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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,

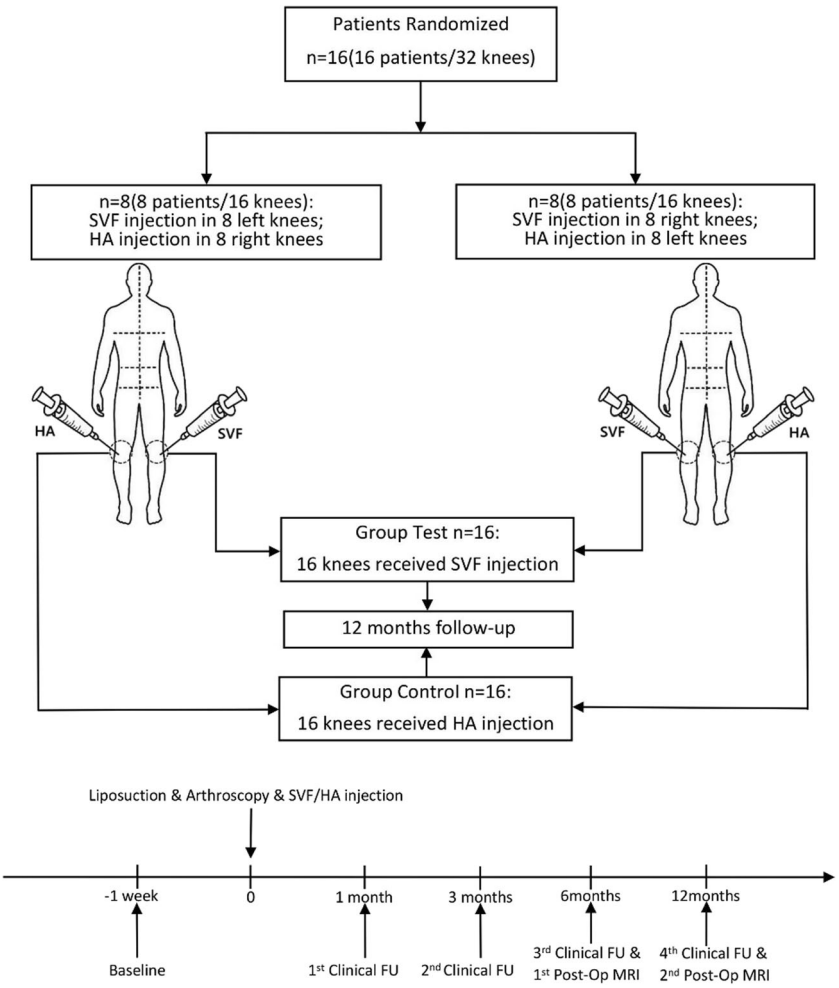

Fig. 1 Study flowchart

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

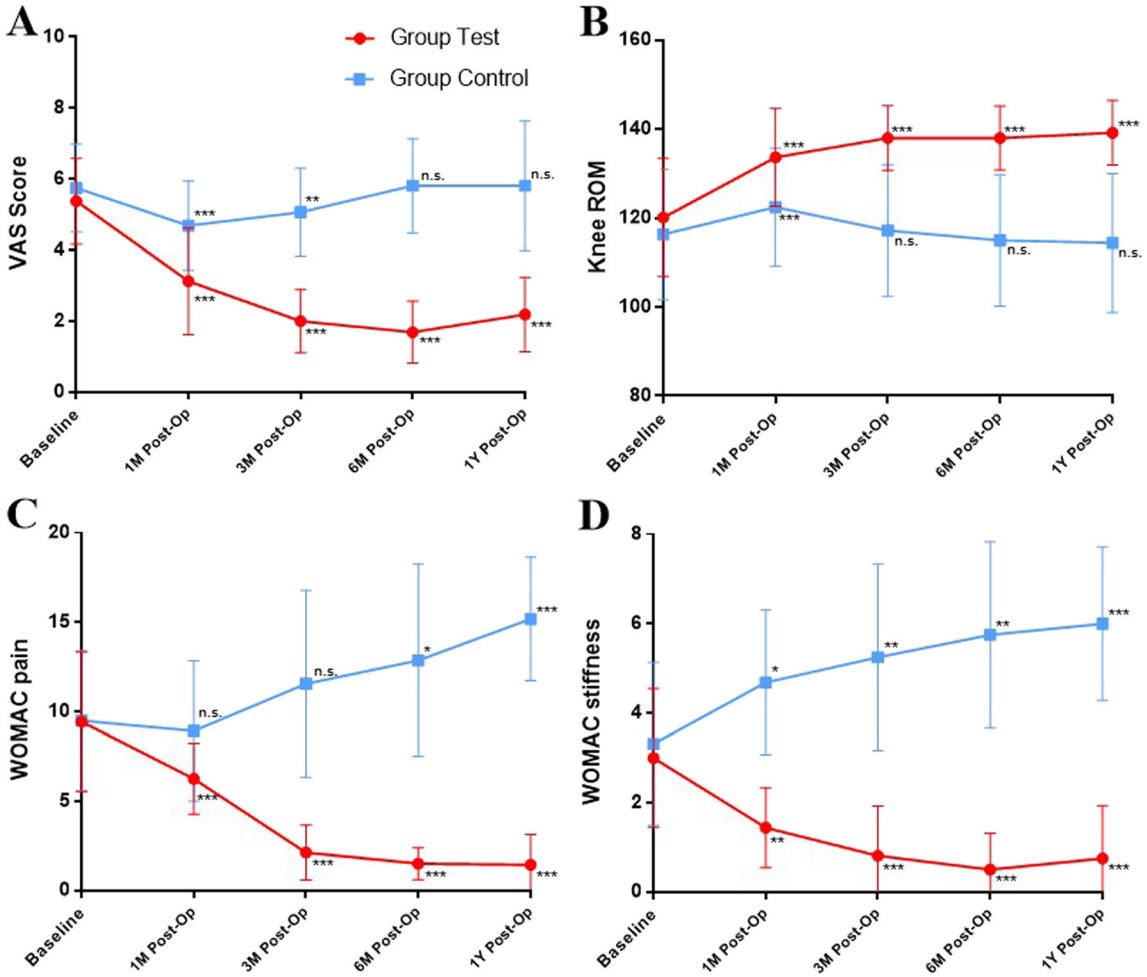

Fig. 2 Changes of VAS, WOMAC score, and knee ROM in two groups during 12-months follow-up. Values in graphs are expressed as mean ± SD in vertical bars, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, non-significant ($P > 0.05$). All values were compared with