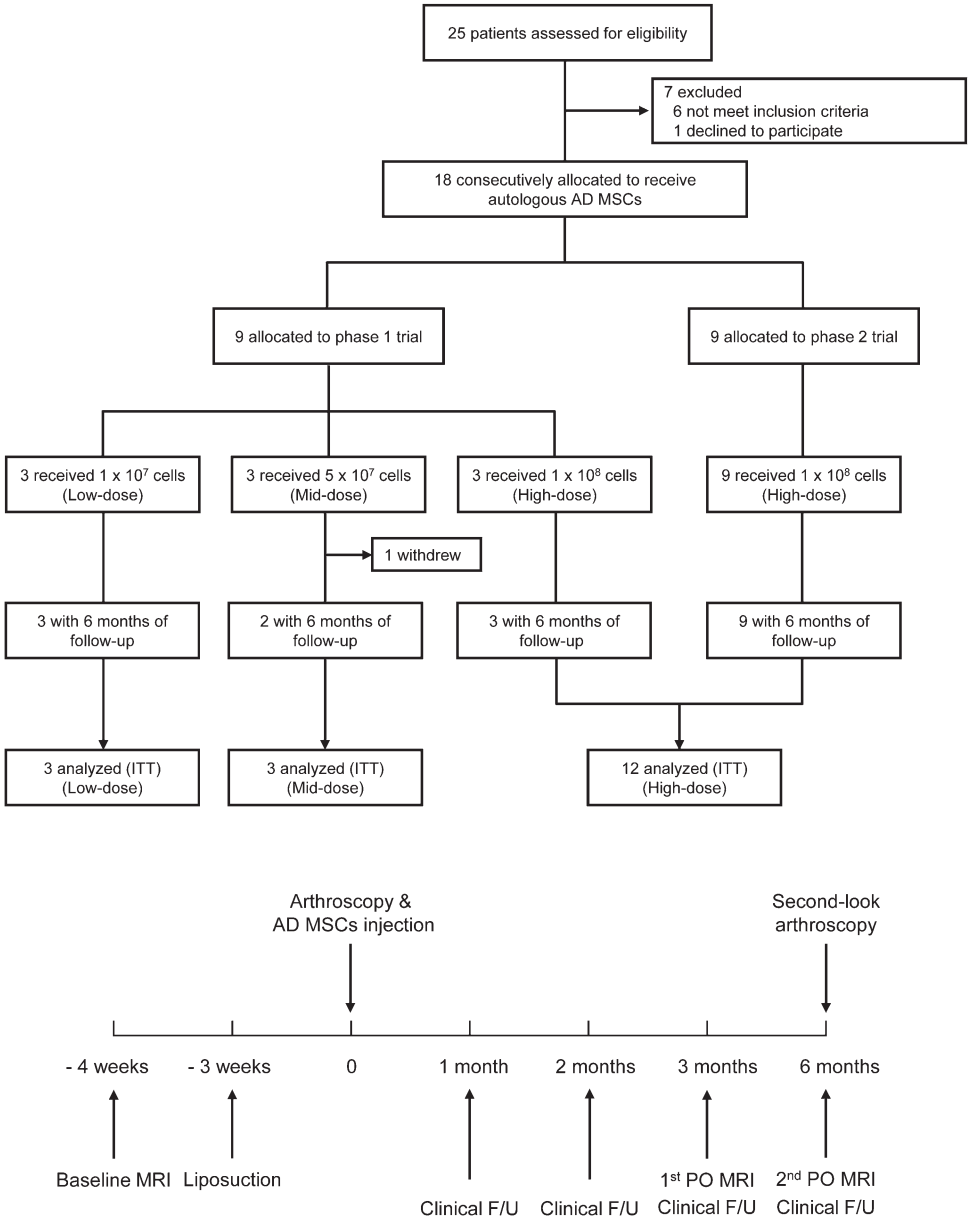

Figure 1. Study flow diagram. Abbreviations: AD MSCs, adipose-tissue derived mesenchymal stem cells; ITT, intention-to-treat; MRI, magnetic resonance imaging.

of regenerated cartilage in each compartment had a slight tendency to become isointense over 6 months in the high-dose group but without a statistical significance (Supporting Information Table 7).

The cartilage volume also increased gradually over time till 6 months both in the medial femoral and tibial condyles in the high-dose group (Fig. 3B; Supporting Information Table 8); from 3,313.7 ± 304.1 mm^3 to 3,780.6 ± 284.4 mm^3 in the medial femoral condyle (14% increase; $p = .044$) and from 1,157.5 ± 145.8 mm^3 to 1,407.7 ± 150.5 mm^3 in the medial tibial condyle (22% increase; $p = .047$). Meanwhile, patients in the low-dose group temporarily also showed increased cartilage volume from 3,315.0 ± 104.3 mm^3 to 3,959.7 ± 55.9 mm^3 at 3 months (27% increase; $p = .026$) in the medial femoral condyle. The cartilage volume of the lateral femoral and tibial condyles and the patella did not change in all dose groups over 6 months).

Second-Look Arthroscopy

As a gold standard for articular cartilage assessment, arthroscopy before and 6 months after AD MSCs injection demonstrated findings consistent with clinical and radiological outcomes. Macroscopically, regenerated cartilage formed in the most severely degenerated area with ICRS grade 3 in the medial femoral and tibial condyles, whereas it was hardly seen in the less severely degenerated area in the lateral compartment and the patella (Fig. 4A–4C). Regenerated cartilage looked glossy white with a smooth surface. With a probe, it felt firm like healthy articular cartilage in the medial femoral condyle, whereas it was less firm in the medial tibial condyle. No loose body, hypertrophy, or abnormal calcification was identified.

The size of cartilage defect measured with a calibrated probe demonstrated a significant reduction of the cartilage

Table 1. Baseline characteristics of patients in the low-, mid-, and high-dose groups

	Low-dose ($n = 3$)	Mid-dose ($n = 3$)	High-dose ($n = 12$)
Cells injected, No.	1×10^7	5×10^7	1×10^8
Age, mean (SD) (years)	63 (8.6)	65 (6.6)	61 (6.2)
Sex, No. (%)			
Male	1 (33.3)	0	2 (16.7)
Female	2 (66.7)	3 (100.0)	10 (83.3)
Height, mean (SD) (cm)	157 (6.7)	156 (1.4)	157 (4.8)
Weight, mean (SD) (kg)	64 (3.5)	68 (5.1)	64 (7.5)
Body-mass index, mean (SD)[a]	26 (1.0)	28 (2.1)	26 (2.1)
Symptom duration, mean (SD), (m)	63 (50.7)	144 (86.5)	117 (135.2)
Activity level (I:II:III:IV), No. (%)[b]			
I	0	0	0
II	0	0	2 (16.7)
III	2 (66.7)	3 (100.0)	7 (58.3)
IV	1 (33.3)	0	3 (25.0)
Functional status (I:II:III:IV), No. (%)[c]			
I	0	0	0
II	0	1 (33.3)	1 (8.3)
III	3 (100.0)	2 (66.7)	11 (91.7)
IV	0	0	0
Previous treatment history, No. (%)[d]			
Surgery	0	0	0
Pharmaceutical	0	1 (33.3)	6 (50.0)
Physiotherapy	0	0	1 (8.3)
Kellgren-Lawrence grade, No. (%)[e]			
Grade 3	2 (66.7)	2 (66.7)	8 (66.7)
Grade 4	1 (33.3)	1 (33.3)	4 (33.3)
Baseline WOMAC score, mean (SD)[f]	43 (22.0)	69 (10.2)	54 (17.9)
Baseline VAS pain score, mean (SD)[g]	70 (17.3)	78 (2.9)	80 (7.5)
Baseline KSS, mean (SD)[h]			
Knee score	41 (11.7)	35 (16.9)	47 (8.8)
Function score	60 (10.0)	57 (11.5)	71 (9.0)
Cartilage defect, mean (SD) (mm^2)[i]	407 (174.1)	535 (31.2)	498 (103.0)

Abbreviations: WOMAC, Western Ontario and McMaster Universities Osteoarthritis index; VAS pain, visual analog scale for pain; KSS, the Knee Society Score.
[a]Calculated as weight in kilograms divided by height in meters squared.
[b]Activity level I indicates high competitive sportsman/woman; II, well-trained and frequently sporting; III, sporting sometimes; IV, nonsporting.
[c]Functional status I indicates "I can do everything that I want to do with my joint"; II, "I can do nearly everything that I want to do with my joint"; III, "I am restricted and a lot of things that I want to do with my joint are not possible"; IV, "I am very restricted and I can do almost nothing with my joint without severe pain and disability."
[d]Each patient was asked whether he/she received surgery (yes or no), pharmaceutical treatment history during last 2 months (yes or no), and physical therapy during last 1 month (yes or no).
[e]Kellgren-Lawrence grade 3 indicates multiple moderate-sized osteophytes, definite narrowing of the joint space, some sclerosis, and possible deformity of bone contour; and grade 4, large osteophytes, marked narrowing of the joint space, severe sclerosis, and definite deformity of bone contour.
[f]WOMAC score evaluates osteoarthritis of the knee. Total scores can range from 0 to 96; higher scores indicate more severe disease.
[g]VAS pain assesses present knee pain with visual analog scale ranging from 0 to 10.
[h]KSS is a measure of functional ability of the knee reported as the two scores, knee socre and function score.
[i]Cartilage defect means the defect in the medical femoral condyle of each participant.

Table 2. Summary of adverse events

	Low-dose ($n = 3$)	Mid-dose ($n = 3$)	High-dose ($n = 12$)
Patients with AEs[a]			
All	2 (67%)	2 (67%)	5 (42%)
Treatment-related	0	0	0
Patients with SAEs[b]			
All	1 (33%)	0	0
Treatment-related	0	0	0

Abbreviations: AE, adverse event; SAE, serious adverse events.
[a]An AE is defined as any undesired medical incident which is not necessarily in cause-and-effect relationship to the treatment.
[b]A SAE is defined as any undesired medical incident which results in death, is life threatening, requires hospitalization, causes disability, or results in a congenital abnormality or birth defect.

defect from $1{,}225.7 \pm 282.8$ mm^2 to 837.8 ± 278.9 mm^2 in the medial femoral condyle (32% decrease; $p = .003$) and from 352.3 ± 77.6 mm^2 to 126.3 ± 43.8 mm^2 in the medial tibial condyle (64% decrease; $p = .008$) in the high-dose group (Fig. 4D). The size of cartilage defect in the lateral femoral and tibial condyle and the patella did not change in all dose groups over 6 months (Supporting Information Table 9).

The ICRS grade of the cartilage defect significantly improved in the medial femoral and tibial condyle in the high-dose group at second-look arthroscopy (Fig. 4E). No significant change was found in the lateral femoral and tibial condyles, and the patella did not change in all dose groups (Supporting Information Table 10).

Histological Outcomes

Generally, biopsy specimens from the medial femoral condyles had no articular cartilage before injection (ICRS 3C) (Fig. 5A). At 6 months after injection, articular cartilage with a thick, glossy white matrix and smooth surface was regenerated and was well-integrated with the subchondral

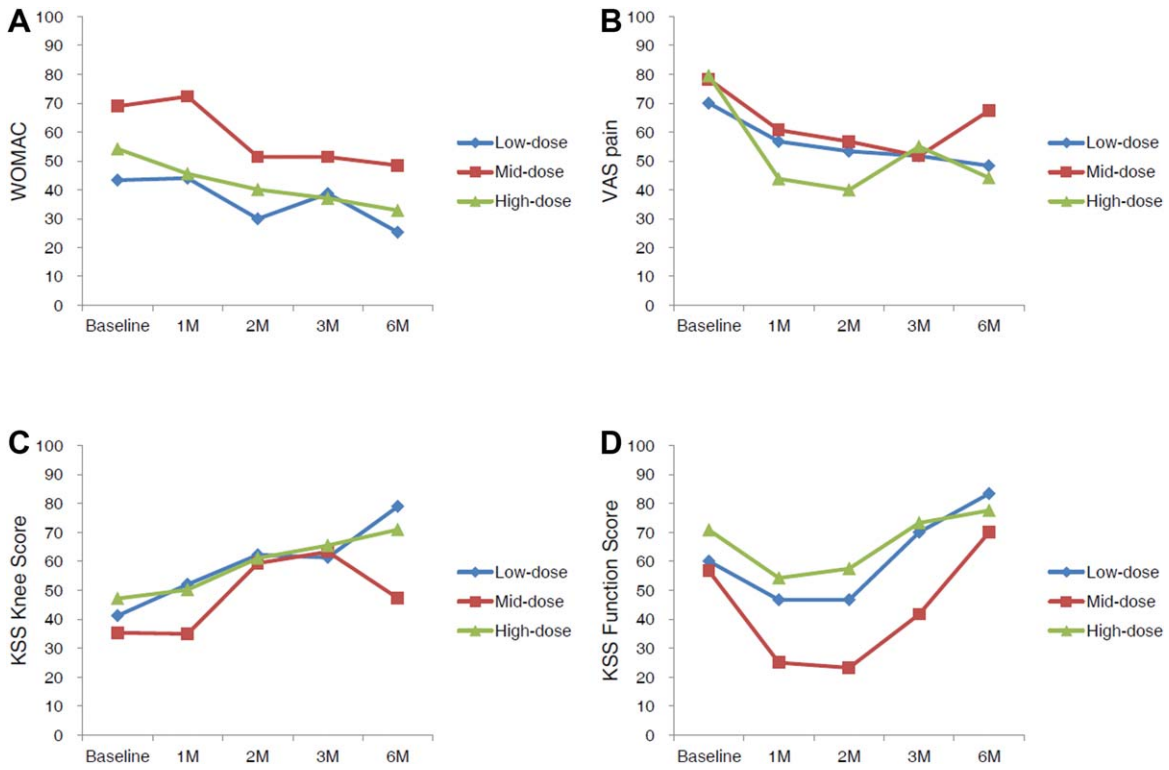

Figure 2. Changes of WOMAC, VAS for knee pain, and KSS knee and function score during 6 months after intra-articular injection of adipose derived mesenchymal stem cell (AD MSCs). **(A):** The WOMAC score. It showed a tendency of improvement in all dose groups over 6 months. However, the statistical significance was found in the high-dose group only. **(B):** Knee pain also showed a decreasing tendency over time but with the statistical significance only in the high-dose group. **(C):** KSS knee score similarly improved during 6 months in all dose group. The statistical significance was found in the high-dose group. **(D):** KSS function score showed a tendency of initial decrease and recovery after 2 months in all dose groups. The initial decrease was due to non-weight bearing for first 2 months after injection. Abbreviations: KSS, knee society clinical rating system score; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index.

bone. In the lower half of the middle zone and the deep zone, safranin O and type II collagen positive hyaline-like cartilage was clearly demonstrated, whereas type I collagen positive fibrocartilage was identified in the superficial and the upper half of the middle zone. Collagen fibrils in the superficial and middle zone run parallel and oblique to articular surface, respectively, whereas those in the deep zone run vertically. Chondrocytes are flattened in the superficial zone and round in the middle and deep zones similar to those in the deep zone of hyaline cartilage. Small chondrocytes are also found in the in the calcified cartilage zone. However, typical columnar chondrocytes or tide mark is not definite, suggesting that maturation is still in process [37]. In some patients with ICRS 3B before injection, hyaline-like articular cartilage similar to Figure 5A was also regenerated (Fig. 5B). Meanwhile, relatively thin fibrocartilage with minimal safranin O and type II collagen positive matrix was formed in the worst case (Fig. 5C). Additional histological data are available in the Supporting Information Figure.

The ICRS II scores changed significantly after AD MSCs injection in four parameters: surface architecture, and surface, mid, and overall assessments (Supporting Information Table 11). The mean thickness of articular cartilage increased from 0.4 ± 0.3 mm before injection, which increased to

Figure 3. Radiological evaluation of articular cartilage regeneration in the medial and femoral condyles after intra-articular injection of adipose derived mesenchymal stem cells (AD MSCs). **(A):** Sagittal and coronal MRIs of the medial femoral and tibial condyles before, 3, and 6 months after AD MSCs injection. Cartilage defects in the medial femoral condyle (green arrows in the upper row) and in the medial tibial condyle (yellow arrows in the lower row) are identified as signal voids between the two condyles. In the low-dose group, no significant changes are identified after injection at 3 months. Small cartilage island is barely noticed in the medial femoral condyle at 6 months. In the mid-dose group, thin and irregular regenerated cartilages can be seen both in the medial femoral and tibial condyles at 3 months. While regenerated cartilages thicken and enlarge more over next 3 months, they seem to be still thin, irregular, and of hyperintensity. In the high-dose group, regenerated articular cartilages can be found both in the medial femoral and tibial condyles at 3 months which are still thin but relatively smooth compared with those in the mid-dose group. At 6 months, regenerated cartilage became thicker, smoother, and mature with isointensity with surrounding cartilage in the both condyles. Cartilage defect in the medical femoral condyle significantly decreased at 6 months in the high-dose group. Meanwhile, cartilage defect in the medial tibial condyle decreased at 3 and 6 months in the high-dose group. **(B):** Changes of articular cartilage volume over 6 months after AD MSC injection in the medial femoral condyle (green in the upper row; right knee viewed from the above) and in the medial tibial condyle (orange in the lower row; right knee viewed from the below) in the high-dose group. The void seen at the baseline before injection (the left column) was gradually filled at 3 months (the middle column) and 6 months (the right column) in the medial femoral and tibial condyles. Articular cartilage volume in the medial femoral (the upper right graph) and tibial condyles (the lower right graph) significantly increased in the high-dose group. Abbreviation: MRI, magnetic resonance imaging.

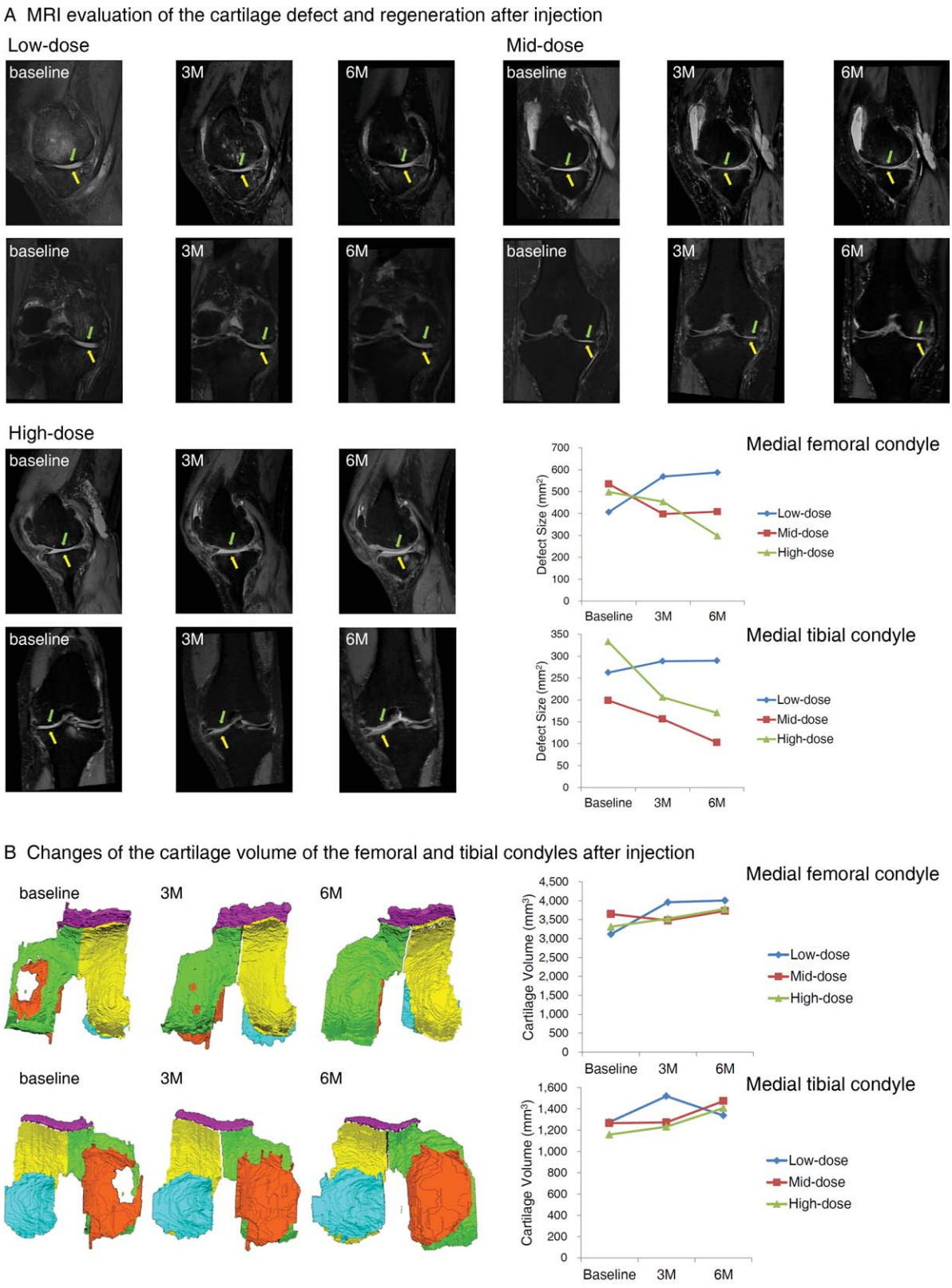
A MRI evaluation of the cartilage defect and regeneration after injection
Low-dose
Mid-dose
High-dose
baseline
3M
6M
Medial femoral condyle
Medial tibial condyle
Defect Size (mm²)
Low-dose
Mid-dose
High-dose
Baseline
3M
6M
B Changes of the cartilage volume of the femoral and tibial condyles after injection
Cartilage Volume (mm³)

Figure 3.

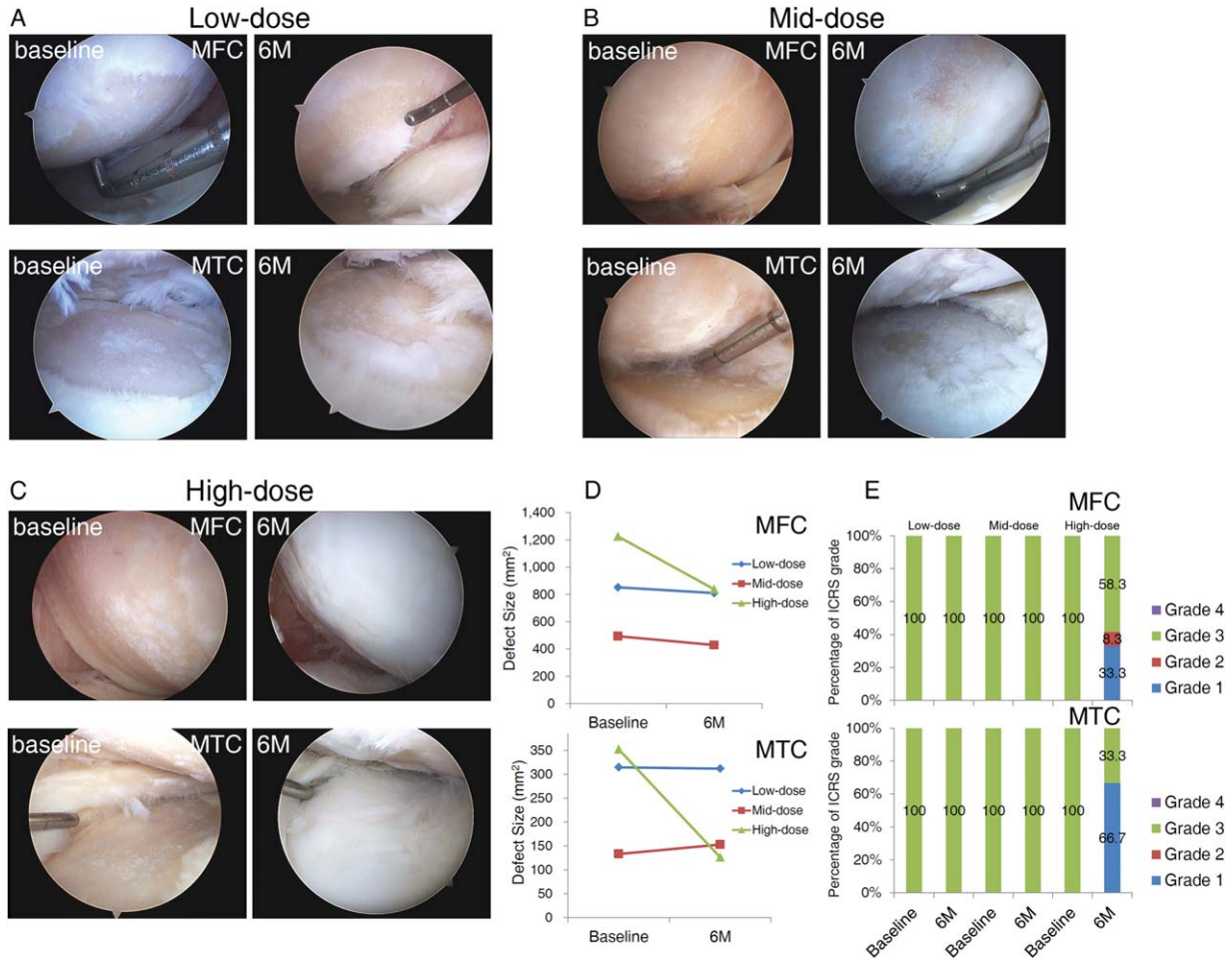

Figure 4. Arthroscopic evaluation of articular cartilage regeneration in the medial and femoral condyles after intra-articular injection of adipose derived mesenchymal stem cells (AD MSCs). **(A):** Arthroscopic finding shows large denuded medial femoral and tibial condyles (international cartilage repair society [ICRS] grade 3) before injection. After 6 months, while small cartilage islands are newly formed in both condyles, the majority of denuded both condyles are not covered. **(B):** Subchondral bones are exposed with nearly complete absence of articular cartilage in both condyles prior to injection. At 6 months, relatively moderate-sized newly formed white cartilage is visible in the medial femoral condyles. Multiple tiny cartilage patches are formed around it. **(C):** Complete absence of articular cartilage (ICRS grade 3) in both condyles before injection. Six months after injection of AD MSCs, a thick, glossy white, and firm hyaline-like cartilage is regenerated and covers the majority of cartilage defects in the medial femoral and tibial condyles. **(D):** Size change of the cartilage defect of the medial femoral and tibial condyles significantly decreased in the high-dose group 6 months after injection, but not in the low- and mid-dose group. **(E):** The ICRS grade of the cartilage defect significantly improved in the medial femoral and tibial condyles in the high-dose group whereas no significant change was found in the lateral femoral and tibial condyles, and the patella did not change in all dose groups (Supporting Information Table 10). Abbreviations: MFC, medial femoral condyle; MTC, medial tibial condyle.

1.6 ± 0.8 mm after injection (300%; $p = .004$). The mean thickness of regenerated cartilage in four patients who had no cartilage (ICRS grade 3C) before injection was also 1.6 ± 0.5 mm.