baseline. **a** VAS score. **b** Knee ROM. **c**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

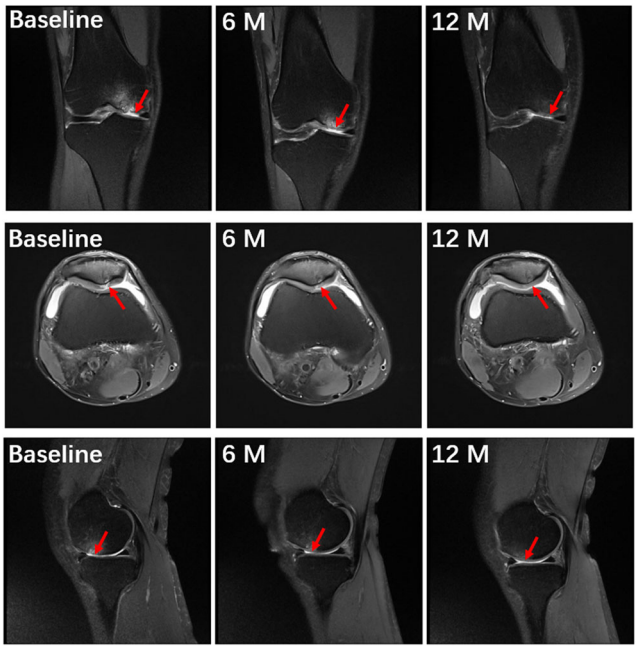

Fig. 3 Magnetic resonance imaging scans of three SVF-treated knees from baseline to 6 and 12-months follow-up showed complete repair and filling of the defects, as well as good integration with the adjacent cartilage and underlying bone in the coronal, transverse and sagittal planes (red arrows)

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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