Discussion

This proof-of-concept trial reached its predetermined primary outcomes, that is, intra-articular injection of AD MSCs into osteoarthritic knee was not associated with apparent adverse events, but improved function of the knee measured with WOMAC over 6 months of follow-up. Patients in the high-dose group demonstrated significantly improved WOMAC score with a clinically meaningful pain reduction which is approximately 30% from the baseline [38]. Evaluation with MRI and second-look arthroscopy identified regenerated articular cartilage consistently in the high-dose group. Histological evaluation revealed that regenerated cartilage had a thick, glossy white matrix with a smooth surface, and was well-integrated with the subchondral bone. In the upper half of the middle and the deep zones, safranin O and type II collagen positive hyaline-like cartilage was clearly demonstrated, whereas type I collagen positive fibrocartilage was identified in the superficial and the upper half of the middle zones. Patients in the mid-dose group showed improvement in some clinical outcomes, but those in the low-dose group did not show improvement in most outcome measures. These results would be due to regeneration of articular cartilage as well as via paracrine effects, and that the effects were closely related to the number of injected AD MSCs.

We agree that osteoarthritis is a mesenchymal disease, that is to say, a condition in which the activity, phenotype, or mobilization of MSC population is altered, leading to an absence of repair and increased degeneration [39]. In osteoarthritis, MSCs are depleted and have reduced proliferative capacity and reduced ability to differentiate [40]. Therefore, provision of an adequate number of healthy and functional MSCs would be helpful for enhancing repair or inhibit the progression of cartilage loss [18]. Potential mechanisms of MSCs for the treatment of osteoarthritis are believed through two ways. One is direct differentiation into chondrocytes, and the other is paracrine effects of secreted bioactive materials [39, 41]. Early studies have focused the differentiation potentials of MSCs which were examined with small surgically created chondral defects in animal models [15, 42]. Recent studies also showed that MSCs contributed to the repair of damaged articular cartilage through homing, engraftment, and production of cartilage matrix [16, 18, 43] in osteoarthritis models. Differentiation of delivered MSCs into chondrocytes appeared to be induced by the local environment of the homing site [43, 44]. Meanwhile, a surging paradigm suggests that direct differentiation might not be the only mechanism, but paracrine effects through secretion of bioactive materials should involve [39, 45]. MSCs are known to stimulate chondrocytes to proliferate and synthesize extracellular matrix [46–48], to induce anti-inflammatory cytokine production [44, 49–51], and to possess immunomodulatory properties [52, 53]. These studies together suggest that MSCs modulate inflammation and provide environment for tissue regeneration either by direct secretion of bioactive materials or by controlling cytokine and growth factor production from endogenous cells [41, 49, 54–57]. The results of this study provide robust evidences for both mechanisms. Regeneration of hyaline-like articular cartilage after injection is clearly demonstrated in this study by MRI, arthroscopy, and histology. Evidences of previous studies showing that injected cells participated in regeneration of articular cartilage suggest that injected MSCs rather than endogenous cells

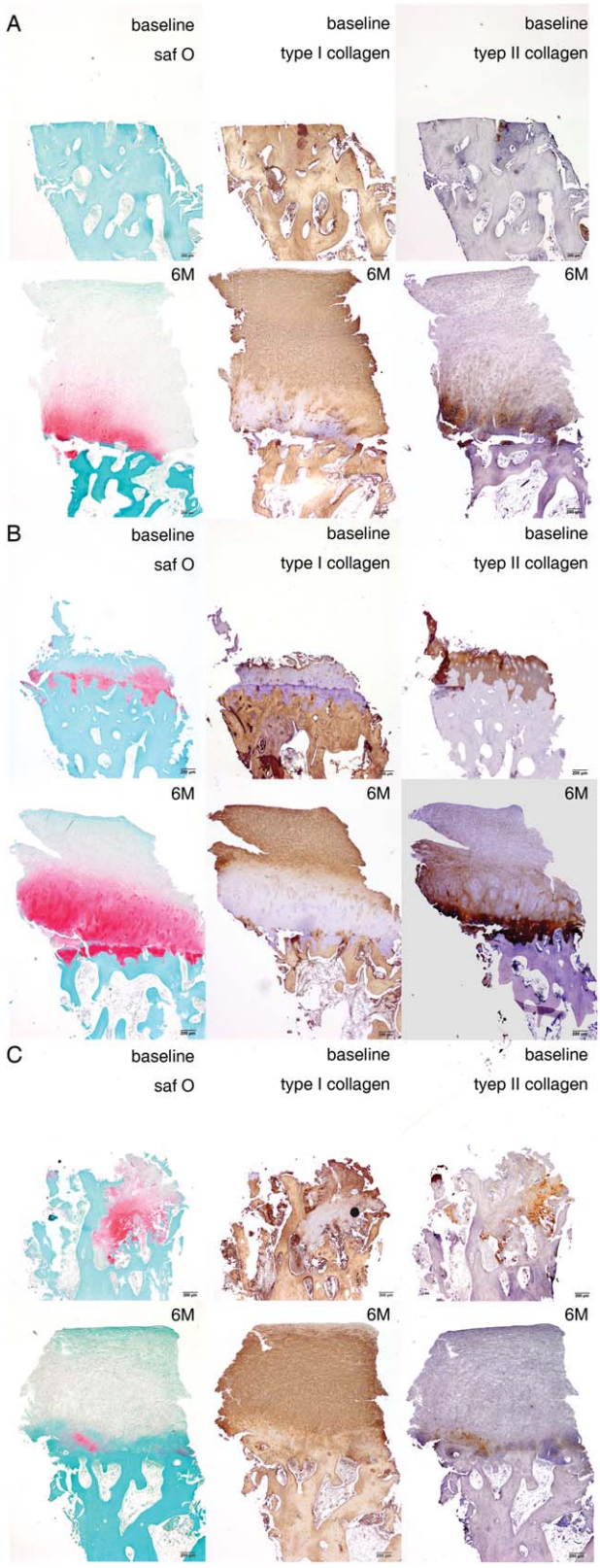

Figure 5.

Figure 5. Histological evaluation of regenerated articular cartilage of biopsy from the medial femoral condyle after intra-articular injection of adipose derived mesenchymal stem cells (AD MSCs). **(A):** A typical biopsy sample from the medial femoral condyle of a patient with international cartilage repair society (ICRS) grade 3C in the high-dose group at the baseline and 6 months after AD MSCs injection stained with safranin O and anti-type I and II collagen antibodies. Whereas no articular cartilage is seen at the baseline, a thick, hyaline-like cartilage with a smooth surface is regenerated and integrated with the subchondral bone 6 months after injection. In the superficial and the upper half of the middle zones, regenerated cartilage is composed of type I collagen and minimally contain type II collagen. Collagen fibrils in the superficial zone run parallel to articular surface while those in the middle zone are aligned obliquely. Safranin O and type II collagen is stained mostly in the lower half of the middle and the deep zones. Collagen fibrils in these zones run vertically. Typical columnar chondrocytes or tide mark is not definite. However, chondrocytes are flattened in the superficial zone, and round in the middle and deep zones similar to those in the deep zone of hyaline cartilage. Small chondrocytes are also present in the in the calcified cartilage zone. **(B):** Another biopsy sample from the medial femoral condyles of ICRS grade 3B at the baseline. At 6 months after injection, articular cartilage is regenerated similar to (A). Regenerated cartilage also has a smooth surface and showed relatively more positive safranin O and type II collagen staining. **(C):** Biopsy samples of the worst case with ICRS grade 3C at the baseline. At 6 months after injection, a relatively thin fibrocartilage is formed. Yet, the surface of regenerated cartilage is smooth, and demonstrated safranin O and type II collagen positive matrix in the deep zone. Abbreviation: saf O, safranin O.

recruited by paracrine mechanism were supposed to regenerate articular cartilage [16, 43, 58] while we did not track. Furthermore, as even few MSCs could trigger paracrine effects [44], better clinical and structural results in the high-dose group should more support that regeneration occurred mainly via direct differentiation. However, improved clinical outcomes not only in the high-dose group but also in the low- and mid-dose group suggest that paracrine effects should also work. Nevertheless, we still do not have enough knowledge about details; when and how much each mechanism contributes, which mechanism is more important to patients with different conditions, optimal cellular dose and condition for each mechanism, and so on. Additional researches need to be done for elucidation of these questions.

We used AD MSCs in this trial with already proven safety [22]. In comparison with bone marrow MSCs, AD MSCs have several advantages including feasibility of harvesting in a large amount by a simple, repeatable, and minimally invasive method, the highest frequency of MSCs [59], easy and rapid expansion in culture, and higher passage cells still retaining stem cell phenotypes and pluripotency [60]. However, the main benefits of AD MSCs are that they have less effect of age or morbidity of patients on quality in contrast to bone marrow MSCs [40, 61–64]. Despite some concerns about inferior chondrogenic potential of AD MSCs [65, 66], several experimental studies showed that AD MSCs reduced hypertrophy and dedifferentiation of chondrocytes [67], inhibit synovial thickening, and protect against joint destruction [68], and decreased the development and progression of osteoarthritis [69, 70]. The results of this study are consistent with previous experimental studies and suggest that AD MSCs are an appealing source for the treatment of osteoarthritis.

Most previous studies that investigated potentials of MSCs for regeneration of articular cartilage have used acute chondral defects models through surgical implantation [13, 15, 17, 71, 72]. Those defects are usually small with defined dimensions, surrounded by relatively normal cartilage and thus would simulate cartilage injury caused by trauma. However, cartilage lesions associated with osteoarthritis are chronic, large, complex in shape and thickness, and surrounded by degenerative cartilage. Therefore, alternate strategies other than direct implantation would be more appropriate [16–18, 73]. MSCs are known to home and are preferentially attracted to diseased tissue rather than to intact tissue [58, 74–76]. Using this homing ability, some authors demonstrated that intra-articularly injected MSCs attached to cartilage defect, proliferated, and participated in regeneration of articular cartilage [16, 17, 43], decreased synovial fluid concentration of prostaglandin E2 [50], and retard the progression of osteoarthritis [18, 77]. A few case reports in human also described encouraging early clinical outcomes of intra-articular injection of bone marrow MSCs [19–21]. In line with previous experimental studies and clinical case reports, this study demonstrated a great promise of intra-articular injection of AD MSCs with details of clinical, radiological, arthroscopic, and histological results. Current medical treatment for osteoarthritis are commonly associated with gastrointestinal, hepatic, renal, or cardiac side effects [78], and surgery is inevitably invasive no matter how minimal it is. This makes intra-articular injection a valuable option, especially in the elderly. Considering very low incidence of infection, 0.002% [79], and feasibility of the procedure, intra-articular injection of MSCs would be a valuable therapy for osteoarthritis if evidences accumulate. One of important findings in this study is that most of regenerated cartilage was found in the medial femoral and tibial condyles, both of which were the most severely degenerated site in the knee. The results are consistent with studies reporting that injected cells adhere diseased rather than intact articular cartilage [18, 80, 81]. Also these results would confirm the homing ability of AD MSCs that actually work in human osteoarthritis. Meanwhile, little change was found in the other compartments such as the lateral femoral and tibial condyles, and patella in which less degenerated cartilage existed. Considering that earlier injection of MSCs during the progression of osteoarthritis would be more beneficial [16], investigations for enhancing homing and engraftment of MSCs not only to as most degenerated location but also to less degenerated site should be necessary.

Patients in the high-dose group showed significantly improved outcomes in most clinical, radiological, and arthroscopic measures whereas those in the low- and mid-dose group did not. These results suggested that a sufficiently adequate number of MSCs should be delivered to the lesion for the best results. The importance as well as concerns of the cell dose has been raised by several authors [58, 65, 82]. Some reported that injection of 1.0×10^7 MSCs generated free bodies of scar tissue in the rat knee [58], whereas others reported insufficient numbers of applied cells showed inferior results [65]. Therefore, the optimal cell dose needed to be clarified for achieving efficacy balanced with safety. This study showed that at least the total number of 1.0×10^8 MSCs per injection would be a prerequisite for consistently good results. Nevertheless, they might not be the best results; regenerated cartilage did not completely cover the original defect of the medial femoral and tibial condyles even in the high-dose group, and there were little changes in the other compartments. Therefore, further studies would be necessary for optimal results, and repeated injections at intervals could be a good option.

There are some limitations of the study. First, there is no control in the study. A larger scale study with an appropriate control would be necessary for clinical application. Second, while regeneration of articular cartilage was clearly identified with MRI, arthroscopic, and histological measures, the 6-month of follow-up would be short especially for the assessment of clinical outcomes as certain clinical outcomes such as VAS pain in the mid-dose group increased at the final follow-up. Further study with longer follow-up would be necessary. Third, the results in the high-dose group might not be the best. As increasing the number of injected cells more may be practically difficult and would raise concerns such as fibrous foreign body formation, another approach including repetition of the injection and enhancement of homing ability of MSCs would be more promising. Fourth, the period of non-weight bearing after injection would not be optimized. As a proof-of-concept study, we focused more on regeneration of articular cartilage than on early return to daily activity. Thus, we recommended non-weight bearing with only toe-touch for 8 weeks that may be similar with the period used in other treatments for cartilage regeneration [83, 84]. Whereas this prolonged period of non-weight bearing might allow some native repair, it decreased and delayed recovery of the knee function after injection as evidenced by initial decline of the function score of KSS (Fig. 2D). Therefore, an optimal rehabilitation protocol for intra-articular injection of MSCs needs to be further investigated. Fifth, clinical researches need to use a validated questionnaire that is specific for the condition being studied. While WOMAC is

a widely used, validated self-administered instrument specifically designed to evaluate knee and hip osteoarthritis [24], it might not be specific for evaluating patients after intra-articular injection of AD MSCs which has never been studied before. Finally, the quality of regenerated cartilage would be not optimal as demonstrated in the histological results. Further investigations for enhancing chondrogenic differentiation would be necessary for better results.

Conclusions

In summary, intra-articular injection of 1.0×10^8 AD MSCs into the osteoarthritic knee improved function and pain of the knee joint without causing adverse events. Radiological, arthroscopic, and histological measures consistently demonstrated decreased of articular cartilage defects by regeneration of hyaline-like articular cartilage. These results are promising to encourage large randomized clinical trials, and we are cautiously optimistic about this new step for the treatment of osteoarthritis of the knee.

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Author Contributions

C.H.J.: conception and design, collection and/or assembly of data, data analysis and interpretation, and manuscript writing; Y.G.L. and W.H.S.: collection and/or assembly of data, analysis of radiographic data, and administrative support; H.K.: collection and assembly of histological samples and administrative support; J.W.C.: collection, assembly, and analysis of MRI data; E.C.J.: harvest adipose tissue and administrative support; J.E.K.: data analysis of histological samples; H.S.: data analysis and interpretation of the cartilage volume measures; J.S.S.: collection and/or assembly of clinical and arthroscopic data and administrative support; I.S.S. and J.C.R.: conception and design, financial support, and provision of study material or patients; S.O.: statistical data analysis; K.S.Y.: conception and design, data analysis and interpretation, manuscript writing, and final approval of manuscript.

Disclosure of Potential Conflicts of Interest

K.S.Y. reported receiving research grant and travel support from K-STEM CELL. E.C.J. reported receiving research grant from K-STEM CELL. I.S.S. and J.C.R. are employees of K-STEM CELL and reported owning stock and/or stock options. C.H.J. reported receiving grants from the National Research Foundation funded by the Korean Ministry of Education and Science Technology. K-STEM CELL is a company manufacturing stem cells and researching their application. No other authors reported any financial disclosures.

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Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis

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Abstract
Purpose In the present study, the clinical outcomes and second-look arthroscopic findings of intra-articular injection of stem cells with arthroscopic lavage for treatment of elderly patients with knee osteoarthritis (OA) were evaluated.
Methods Stem cell injections combined with arthroscopic lavage were administered to 30 elderly patients (≥65 years) with knee OA. Subcutaneous adipose tissue was harvested from both buttocks by liposuction. After stromal vascular fractions were isolated, a mean of 4.04×10^6 stem cells (9.7 % of 4.16×10^7 stromal vascular fraction cells) were prepared and injected in the selected knees of patients after arthroscopic lavage. Outcome measures included the Knee Injury and Osteoarthritis Outcome Scores, visual analog scale, and Lysholm score at preoperative and 3-, 12-, and 2-year follow-up visits. Sixteen patients underwent second-look arthroscopy.
Results Almost all patients showed significant improvement in all clinical outcomes at the final follow-up examination. All clinical results significantly improved at 2-year follow-up compared to 12-month follow-up ($P < 0.05$). Among elderly patients aged >65 years, only five patients demonstrated worsening of Kellgren–Lawrence grade. On second-look arthroscopy, 87.5 % of elderly patients (14/16) improved or maintained cartilage status at least 2 years postoperatively. Moreover, none of the patients underwent total knee arthroplasty during this 2-year period.
Conclusion Adipose-derived stem cell therapy for elderly patients with knee OA was effective in cartilage healing, reducing pain, and improving function. Therefore, adipose-derived stem cell treatment appears to be a good option for OA treatment in elderly patients.
Level of evidence Therapeutic case series study, Level IV.

Keywords Mesenchymal stem cell · Arthroscopic lavage · Knee osteoarthritis

Introduction

Osteoarthritis (OA) is the most common musculoskeletal disorder [3]. Synovial inflammation, in particular, can affect joint homoeostasis [5] and is associated with pain and OA disease progression [31]. The current treatments for OA are not regenerative and have little impact on the progressive degeneration of joint tissues. Clinical interventions are primarily symptomatic and focus on pain reduction and inflammation control through nonsteroidal anti-inflammatory drugs and ultimately with total joint replacement [4]. Few options are currently available for elderly patients with moderate to severe arthritis. Most approaches are palliative and address symptoms rather than influencing the biochemical environment of the joint or disease process.

Because of their multilineage potential, immunosuppressive activity, limited immunogenicity, and relative ease of growth in culture, mesenchymal stem cells (MSCs) are an attractive option for clinical use. Therefore, MSCs have been suggested for use in the cell-based treatment of cartilage lesions. In our previous study, 25 patients affected by a knee degenerative condition were treated with infrapatellar

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fat pad-derived MSC therapy by intra-articular injections and assessed at a 16.3-month follow-up [17]. The results indicated that this procedure was safe and helps in reducing pain and improving function in patients with knee OA. In a subsequent study involving the use of stem cell therapy [18], we obtained good clinical and radiological results at 2 years of follow-up. However, changes in clinical and MRI scores were positively related to the number of cells injected, indicating that treatment efficacy improved with an increase in the number of cells injected. Therefore, in the present study, we used buttock subcutaneous fat tissue as the source for stem cells because a sufficient quantity of adipose tissue was available in this region. Moreover, there are wide variations in the amount of fat in the infrapatellar fat pad, but we were able to obtain a consistent volume of buttock subcutaneous fat tissue in all patients. Therefore, we obtained fat from the gluteus region rather than the infrapatellar fat pad in the present study.

Intra-articular insertion of adipose tissue-derived stem cells with arthroscopic lavage was believed to improve articular cartilage status and decrease pain for a long period in elderly patients with knee OA. Thus, in the present study, the potential treatment of OA symptoms with MSCs and arthroscopic lavage was evaluated using clinical results and second-look arthroscopic findings.

Materials and methods

Between November 2010 and January 2011, 30 stem cell injections combined with arthroscopic lavage were administered to elderly patients (≥65 years) with knee OA. Thirty patients [5 men and 25 women; mean age, 70.3 (range 65–80) years], in whom previous nonsurgical treatment (undergoing nonoperative management options such as physical therapy and nonsteroidal anti-inflammatory drugs, for a minimum of 3 months) had failed and who had refused to undergo prosthetic replacement, underwent stem cell therapy as a salvage procedure, which was performed one senior surgeon (Y.G.K.) at the authors' institute. Eligibility requirements were age ≥65 years and diagnosis of idiopathic or secondary knee OA [Kellgren–Lawrence [12] (K–L) grade 2 or 3 OA in multiple compartments, including the medial or lateral tibiofemoral joint compartments or the patellofemoral compartment]. Patients were excluded if they met at least one of the following criteria: diagnosis with K–L [12] grade 4 OA or inflammatory or postinfectious arthritis, previous arthroscopic treatment for knee OA, previous major knee trauma, intra-articular hyaluronic acid or corticosteroid injection in the preceding 3 months, mechanical pain caused by meniscal tears (including flap tears, bucket-handle tears, and complex tears), and inability to provide informed consent. Three doctors, who were blinded to the grading results of the other examiners, performed K–L grading in all the patients. The knee joint is typically evaluated using an extended knee radiograph, which is a bilateral anteroposterior image acquired while the patient is in a weight-bearing condition, with both the knees completely extended.

Collection of subcutaneous adipose tissue

Subcutaneous adipose tissues were harvested from the patients' buttocks by liposuction, as described previously [13, 15]. One day before the arthroscopic surgery, we harvested the adipose tissue through liposuction using a tumescent solution. The patient was placed in the prone position under intravenous sedation. After surgical preparation, a hollow blunt-tipped cannula was introduced into the subcutaneous space through a small incision, and subcutaneous adipose tissue was infiltrated with a tumescent solution to minimize blood loss and tissue contamination by peripheral blood cells prior to aspiration, which consisted of 0.9 % saline solution (500 mL) supplemented with 2 % lidocaine (10 mL), 8.4 % sodium hydrogen carbonate (4 mL), and 0.1 % epinephrine (0.7 mL). The liposuction material was aspirated by gentle suction. We aimed to routinely collect 140 cc of liposuctioned adipose tissue, of which 120 cc was used for the injection, and 20 cc was subjected to laboratory analysis to examine the plastic-adherent cells that form colony forming units-fibroblast (CFU-F) and confirm the multilineage differentiation of adipose-derived stem cells.

Isolation of stromal vascular fraction and MSCs from subcutaneous adipose tissue

In the operating room, adipose tissue (120 cc) was suspended in phosphate-buffered saline (PBS), placed in a sterile box, and transported to a laboratory. Mature adipocytes and connective tissues were separated from the stromal vascular fraction by centrifugation, as reported by Zuk et al. [35]. The volume of the stromal vascular fraction is usually less than 0.1 cc. Prior to insertion, bacteriologic tests were performed to ensure the absence of contamination in the samples, and the viability of cells was assessed using the methylene blue dye exclusion test. The remaining 20 cc of adipose tissue was processed by the same method and used for cell analysis.

Assessment of plastic-adherent cells that form CFU-F and immunophenotyping of adipose-derived stem cells

To evaluate the frequency of mesenchymal-like progenitors in patient stromal vascular fraction, cells were cultured

in T-25 flasks at a final concentration of 16 cells/cm^2. Colonies consisting of ≥50-cell aggregates were scored under an optical microscope, and the immunophenotype of adipose-derived stem cells was analysed by flow cytometry (FACS). MSC marker phenotyping was performed as previously described [20].

Confirmation of multilineage differentiation of adipose-derived stem cells

Adipose-derived stem cells were plated at 2×10^3 cells/cm^2 in DMEM containing 10 % FBS and allowed to adhere for 24 h. The culture medium was then replaced with specific inductive media to determine the adipogenic, osteogenic, and chondrogenic differentiation potential, as previously reported [20].

Arthroscopic lavage and implantation of MSCs

Patients received arthroscopic lavage under spinal anaesthesia with the use of a tourniquet. The orthopaedic surgeon evaluated the medial, lateral, and patellofemoral joint compartments; graded the articular lesions according to the International Cartilage Repair Society (ICRS) Cartilage Injury Evaluation Package; and irrigated the compartment with at least 1 L of saline. While performing arthroscopic lavage on the 30 patients, we noted that the cartilage status in the medial compartment was grade II in two patients, grade III in 15 patients, and grade IV in 13 patients. Further, the cartilage status in the lateral compartment was grade II in ten patients, grade III in 11 patients, and grade IV in nine patients. In addition, the cartilage status in the patellofemoral compartment was grade II in eight patients, grade III in 14 patients, and grade IV in eight patients. The following treatments were not performed: synovectomy; excision of degenerative tears of the menisci or osteophytes that prevented full extension, and abrasion or microfracture of chondral defects. Because we excluded patients who experienced mechanical pain caused by a meniscal tear, only 16 patients exhibiting degenerative meniscal tears that did not cause mechanical knee pain were included in the study. After arthroscopic lavage, a mean of 4.2×10^7 stromal vascular fraction cells were prepared with approximately 3.0 mL of platelet-rich plasma (PRP). The stromal vascular fraction cells were injected into the most severe cartilage defect area in the selected knees of patients under arthroscopic guidance. Immediately after arthroscopic lavage, the affected knee was placed in a cylinder splint for 24 h. No analgesics, anti-inflammatory drugs, or immunosuppressive drugs were administered or permitted after the procedure for 3 months.

For PRP preparation, a 30-mL venous blood sample (collected in a bag containing 4 mL of sodium citrate) was collected for every lesion that was treated. The complete peripheral blood count was determined using the first blood sample collected. Thereafter, the samples were centrifuged twice (at 1,800 rpm for 15 min to separate the erythrocytes, and then at 3,500 rpm for 10 min to concentrate the platelets) to yield 6 mL of PRP. The total number of platelets per microlitre in the PRP was a mean of 500 % times greater than that in the whole blood, and an average of 1,280,000/μL platelets were administered at the lesion sites during every injection. Prior to injection in all cases, calcium chloride was added to the PRP unit to activate the platelets. All the procedures were performed in the same laboratory setting, and all open procedures were performed in an A-class sterile hood.

Clinical assessment

Clinical outcome was evaluated using the Lysholm score [16], the Knee injury and Osteoarthritis Outcome Score (KOOS) [30], and visual analog pain score (VAS) on a 10-point scale (0–10) for pain (0 = no pain; 10 = worst possible pain). Patients were evaluated preoperatively as well as postoperatively at 3-, 12-month, and 2-year follow-up visits. At the 2-year follow-up, patients also completed a questionnaire intended to assess their satisfaction with the treatment. Radiographic evaluation included the standing weight-bearing anteroposterior view, lateral view, skyline view, and full-length anteroposterior view.

Second-look arthroscopy

Among the 30 patients who received stem cell therapy, 16 underwent second-look arthroscopy by one surgeon at our hospital. The indications for second-look arthroscopy were as follows: (1) asymptomatic patients, to evaluate the healing status of degenerative cartilage, and (2) patients who complained of knee pain at follow-up. The healing status of degenerative cartilage was classified as very positive, positive, neutral, or negative in the most severe cartilage defect area of the knee. We noted the presence of severe cartilage lesions at the medial compartment in 9, at the lateral compartment in 4, and at the patello-femoral compartment in three patients. "Very positive" was considered when a remarkable change was noted throughout the degenerative cartilage with good integration to adjacent normal articular surface and normal gross appearance. "Positive" was considered when newly forming cartilage tissue was found to partially cover the degenerative cartilage compared to that noted preoperatively. "Neutral" was considered when an uncertain change was noted over 2 years compared to the preoperative status. "Negative" was considered when progression of degenerative cartilage was noted compared to preoperative status. The

examinations were performed during second-look arthroscopy by all members of the surgical team (Y.G.K., S.K.K., and Y.J.C.). The observation was confirmed only once a consensus was reached among all the three surgeons.

This study was approved by the Research Ethics Board of Yonsei Sarang Hospital (registration number 10-R03-05), and written informed consent was obtained from all participants.

Statistical analysis

Statistical analysis was performed using SPSS software version 12.0.1 (SPSS Inc., Chicago, Illinois), with significance defined as $P < 0.05$. Descriptive statistics were calculated as mean ± standard deviation. The normality of distribution was checked using the Shapiro–Wilk test. Our data followed normal distribution because the probability of the Shapiro–Wilk test was $P > 0.05$ and the number of patients was 30. The principal dependent variables of clinical outcomes were KOOS, Lysholm score, and VAS at the last follow-up. The paired *t* test was conducted to evaluate changes in preoperative and serial follow-up values. We analysed the association of factors—patient characteristics and radiological grade of OA—with clinical outcomes. Mean values were used as standard values for dividing patients according to age and K–L grade. Differences between groups were analysed using the independent *t*-test.

Results

Cell isolation and characterization of adipose-derived stem cells

We evaluated the capacity of human subcutaneous adipose tissue to generate mesenchymal progenitors using the CFU-F. Thus, after isolation, adipose-derived stem cells represented a mean of 9.7 % of stromal vascular fraction cells (range 6.8–12.4 % of stromal vascular fraction cells). After the stromal vascular fractions were isolated, a mean of 4.0×10^6 stem cells (9.7 % of 4.2×10^7 stromal vascular fraction cells) were prepared. FACS characterization indicated positive expression of the surface markers CD90 (99.8 %) and CD105 (88.9 %) and negative expression of CD34 (12.0 %) and CD14 (1.2 %), as shown previously (Fig. 1a) [35]. Adipose-derived stem cells treated with conditioned media demonstrated characteristics of adipogenic, osteogenic, and chondrogenic differentiation after staining (Fig. 1b).

Clinical outcomes at follow-up

The mean Lysholm score significantly increased from 54.3 ± 15.4 to 74.2 ± 13.4 ($P < 0.05$). The mean VAS decreased from 4.7 ± 1.6 preoperatively to 1.7 ± 1.4 at 2-year follow-up ($P < 0.05$). The median KOOS from preoperative to 2-year follow-up assessments is summarized in Fig. 2. Moreover, all clinical results significantly improved at 2-year follow-up compared to those at 1-year follow-up ($P < 0.05$). With regard to overall patient satisfaction with the operation, 16 patients reported their satisfaction as excellent (53 %), 7 as good (23 %), 4 as fair (13 %), and 3 as poor (10 %). At 2-year follow-up, the K–L grade in five patients increased by one grade. The K–L grade in two patients increased from grade 2 to 3, whereas the K–L grade in three patients increased from grade 3 to 4. However, no patients underwent a second operation such as total knee arthroplasty. No major complications associated with arthroscopic lavage and liposuction, either intraoperatively or postoperatively, were observed in this series. In three patients, slight knee pain was experienced in the first week after the stem cell injection, which resolved spontaneously in two patients in 1 week with no medication and resolved after 2 weeks in the other patient with anti-inflammatory drug medication.

Associations between patient characteristics and outcomes

A statistically significant association was observed between patients' age and mean improvement from baseline in all KOOS subscales to 2-year follow-up ($P < 0.05$; Fig. 3), and a statistically significant association was observed between K–L grade 2 and higher Lysholm score improvement ($P = 0.002$; Table 1). No other parameters showed a statistically significant association.

Second-look arthroscopy

At a minimum follow-up of 24 months (median 25.0 months; range 24–26 months), 16 patients treated with MSC therapy underwent second-look arthroscopy, including 12 who were asymptomatic to evaluate the cartilage status, and 4 subsequent symptomatic patients with recurrent knee joint pain to plan further treatment. We explained the purpose of second-look arthroscopy to patients before surgery and received written consent. On second-look arthroscopy, 3 knees (all were asymptomatic) were rated "very positive" and 7 were rated "positive" (1 was symptomatic and 6 were asymptomatic). Four knees were rated "neutral" (2 each were symptomatic and asymptomatic), and the other 2 patients experienced failed healing (1 each was symptomatic and asymptomatic; Table 2). The differences between parameters of four groups were not significant.

The findings of a 67-year old woman during the first and second arthroscopy procedure showed marked changes in cartilage defects of the medial femoral condyle (Fig. 4).

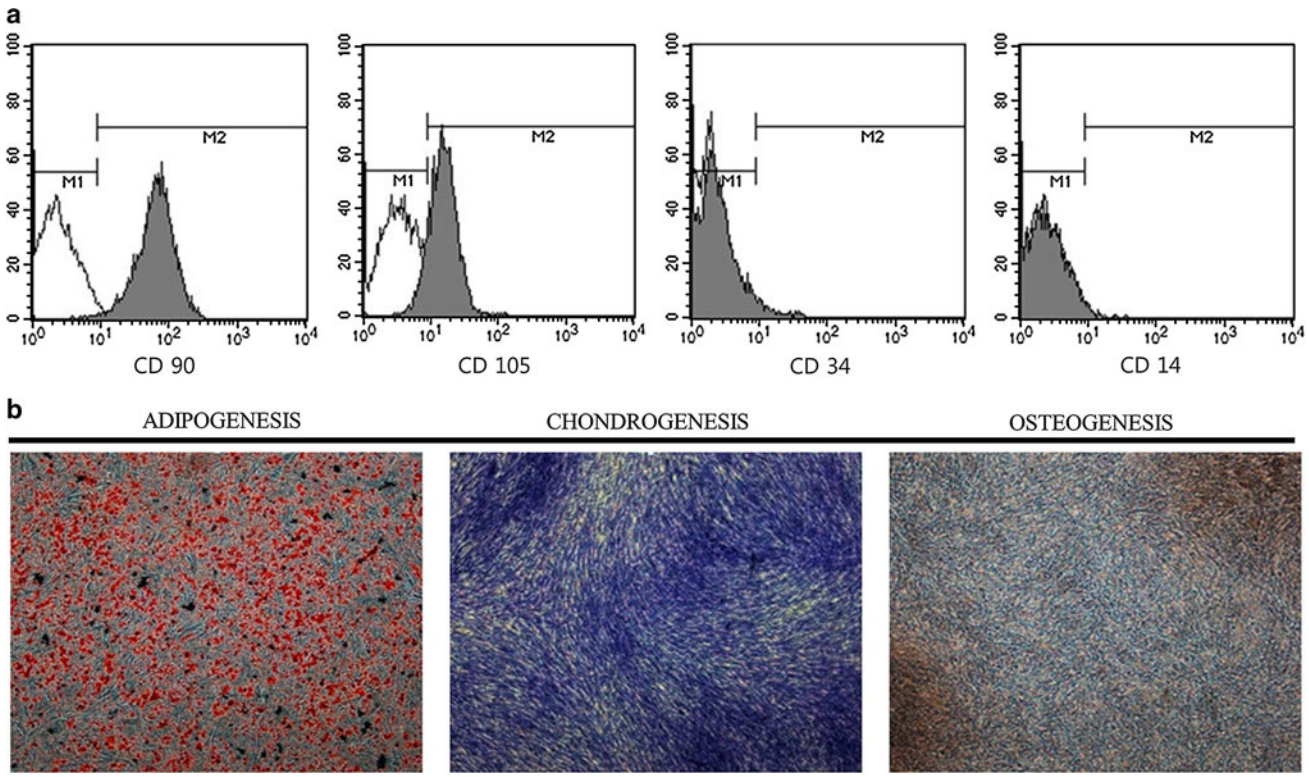

Fig. 1 Phenotypic characterization and differentiation potential of adipose-derived stem cells. **a** adipose-derived stem cells were isolated from stromal vascular fraction and then tested for mesenchymal surface markers (CD105 and CD90) and hematopoietic and endothelial markers (CD34 and CD14) by flow cytometry. **b** The differentiation potential of adipose-derived stem cells toward the adipogenic, chondrogenic, and osteogenic lineage was confirmed by Oil Red O, toluidine blue, and Von Kossa's method. Cells were cultured in normal medium for 2 weeks and then histochemically stained

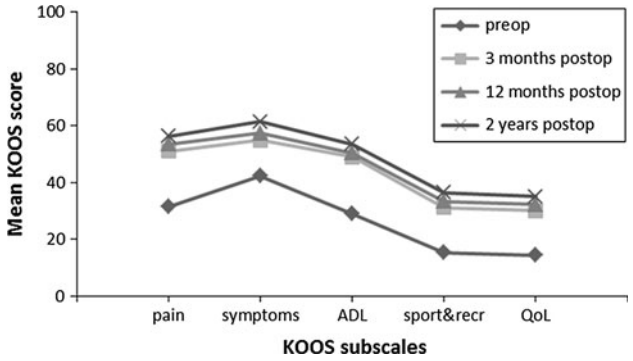

Fig. 2 KOOS profiles prior to and up to 2 years after stem cell therapy. Mean KOOS scores ($n = 30$) at the preoperative, 3-, 12-month and 2-year assessments after MSC therapy. At all follow-up point, differences in the values were statistically significant ($P < 0.05$) compared with the preoperative status. *ADL* activities of daily living, *sports/recr* sports and recreation, *QoL* quality of life

Discussion

The most important finding of the present study was that adipose-derived stem cell therapy was effective in cartilage healing, reducing pain, and improving function in elderly patients with knee OA. Additionally, among elderly patients aged >65 years, only 5 patients demonstrated worsening of the K–L grade. On second-look arthroscopy, 87.5 % of elderly patients (14/16) improved or maintained cartilage status at least 2 years postoperatively. Moreover, no patient underwent total knee arthroplasty during this 2-year period. Therefore, stem cell injection appears to be a good option for OA treatment in elderly patients. The results of stem cell injection with arthroscopic lavage were excellent at the final follow-up and showed improvement compared to those at 3 and 12 months. This finding indicates that even if the effect of arthroscopic lavage is eliminated, good results are achieved over medium-term follow-up. We acknowledge that arthroscopic lavage could be at least partly responsible for the improved clinical outcomes. However, arthroscopic lavage has only very short-term clinical effects in patients with advanced knee OA [26]; in the present study, clinical improvements persisted for more than 2 years (until at least the final follow-up at 24 months), and the second-look arthroscopy findings in these patients indicated that 87.5 % of elderly patients (14/16) improved or maintained cartilage healing status at 2 years postoperatively. Because the cartilage of OA

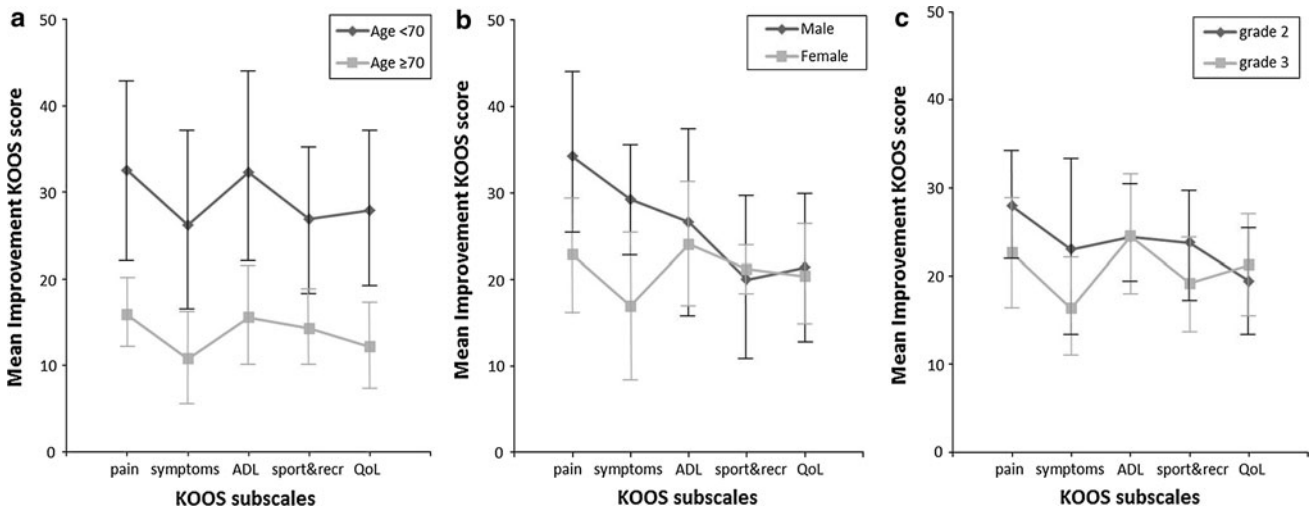

Fig. 3 Associations between patient characteristics and mean improvement from baseline in Knee injury and Osteoarthritis Outcome Score (KOOS) subscales to 2-year follow-up: **a** age (<70 vs >70), **b** sex (male vs female), **c** Kellgren–Lawrence grade (2 vs 3). *ADL* activities of daily living, *sports/recr* sports and recreation, *QoL* quality of life. A statistically significant association was only observed between patients' age and mean improvement from baseline in all KOOS subscales to 2-year follow-up

Table 1 Associations between patient characteristics and mean improvement of clinical outcomes, preoperatively to 2-year follow-up

Parameters	Age (years)		Sex		K–L[a]	
	<70	≥70	Male	Female	2	3
Lysholm score (SD)	23.8 (15.5)	15.5 (10.6)	9.2 (10.0)	22.1 (13.7)	29.0 (12.7)[†]	13.9 (11.4)
VAS (SD)	−3.3 (2.1)	−2.7 (1.2)	−3.2 (1.6)	−3.0 (1.8)	−3.1 (1.7)	−2.9 (1.8)

[†] Significant difference between both groups ($P < 0.05$)

[a] Radiological findings of osteoarthritis described by Kellgren and Lawrence

Table 2 Second-look patient demographics and general findings

Cartilage healing status (patient number, %)	Very positive (3, 18.7 %)	Positive (7, 43.8 %)	Neutral (4, 25.0 %)	Negative (2, 12.5 %)
Age, years (± SD)	70.3 ± 7.6	69.0 ± 2.4	76.0 ± 7.3	72.0 ± 2.8
Gender (M/F)	0/3	2/5	1/3	0/2
K–L[a] at 2nd look (II/III/IV)	1/2/0	2/5/0	1/2/1	0/1/1
Reason for 2nd look (evaluation/pain)	3/0	6/1	2/2	1/1
Follow-up period, months (± SD)	25.0 ± 0	24.9 ± 0.4	25.3 ± 0.96	24.5 ± 0.71

The differences between parameters of four groups were not significant ($P > 0.05$)

[a] Radiological findings of osteoarthritis described by Kellgren and Lawrence

patients has diffuse degenerative lesions, the grading system of severe lesions used by certain classifications such as Outerbridge's classification [24] or the ICRS grade does not seem to be appropriate to describe the change in the cartilage status in OA patients. Therefore, we believe that the identification of the change in cartilage status is difficult using Outerbridge's classification [24] or the ICRS grade. Thus, a different method for the classification of regeneration, which was used in the present study, is essential to classify the change in the cartilage status.

Previous studies have shown that the outcomes of chondrocyte transplantation in patients aged >40 years were inferior compared to those previously noted in younger populations, and the failure rate at medium-term follow-up was also comparatively higher [19]. However, in the present study, the combination of MSC therapy and PRP was found to be effective in elderly patients with knee OA. We believe that although the chondrocytes of older patients have lower activity, the adipose-derived stem cells in elderly patients have sufficient stem cell activity, as noted during our

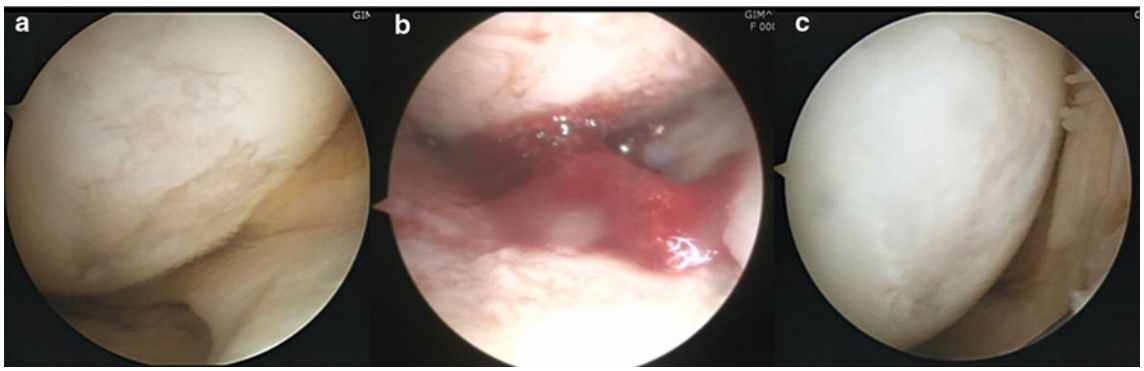

Fig. 4 **a** Intraoperative arthroscopic finding showing a cartilage defect in the medial femoral condyle (MFC). **b** Intraoperative arthroscopic finding showing insertion of stem cells with PRP. **c** Second-look arthroscopy revealed that the cartilage defect was completely covered with smooth tissues, which was considered to be cartilage. This finding of a remarkable change throughout the degenerative cartilage with good integration with the adjacent normal articular surface and normal gross appearance was defined as "very positive"

characterization of adipose-derived stem cells. Moreover, although chondrocytes are generally believed to develop only through tissue-specific differentiation, stem cells are believed to develop through tissue-specific differentiation as well as a powerful paracrine effect.

We previously reported that infrapatellar fat pad-derived MSC therapy with intra-articular injections is safe and aids in reducing pain and improving function in patients with knee OA [17]. Over a long-term follow-up period [18], both clinical assessments and MRIs indicated that MSC therapy involving the intra-articular injection of MSCs into the knee is effective for reducing pain and improving function in patients with knee OA; furthermore, 2-year follow-up results were better than short-term results. Because further analysis indicated that clinical and radiological results improved as the number of injected cells increased, we changed our source of stem cells to obtain a greater number. Therefore, in the present study, a larger number of cells were obtained by using subcutaneous adipose tissue from the buttock (mean number of stem cells, 4.0×10^6), compared with that from the infrapatellar fat pad, from which MSCs were derived and a mean number of 1.2×10^6 stem cells were obtained. Moreover, although there are wide variations (range 6.4–13.1 g) in the amount of fat in the infrapatellar fat pad, 120 cc of buttock subcutaneous fat tissue could be consistently obtained in all the patients.

A recent study demonstrated that adipose tissue contains multipotent stem cells [25], or adipose-derived stem cells, which can be easily purified after digestion of fat and selection by adhesion onto plastic from the very heterogeneous crude stromal fraction. Adipose tissue is the subject of great interest as a therapeutic cell source because the cells are obtained from adults, thereby avoiding ethical concerns, and from tissue that is abundant and easy to obtain, even compared with bone marrow where sampling requires general anaesthesia. Additionally, because the frequency of adipose-derived stem cells in adipose tissue is much higher than that of MSCs in bone marrow, many cells can be obtained without a large number of passages, thus greatly decreasing the risk of culture-induced chromosomal abnormality or senescence [33].

Adipose tissue is composed of two main cell populations: mature adipocytes and the stromal vascular fraction. The latter is a heterogeneous fraction including preadipocytes, endothelial cells, smooth muscle cells, pericytes, macrophages, fibroblasts, and adipose-derived stem cells, which share several characteristics with bone marrow stem cells [29, 32]. Adipose-derived stem cells are promising candidates in a broad range of innovative therapies, ranging from regenerative medicine to tissue engineering, in autoimmune pathologies. Moreover, the use of stromal vascular fraction or adipose-derived stem cells has been proposed in several chronic pathologies such as Crohn's disease [10], autoimmune pathologies (e.g., multiple sclerosis) [27], and allergic pathologies. Their effectiveness against these pathologies can be explained by the immunoregulatory and anti-inflammatory activities of adipose-derived stem cells or nonexpanded stromal vascular fraction cells [27]. Unfortunately, since the majority of scientific studies have focused on in vitro-expanded adipose-derived cells, relatively little is known about the potential clinical effects of the whole lipoaspirate, which contains numerous cell populations besides MSCs. Recently, adipose-derived stem cells have been identified as a new option for the treatment of osteochondral lesions, and the injection of MSCs with marrow stimulation treatment has been proposed for the treatment of such cases in our institute [14]. Moreover, Desando et al. [9] reported that the healing proprieties of adipose-derived stem cells, including the promotion of cartilage and menisci repair and attenuation of inflammatory events in the synovial membrane, may facilitate the inhibition of OA progression.

In previous studies using bone marrow-derived MSCs for the treatment of cartilage defects, culture expansion of MSCs was performed to obtain a large number of cells [2, 11]. However, MSC culture expansion is costly, time-consuming, and carries some risk of contamination. In addition, MSC properties may be altered during culture by various elements of the local microenvironment that can affect MSC differentiation [7, 28]. In the present study, we could extract approximately 4.0×10^6 stem cells without culture (9.7 % of the 4.2×10^7 cells in the stromal vascular fraction). Consistent with our results, De Toni et al. [8] reported that adipose-derived stem cells represent 6.4 % of nucleated cells in the normal vascular fraction in adipose tissue, whereas MSCs represent only 0.0005 % of nucleated cells in the human bone marrow, which is a considerable difference. The advantages of our methods are that MSCs can be harvested in a minimally invasive manner and are easily isolated; in addition, an important advantage of this procedure is that, since time-consuming in vitro cell culture is not required, all procedures can be performed with a single admission.

Although the primary effects of stem cell treatment are generally believed to occur through tissue-specific differentiation [6, 23], new data suggest that the therapeutic potential of these cells may be related to their paracrine effect [6, 21]. Following second-look arthroscopy, only 10/16 patients in our cohort demonstrated cartilage formation. However, regardless of cartilage formation, almost all patients demonstrated improved clinical symptoms. In the some case, although radiological and second-look findings indicated worsening, the patient showed excellent clinical outcome and high satisfaction with her results. Thus, the main effect of this therapy appears to be the paracrine effect. Several studies have shown that MSCs can modulate the functions of adaptive immune system cells such as T cells and B cells [1]. Other studies have shown that these stem cells are also able to induce expression of anti-inflammatory mediators, such as IL-10 and IL-12p40, in macrophages [22]. Our stem cell therapy may act primarily through a long-lasting anti-inflammatory effect.

In this study, the effect of MSC insertion was maintained for 2 years. MSC therapy may be a new option for elderly patients who are not fully indicated for total knee arthroplasty. Menno et al. [34] reported that a single injection of adipose-derived stem cells into the knee joints of mice with early-stage collagenase-induced OA inhibits synovial thickening, formation of enthesophytes associated with ligaments, and cartilage destruction. Additionally, in contrast to early treatment, late injection of adipose-derived stem cells after OA induction showed no significant effect on synovial activation or joint pathology. Similar to the preceding findings, in our study, patients aged <70 years and K–L grade two patients achieved greater improvement in clinical outcomes than those aged >70 years or those with K–L grade 3. Therefore, we believe that MSCs may be particularly useful for delaying total knee arthroplasty in younger patients and cases of less severe OA.

The present study has some limitations. First and most importantly, our data lack quantitative evidence. MRI examination and biopsy should be performed. Second, this study is a level IV study, and therefore, no control group was included. Although we had earlier proved the effect of infrapatellar fat pad-derived adipose stem cells, we altered the source of the cells and used a novel method of arthroscopic-guided injection in the present study. Thus, we performed a new study without having a group for comparison, such as a pilot study. As the present study was designed as a pilot study, only patients aged >65 years who did not wish to undergo total knee arthroplasty were included. An additional study with a comparative design in patients with an early stage of OA will be performed. Third, our treatments were delivered during a single injection, although the possibility exists that optimal results can only be obtained by giving patients >1 injection within a certain time period. Fourth, only the effects of simultaneous treatment with both stem cells and PRP were focused on in the present study; additional work is needed to measure the effects of pure stem cell injections, distinguish the effects of stem cells from those of PRP, and determine the proper use of costimulators. Finally, the major limitation of the current study is the lack of matched control groups that would facilitate determining the efficacy of the stem cell therapy.

The clinical relevance of this study is that adipose-derived stem cell treatment may be a useful therapy for knee OA. Therefore, adipose-derived stem cell treatment appears to be a good option for OA treatment in elderly patients.

Conclusions

Adipose-derived stem cell therapy for elderly patients with knee OS was effective in cartilage healing, reducing pain, and improving function. Therefore, adipose-derived stem cell treatment appears to be a good option for OA treatment in elderly patients.

Conflict of interest None.

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Comparative Outcomes of Open-Wedge High Tibial Osteotomy With Platelet-Rich Plasma Alone or in Combination With Mesenchymal Stem Cell Treatment: A Prospective Study

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Purpose: This study compared the clinical results and second-look arthroscopic findings of patients undergoing open-wedge high tibial osteotomy (HTO) for varus deformity, with or without mesenchymal stem cell (MSC) therapy. **Methods:** This prospective, comparative observational study was designed to evaluate the effectiveness of MSC therapy. The patients were divided into 2 groups: HTO with platelet-rich plasma (PRP) injection only (n = 23) or HTO in conjunction with MSC therapy and PRP injection (n = 21). Prospective evaluations of both groups were performed using the Lysholm score, Knee Injury and Osteoarthritis Outcome Score (KOOS), and a visual analog scale (VAS) score for pain. Second-look arthroscopy was carried out in all patients at the time of metal removal. **Results:** The patients in the MSC-PRP group showed significantly greater improvements in the KOOS subscales for pain (PRP only, 74.0 ± 5.7; MSC-PRP, 81.2 ± 6.9; $P < .001$) and symptoms (PRP only, 75.4 ± 8.5; MSC-PRP, 82.8 ± 7.2; $P = .006$) relative to the PRP-only group. Although the mean Lysholm score was similarly improved in both groups (PRP only, 80.6 ± 13.5; MSC-PRP, 84.7 ± 16.2; $P = .357$), the MSC-PRP group showed a significantly greater improvement in the VAS pain score (PRP only, 16.2 ± 4.6; MSC-PRP, 10.2 ± 5.7; $P < .001$). There were no differences in the preoperative (PRP only, varus 2.8° ± 1.7°; MSC-PRP, varus 3.4° ± 3.0°; $P = .719$) and postoperative (PRP only, valgus 9.8° ± 2.4°; MSC-PRP, valgus 8.7° ± 2.3°; $P = .678$) femorotibial angles or weight-bearing lines between the groups. Arthroscopic evaluation, at plate removal, showed that partial or even fibrocartilage coverage was achieved in 50% of the MSC-PRP group patients but in only 10% of the patients in the PRP-only group ($P < .001$). **Conclusions:** MSC therapy, in conjunction with HTO, mildly improved cartilage healing and showed good clinical results in some KOOS subscores and the VAS pain score compared with PRP only. **Level of Evidence:** Level II, prospective comparative study.

Globally, osteoarthritis (OA) is the most common cause of knee pain. Arthritis of the knee joint commonly affects the medial compartment and is associated with misalignment, thereby placing a greater load on the affected compartment.[1] High tibial osteotomy (HTO) is a treatment option for younger and/or physically active patients who have OA of the medial compartment of the knee. HTO was originally devised to treat varus OA by decreasing pressure on the medial compartment.[2] In this regard, several studies have reported remodeling of the articular cartilage after HTO and attributed improvements to reduced contact stress by altering the weight-bearing axis.[2-5] However, HTO alone induces partial remodeling of the articular cartilage,[3] and therefore additional procedures, such as stem cell transplantation, may further enhance articular cartilage healing in OA patients.

Intra-articular injection of mesenchymal stem cells (MSCs) was reported to be effective for reducing pain in patients with knee OA.[6,7] In a previous study, postoperative magnetic resonance imaging studies also showed notable improvements in medial femoral condyle cartilage defects. On the basis of these findings, stem cell injection was used to achieve greater cartilage remodeling and better clinical results after HTO surgery.

The purpose of this study was to compare the clinical results and second-look arthroscopic findings in patients undergoing open-wedge HTO for varus deformities, with or without MSC therapy. MSC

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therapy with platelet-rich plasma (PRP), in conjunction with HTO, was hypothesized to provide improved cartilage healing and clinical results compared with injection of PRP only.

Methods

This prospective, comparative observational study was designed to evaluate the effectiveness of MSC therapy. Study protocols were approved by the local ethics committee, and all patients provided written informed consent. From January to October 2011, 44 patients who met the following inclusion criteria were enrolled in this study. The inclusion criteria for surgical treatment reflected those outlined in the literature for this procedure: (1) age younger than 60 years, (2) radiographs showing grade III or lower Kellgren-Lawrence symptomatic isolated medial knee compartment OA, (3) failure of conservative treatment, and (4) absence of additional cartilaginous procedures (autologous chondrocyte transplantation, microfracture). Patients were excluded if they did not consent to undergo a second operation for plate removal and second-look arthroscopy and could not be evaluated at either the 1- or 2-year postoperative visit. In addition, patients were excluded if they had undergone previous cartilage procedures, such as microfracture or chondroplasty, for chondral lesions of the medial femoral condyle because the intention was to examine the effect of MSC therapy on cartilage healing. Patients were also excluded if they met at least 1 of the following criteria: severe cartilage lesions of the lateral compartment or patellofemoral compartment, as observed using preoperative magnetic resonance imaging; inflammatory or postinfectious arthritis; previous arthroscopic treatment for knee OA; previous major knee trauma; intra-articular hyaluronic acid or corticosteroid injection within the preceding 3 months; mechanical pain caused by meniscal tears (including flap tears, bucket-handle tears, and complex tears); chronic anterior cruciate ligament/posterior ligament instability; or inability to provide informed consent.

Patients were randomized into either the PRP-only group or the MSC-PRP group. Simple randomization methods were used in which each patient, when enrolled in the trial, was asked to choose either of 2 identical envelopes with either the PRP-only or MSC-PRP group indicated inside. The randomization process was conducted by a hospital staff member blinded to the patients' data. Patients, however, were not blinded to the interventional method (liposuction) used. A total of 52 patients were enrolled, with 26 knees comprising each group.

The patients were prospectively evaluated by physiotherapists using the Lysholm score,[8] the Knee Injury and Osteoarthritis Outcome Score (KOOS),[9] and a 100-point visual analog scale (VAS) score for pain (0, no pain; 100, worst possible pain). Patients were evaluated preoperatively and postoperatively at 3 months, at 1 year, and at the last follow-up visit (mean, 24.4 months; range, 24 to 25 months). Before surgery, radiographs of the knee joints were obtained, including an anteroposterior (AP) view, a true lateral view at 30° of knee flexion, and an AP long-leg weight-bearing view. To investigate the mechanical effects of HTO, the femorotibial angle (FTA) and percentage of mechanical axis[10] were measured using standing AP radiographs taken immediately before surgery and after surgical removal of the plate. The FTA was determined as the angle between the femoral and tibial shaft axes on the standing AP radiographs.

Collection of Subcutaneous Adipose Tissue

Subcutaneous adipose tissue was harvested from both buttocks of each patient. One day before HTO, adipose tissue was harvested by tumescent liposuction, with the patient under local anesthesia.[11] Routinely, 140 mL of adipose tissue that had undergone liposuction was collected; 120 mL was used for the injection. The remaining 20 mL was subjected to laboratory analyses to assess the plastic-adherent cells that formed colony-forming unit fibroblasts and to confirm the multilineage differentiation of the adipose-derived stem cells (ADSCs).

Isolation of Stromal Vascular Fraction and MSCs From Subcutaneous Adipose Tissue

In the operating room, adipose tissue (120 mL) was suspended in phosphate-buffered saline solution, placed in a sterile box, and transported to a laboratory. Mature adipocytes and connective tissue were separated from the stromal vascular fraction (SVF) by centrifugation, as reported by Zuk et al.[12] The volume of the SVF was usually less than 1.0 mL. For injection, SVF cells were prepared with approximately 3.0 mL of PRP. Before injection, bacteriologic tests were performed to ensure the absence of sample contamination, and the cell viability was assessed by methylene blue dye exclusion.

Assessment of Plastic-Adherent Cells That Form Colony-Forming Unit Fibroblasts and Immunophenotyping of ADSCs

To evaluate the frequency of mesenchymal-like progenitors in patients' SVF, cells were cultured in T-25 flasks at a final concentration of 16 cells/cm^2. Colonies consisting of 50-cell aggregates or greater were scored under an optical microscope, and the immunophenotypes of the ADSCs were analyzed by flow cytometry (fluorescence-activated cell sorting). MSC marker phenotyping was performed as previously described.[13]

Confirmation of Multilineage Differentiation of ADSCs

ADSCs were plated at 2×10^3 cells/cm^2 in Dulbecco's modified Eagle medium containing 10% fetal bovine

serum and allowed to adhere for 24 hours. The culture medium was then replaced with specific media to induce adipogenic, osteogenic, and chondrogenic differentiation, as previously reported.[13]

PRP Preparation

For PRP preparation, a 60-mL venous blood sample (collected in a tube containing 4 mL of sodium citrate) was collected from each patient. A complete peripheral blood count was determined. The samples were centrifuged twice (at 1,800 rpm for 15 minutes to separate the erythrocytes and then at 3,500 rpm for 10 minutes to concentrate the platelets) to yield 6 mL of PRP. The PRP was divided into 2 units of 3 mL each. One unit was sent to the laboratory for determination of the platelet concentration and for quality testing (bacteriologic tests); the other was used for the first injection, within 2 hours of preparation.

MSC Implantation and Open-Wedge HTO

The patients were positioned supine on the operating table, and a thigh tourniquet was applied. Before undergoing HTO, each patient underwent arthroscopic surgery. Using arthroscopy, the orthopaedic surgeons (Y-G.K., Y-J.C.) evaluated the medial, lateral, and patellofemoral joint compartments; graded the articular lesions according to the International Cartilage Repair Society Cartilage Injury Evaluation Package[14]; irrigated the compartment with at least 1 L of saline solution; and performed 1 or more treatments, including synovectomy, debridement or excision of the degenerative tears of the menisci, or removal of articular cartilage fragments, chondral flaps, or osteophytes that prevented full extension. After completion of the arthroscopic procedure, the arthroscopic fluid was washed out. In the MSC-PRP group, injection of MSCs plus PRP (isolated 1 day before arthroscopic surgery) was administered under arthroscopic guidance. In the PRP-only group, the injection of PRP alone was performed after the arthroscopic procedure by injection into the medial joint space under arthroscopic guidance.

After injection, HTO was performed according to the technique recommended by the AO International Knee Expert Group.[15] The TomoFix system (Synthes, Solothurn, Switzerland) was used to stabilize the osteotomy, which was performed in a biplanar fashion. Before surgery, the correction angle and open-wedge size were calculated by the operator (Y-G.K. and Y-J.C.), using AP radiographs of the lower extremity (orthoroentgenogram) with the patient in standing (full weight-bearing) position. The aim was to pass the weight-bearing line through a point 62% lateral to the tibial plateau from the medial edge of the medial tibial plateau; the correction angle and size of the open wedge were measured on the orthoroentgenogram before surgery. All measurements were independently calculated by 2 junior surgeons (O-R.K., Y-S.K.), and all osteotomies aimed for mild overcorrection.[16] A β−tricalcium phosphate (Synthes, Bettlach, Switzerland) wedge, corresponding to the open space, was inserted into the osteotomy site. This material is a fully synthetic, resorbable bone graft substitute, consisting of pure β−tricalcium phosphate with a compressive strength similar to that of cancellous bone.

One day after surgery, isometric quadriceps, active ankle, and straight leg−raising exercises began. The patients were allowed to move their knee from 0° to 90° after 2 weeks. Toe-touch weight bearing was allowed for 2 weeks after surgery, followed by partial weight bearing for the next 2 weeks. Full weight bearing was allowed at 4 weeks, after radiographic evaluation of bone consolidation at the osteotomy site.

Second-Look Arthroscopy

For all patients in this study, second-look arthroscopy was performed during metal removal for fixation. The interval between HTO (first intra-articular observation) and removal of the plate (second intra-articular observation) was 14 to 24 months (mean, 19.8 months). All second-look arthroscopies were video recorded (3 to 5 minutes). The examinations were performed during second-look arthroscopy video review by all members of the surgical team, and the findings were confirmed only when a consensus was reached. Chondral lesions were described, according to the Kanamiya grading system,[4] as follows: grade 1, no regenerative change; grade 2, white scattering with fibrocartilage; grade 3, partial fibrocartilage coverage; and grade 4, even fibrocartilage coverage.

Power Calculation and Statistical Analysis

A difference of 15 points in the Lysholm score (1 of the main outcome measures) represented a clinically significant difference between treatment groups. Thus, accepting less than 5% probability of a type I error and a power of 80%, we determined that a total sample size of 22 patients was required for each group. Predicting a 10% dropout rate, we enrolled a total of 52 patients, with 26 knees comprising each group.

Statistical analyses were performed by use of SPSS software, version 12.0.1 (SPSS, Chicago, IL), with significance defined as $P < .05$. The principal dependent variables of the clinical outcomes were the KOOS, Lysholm score, and VAS pain score at the final follow-up. The Fisher exact test and a χ^2 test were used to compare categorical data. Differences between groups were analyzed by use of the Mann-Whitney U test. The Wilcoxon rank sum test was used for within-group analyses (preoperative v postoperative in same group). The Spearman rank order correlation test was used to

Table 1. Overview of Patient Groups

	PRP-Only Group	MSC-PRP Group	*P* Value
No. of patients	23	21	
Male/female sex (n)	6/17	5/16	.53
BMI (kg/m^2)	24.7 ± 3.3	25.7 ± 2.9	.29
Follow-up period (mo)	24.6 ± 6.4	24.2 ± 4.7	.32
Age (yr)	52.3 ± 4.9	54.2 ± 2.9	.48

NOTE. Values are expressed as mean ± standard deviation unless otherwise indicated.
BMI, body mass index.

analyze the correlation between cartilage healing status and patient demographic factors.

Results

Patient Characteristics

The patient demographic data and characteristics are shown in Table 1. Figure 1 shows the trial profile of this study. There were 52 patients recruited into the study, 26 patients in each group. However, 5 patients (2 in the PRP-only group and 3 in the MSC-PRP group) could not be evaluated at either the 1- or 2-year post-operative visit. Second-look arthroscopic data are missing for 1 patient in the PRP-only group and for 2 patients in the MSC-PRP group because they did not consent to undergo a second surgical procedure for plate removal. Finally, for 44 patients (23 in the PRP-only group and 21 in the MSC-PRP group), second-look arthroscopic results and 2-year clinical results were available for the last analysis. There were no significant differences in patient demographic data between the 2 groups.

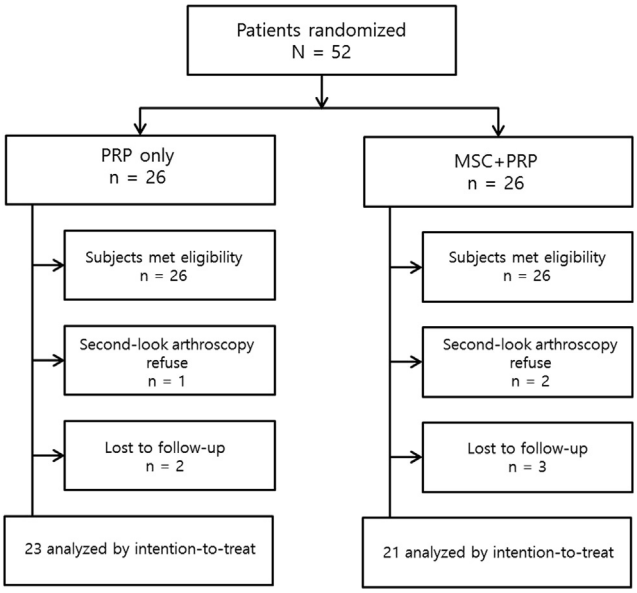

Fig 1. Trial profile of patients randomized in study. The patients were randomized into 2 groups of 26 subjects each; 5 patients were lost to follow-up during the 2-year follow-up and 3 patients refused the second-look arthroscopy.

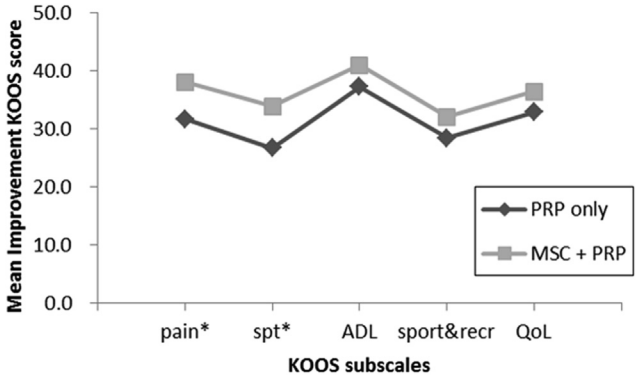

Fig 2. Mean improvement from baseline in KOOS subscales at last follow-up. Asterisks indicate statistical significance ($P <$ .05). (ADL, activities of daily living; QOL, quality of life; sports&rec, sports and recreation; spt, symptoms.)

Cell Isolation and Characterization of ADSCs

The platelet concentrations (mean ± SD) in whole blood and PRP were 208.53 ± 42.9 × 10^3/mL and 1,303.27 ± 375.2 × 10^3/mL, respectively.

After isolation, ADSCs represented 8.5% of the SVF cells (range, 6.8% to 10.2% of SVF cells), or 4.11 × 10^6 stem cells (8.5% of the 4.83 × 10^7 SVF cells) were prepared. Flow cytometry characterization showed positive expression of the CD90 (98.34%) and CD105 (91.23%) surface markers and negative expression of CD45 (2.23%), CD34 (6.45%), and CD14 (2.32%). ADSCs treated with conditioned media showed characteristics of adipogenic, osteogenic, and chondrogenic differentiation, as previously reported.[17]

Clinical and Radiologic Outcomes at Follow-up

The patients in the MSC-PRP group showed a trend toward greater improvements in all of the KOOS subscales, although significant differences were only observed for the pain and symptom subscales at the last follow-up (Fig 2). The MSC-PRP group showed significantly greater improvements in the KOOS pain subscale (PRP only, 74.0 ± 5.7; MSC-PRP, 81.2 ± 6.9; $P <$ .001) and symptom (PRP only, 75.4 ± 8.5; MSC-PRP, 82.8 ± 7.2; $P =$ .006) scores relative to the PRP-only group. The other clinical and radiologic outcomes at the preoperative and final follow-up time points, for both groups, are summarized in Table 2. The mean Lysholm score was also significantly improved in both groups ($P <$ .001), but no differences were seen between the groups ($P =$ .357). Although the mean VAS pain score decreased significantly (i.e., improved) at the final follow-up visit in both groups ($P <$ .001), the MSC-PRP group showed a greater improvement relative to the PRP-only group ($P <$ .001).

The standing AP radiographs taken immediately after implant removal showed improved knee joint mechanics in both groups relative to their preoperative conditions. However, there were no differences in the

Table 2. Clinical and Radiologic Results of Patient Groups

	PRP-Only Group	MSC-PRP Group	*P* Value (95% CI)
Lysholm score			
Preoperative	56.7 ± 12.2	55.7 ± 11.5	.747 (−12.12 to 8.83)
Last follow-up	80.6 ± 13.5	84.7 ± 16.2	.357 (−8.4 to 1.2)
VAS			
Preoperative	45.4 ± 7.1	44.3 ± 5.7	.460 (−0.77 to 0.29)
Last follow-up†	16.2 ± 4.6	10.2 ± 5.7	<.001 (0.23 to 0.98)
WBL (%)			
Preoperative	16.1 ± 5.7	17.7 ± 7.3	.800 (−2.56 to 3.91)
Last follow-up	60.3 ± 3.0	61.1 ± 3.4	.758 (−3.50 to 4.51)
FTA (°)			
Preoperative	Varus 2.8 ± 1.7	Varus 3.4 ± 3.0	.719 (−1.30 to 1.87)
Last follow-up	Valgus 9.8 ± 2.4	Valgus 8.7 ± 2.3	.678 (−1.32 to 1.90)
Initial cartilage status (n)*			.876
Grade 2	1	0	
Grade 3	11	9	
Grade 4	11	12	

NOTE. Values are expressed as mean ± standard deviation unless otherwise indicated.
CI, confidence interval; WBL, weight-bearing line.
*Initial cartilage status was graded by arthroscopy before HTO; the orthopaedic surgeons (Y-G.K., Y-J.C.) evaluated the medial joint compartments and graded the articular lesions according to the International Cartilage Repair Society Cartilage Injury Evaluation Package.
†Significant difference at last follow-up between groups ($P < .05$).

postoperative FTAs ($P = .678$) or weight-bearing lines ($P = .758$) between the groups.

Second-Look Arthroscopy

There were no significant differences in the initial cartilage status between the groups ($P = .876$) (Table 2). However, there was a significant difference between the groups with respect to cartilage healing ($P = .023$) (Fig 3). Second-look arthroscopy, during plate removal, showed that 0 of the 23 knees in the PRP-only group had even fibrocartilage coverage (grade 4), determined arthroscopically. One knee (4.3%) had partial fibrocartilage coverage (grade 3), 11 (47.8%) had white scattering with fibrocartilage (grade 2), and 11 (47.8%) did not show any regenerative changes (grade 1). In contrast, in the MSC-PRP group, 3 knees (14.3%) had even fibrocartilage coverage (grade 4), 8 (38.1%) had partial fibrocartilage coverage (grade 3), 9 (42.9%) had white scattering with fibrocartilage (grade 2), and 1 (4.8%) did not show any regenerative changes (grade 1). Figure 4 shows examples of the arthroscopic photographs used in the patient evaluations.

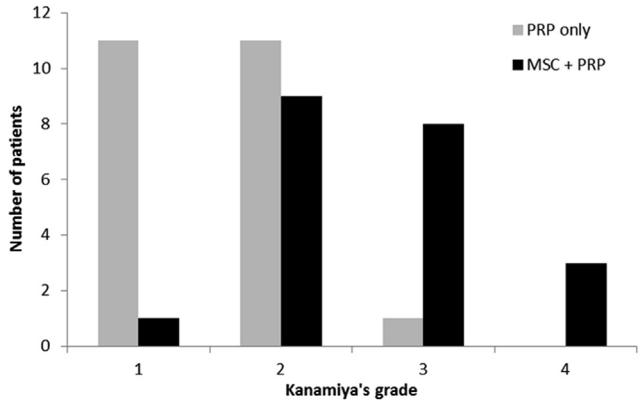

Fig 3. Articular cartilage healing status, using the Kanamiya grading system,[4] during second-look arthroscopy in both groups.

Correlation Between Cartilage Healing Status and Patient Demographic Factors

The correlations between cartilage healing status and other patient demographic factors were analyzed to determine whether there were other reasons for the observed cartilage healing status. However, significant correlations were not found between the cartilage healing status and patient body mass index, age, or radiologic parameters (Table 3).

Discussion

The principal findings of this study were that HTO in conjunction with the use of MSCs plus PRP resulted in good fibrocartilage repair and improved clinical results compared with HTO and PRP only. Importantly, other patient demographic factors, such as age, were not associated with improvements in cartilage healing, suggesting that the improvements were primarily due to MSC injection. Thus these findings support the hypothesis that MSC therapy with PRP, in conjunction with HTO, provided additional benefits for cartilage healing and clinical results compared with injection of PRP only.

HTO has been recommended for treating varus OA to decrease the pressure on the damaged medial compartment of the joint, provide pain relief, and reduce the progression of medial OA.[18] Although HTO theoretically decreases the stress on the load-bearing cartilage in the medial compartment,[2-5] some studies

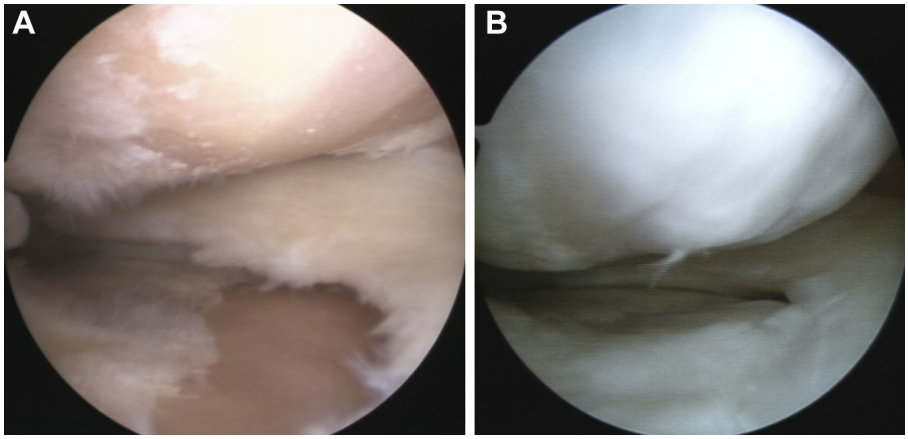

Fig 4. Intraoperative arthroscopic images during first- and second-look arthroscopy. (A) Findings in a 53-year-old woman in the MSC-PRP group. During the first arthroscopy, eburnation of the articular surfaces was found. (B) Marked changes in the cartilage defects of the medial femoral condyle are shown. The articular surface shows an even fibrocartilage coverage at 17 months postoperatively.

have reported that partial remodeling of the articular cartilage occurs with cartilage regeneration after HTO.[19,20] For better chondral defect remodeling, HTO combined with chondral resurfacing has been attempted.[3,21] The most popular chondral resurfacing procedures are marrow stimulation techniques. These techniques involve microfractures that promote cartilage repair by stimulating the bone marrow through the subchondral bone and by producing blood clots containing mesenchymal cells on the articular surface. In a 2-year follow-up study of 38 patients, Sterett and Steadman[21] reported that combining a medial open-wedge HTO with a microfracture in the varus knee was an effective method for decreasing pain and increasing function. However, Mithoefer et al.[22] reported that microfractures effectively improved knee function in all patients during the first 24 months after the microfractures, but the durability of the initial functional improvement was inconsistent. Moreover, in patients with degenerative knee arthritis, the cartilage lesion is diffuse and not focal, meaning that microfractures cannot be applied in all HTO cases. Thus, for cartilage defect remodeling, other options are needed.

MSCs are emerging as powerful tools for cartilage repair because of their ability to differentiate into various connective tissues, including cartilage, bone, and fat.[23,24] The intra-articular injection of MSCs was reported to effectively reduce pain while promoting cartilage regeneration in patients with knee OA.[6,7] On the basis of these previous findings, stem cell injection may be used to achieve greater cartilage remodeling and better clinical results after HTO surgery. Thus, in our study, more patients in the MSC-PRP group achieved partial or even fibrocartilage coverage than in the PRP-only group, showing a clear relation between the cartilage healing status and MSC therapy. Furthermore, the patients in the MSC-PRP group showed statistically significantly better clinical outcomes in the VAS pain score and 2 KOOS subscores compared with patients in the PRP-only group. Although better scores were observed in the group receiving MSC therapy than in the group receiving PRP only, there were no differences between the groups with respect to the Lysholm score and the other KOOS subscores.

Table 3. Correlation Between Cartilage Healing Status and Patient Demographic Factors

	Healing Status	
	Spearman ρ	*P* Value
BMI	0.81	.60
Age	0.09	.56
WBL	0.10	.51
FTA	−0.08	.60

NOTE. Data were calculated using the Spearman rank order correlation test.

BMI, body mass index; WBL, weight-bearing line.

In this study, subcutaneous adipose tissue was used as the stem cell source. Adipose tissue is composed of 2 main cell populations, mature adipocytes and the cells in the SVF. The latter comprise a heterogeneous fraction that includes preadipocytes, endothelial cells, smooth muscle cells, pericytes, macrophages, fibroblasts, and ADSCs, which share several characteristics with bone marrow stem cells.[25,26] ADSCs are promising candidates in a broad range of innovative therapies, ranging from regenerative medicine to tissue engineering. Moreover, the use of ADSCs has been proposed for several chronic diseases, such as Crohn disease,[27] autoimmune pathologies (e.g., multiple sclerosis),[28] and allergic pathologies. The effectiveness against these pathologies can be explained by the immunoregulatory and anti-inflammatory activities of ADSCs and non-expanded SVF cells.[28] Unfortunately, because most studies have focused on in vitro expanded adipose-derived cells, relatively little is known about the potential clinical effects of the whole lipoaspirate, which contains numerous cell populations in addition to MSCs. Recently, ADSCs have been suggested as a new option for the treatment of osteochondral lesions, and the injection of MSCs with marrow stimulation has

been proposed for treating such cases.[29] Moreover, Desando et al.[30] reported that the healing properties of ADSCs, including their promotion of cartilage and meniscus repair and attenuation of inflammatory events in the synovial membrane, may inhibit OA progression. Jurgens et al.[31] evaluated the safety, feasibility, and efficacy of freshly isolated SVF cells and cultured ADSCs in an animal model. They showed the preclinical safety and feasibility of a 1-step surgical procedure for osteochondral defect regeneration using freshly isolated SVF cells and cultured ADSCs. Specifically, they observed similar regeneration induced by either freshly isolated SVF cells or cultured ADSCs.

In OA patients the healing tissue has been shown to be quite different from the surrounding degenerated yellow cartilage. Furthermore, because the cartilage of OA patients has diffuse degenerative lesions, identifying changes in the status of OA patients is difficult. In other words, the grading of severe lesions, used in the Outerbridge classification[32] and the International Cartilage Repair Society grade, does not seem appropriate to describe these changes in the cartilage status of OA patients. Thus the classification of the regenerative progress using the Kanamiya classification,[4] as used in our study, is necessary.

MSC therapy has previously been shown to induce a positive effect in OA treatment through 2 mechanisms, paracrine signaling and end-organ (e.g., cartilage) formation. Paracrine mechanisms likely explain the clinical improvements, whereas cartilage formation explains the differences in cartilage healing status observed between the groups in this study at their final follow-up visit. The MSC therapy method used in this study was a very primitive technique; therefore the method cannot likely be used in isolation. For the application of this technique, several challenges still need to be overcome, including the identification of the optimal sources of stem cells, scaffolds, and growth factors.

Limitations

This study has several limitations. First, the follow-up period was short, and therefore future studies with longer cartilage formation and survival follow-up periods should be undertaken. Second, the stem cells were delivered with a single injection, whereas optimal results may require providing patients with more than 1 injection over time. Third, pathologic examinations of the cartilage properties in each group were not performed. Fourth, the loss of correction influenced the clinical outcome; because patients were not assessed in the standing position, measurement of correction angles in the immediate postoperative period was not performed. Therefore a measure of the influence of correction loss on clinical outcomes was not possible. Fifth, because several patients were excluded because they did not want to undergo plate removal, there might be the problem of selection bias in this study. Sixth, the Kanamiya grading system[4] was a potential limitation because it was not validated with known interobserver and intraobserver variability. Lastly, an additional limitation is the potential for type II errors because of the small sample sizes. Although an a priori power evaluation was conducted to determine the number of participants required for the trial, the calculations were completed using limited data. Therefore the study may suffer from a type II statistical error, resulting from the effects of stem cells on persons with diffuse cartilage lesions. Thus the lack of significant differences in some of the clinical outcome data, with the exception of the pain scores and symptom subscores, was likely because of a type II error. In addition, although statistically significant improvements in some KOOS subscores and in the VAS pain score were observed, they may not reflect clinically significant improvements. Therefore another study will be needed with a larger number of patients.

Conclusions

MSC therapy, in conjunction with HTO, induced mild improvements in cartilage healing and good clinical results in some KOOS subscores and the VAS pain score compared with PRP only.

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