Human adipose-derived mesenchymal progenitor cells plus microfracture and hyaluronic acid for cartilage repair: a Phase IIa trial

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Aim: This study aimed to preliminarily evaluate the safety and efficacy of human adipose-derived mesenchymal progenitor cells (haMPCs) in combination with microfracture and hyaluronic acid (HA) for treating cartilage defects. **Materials & methods:** A total of 30 patients with medial femoro-tibial condylar cartilage defects were randomized into three groups: arthroscopic microfracture group and normal saline injection, arthroscopic microfracture and intra-articular injection of HA, or arthroscopic microfracture in combination with intra-articular injection of HA and haMPCs. **Results & conclusions:** The data demonstrated that intra-articular injection of haMPCs plus microfracture and HA is a safe procedure to improve joint function in patients with knee cartilage defects. These findings provide an impetus for future research on this treatment. ClinicalTrials.gov Identifier: NCT02855073

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Graphical abstract:

Knee osteoarthritis (OA) is a degenerative joint disease, leading to irreversible damage of the cartilage, osteophyte formation, bone reorganization, synovial inflammation and loss of joint function [1,2]. Current treatments focus on relieving symptoms and improving joint function [3,4]. Total joint replacement is proven to be the most effective surgical intervention; however, it is an aggressive procedure and should be reserved for patients whose symptoms have not responded to other treatments [5]. Other treatment strategies include but are not limited to (arthroscopic) microfracture, intra-articular injection of hyaluronic acid (HA) and intra-articular injection of stem cells.

Although clinical efficacy of microfracture [6–10], intra-articular injection of HA [11–20] and stem cells [21–26] have been reported by multiple published studies, these treatments were used mainly in younger patients with low grade OA, making it difficult to evaluate the treatment efficacy in Kellgren Lawrence (KL) grade 3 and KL grade 4 OA.

In addition, many studies have cast doubt on the value of these treatments, indicating that microfracture [27,28] and HA [29] provide no additional benefit over physiotherapy and placebo. Although a knee arthroplasty is predictable and reasonable for high-grade OA, it may not be ideal for all patients. After total knee arthroplasty (TKA), low-impact activities, instead of moderate- or high-impact activities, are recommended [30,31]. Only 20% patients return to high impact sports [32]. Active patients younger than 60 years with symptomatic moderate to severe OA are frequently looking for alternative treatment options, even if only a temporizing measure [33].

In previous studies, various stem cell sources have been used to treat OA because of their chondrogenic and anti-inflammatory properties [34–36]. Among them, adipose-derived stem cells (ADSCs) are easy to collect for clinical application with high isolation yields and are not affected by the patients' age, sex and physiological status [37]. Like stem cells, progenitor cells also tend to differentiate into a specific type of cell and replicate themselves to replace or repair damaged tissues or organs. Results in previous studies suggested that intra-articular injection of human adipose-derived mesenchymal progenitor cells (haMPCs) in animal models promoted regeneration of articular cartilage [38,39].

To date, few studies have used haMPCs in treating cartilage defects in moderate to severely arthritic knees. This study, therefore, aimed to preliminarily evaluate the safety and efficacy of haMPCs in KL grade 3 patients with knee cartilage defects. We conducted a Phase IIa proof-of-concept clinical trial in KL grade 3 patients aged 50–75 with knee cartilage defects to preliminarily evaluate and compare the clinical effects of arthroscopic microfracture, intra-articular injection of sodium hyaluronic acid (Artz, Seikagaku Corporation, Japan), and intra-articular injection of haMPCs (Re-Join[®], Cellular Biomedicine Group, Inc., USA). We assumed that haMPCs in combination with microfracture and HA will provide clinical improvement and promote cartilage regeneration for patients with cartilage defects within arthritic knees.

Patients & methods

A randomized, single-blind, parallel group, Phase IIa proof-of-concept clinical trial to preliminarily evaluate and compare the clinical effects of arthroscopic microfracture, sodium hyaluronic acid and Re-Join[®] (autologous adipose-derived mesenchymal progenitor cells and sodium hyaluronic acid) injection for the patients with cartilage defects within arthritic knees was conducted from July 2016 to December 2018 in two hospitals: Shanghai Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine and Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. The study protocol was approved by the local ethics committees of both institutions (No.22, 2014; Scientific Research Projects Approval Determination of Independent Ethics Committee of Shanghai Ninth People's Hospital affiliated to Shanghai Jiao Tong University School of Medicine and Scientific Research Projects Approval Determination of Independent Ethics Committee of Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine) and registered at clinicaltrials.gov with identifier NCT02855073 (date of registration, July 8, 2016). The methods were carried out in accordance with the relevant guidelines and regulations. Informed consent was obtained according to the Helsinki Declaration.

Patient selection & enrollment

Patients with a clinical diagnosis of knee OA with medial femoro-tibial condylar or trochlear-patellar cartilage defect were assessed for eligibility. Based on the defect area, enrolled patients were separated into two groups. Informed Consent was obtained from all patients before participation in this trial.

After signing the Informed Consent Form, each patient was randomized into one of the three treatment groups: The M group, treated with arthroscopic microfracture and intra-articular injection of normal saline (NS); the MS group, treated with arthroscopic microfracture and intra-articular injection of sodium hyaluronic acid; or the MSR group, treated with arthroscopic microfracture in combination with intra-articular injection of sodium hyaluronic acid and Re-Join. A study flowchart detailing this information is shown in Figure 1. Analgesics were not permitted except for the rescue medications, acetaminophen, at a dose of 4000 mg or less per day.

Inclusion criteria

Eligible patients were between 50 and 75 years of age with clinical diagnosis of knee OA cartilage defects and radiographic changes of grade 3 according to Kellgren–Lawrence criteria in the targeted knee [40] and had an average pain intensity of grade 6 or more on a 10-point visual analog scale (VAS) for at least 4 months. To be included in the study, the patient must have either medial femoro-tibial condylar defect or trochlear-patellar defect

Figure 1. Study flow diagram. (A) Flowchart of the clinical trial. **(B)** Diagrams for each treatment (left) and detailed time points for treatment and follow-ups (right).

HA: Hyaluronic acid; M: Arthroscopic microfracture and normal saline injections; MRI: Magnetic resonance imaging; MS: Arthroscopic microfracture and sodium hyaluronic acid injections; MSR: Arthroscopic microfracture, sodium hyaluronic acid, and Re-Join[®] injections.

and the defect area must have been 2–6 cm² measured by MRI, and the course of knee OA must have been no longer than 10 years.

Exclusion criteria

Patients were excluded if they met at least one of the following criteria: had both medial femoro-tibial condylar and trochlear-patellar cartilage defect; pregnant women or lactating mothers; previous arthroscopic treatment for knee OA; intra-articular HA or corticosteroid injection in the preceding 3 months; previous total meniscectomy,

meniscal allograft, bucket-handle tears and complex tears; secondary arthritis (related to rheumatoid arthritis, spondyloarthritis, previous major knee trauma, inflammatory or postinfectious arthritis, and crystal arthropathies); positive human immunodeficiency (HIV), hepatitis B (HBV) or hepatitis C (HCV) at screening indicative of current of past infection; major malalignment which can affect this study such as uncontrollable cardiovascular diseases, renal diseases, liver diseases, endocrine diseases, cancer or diabetes; participated in other clinical trials within 3 months prior to this study; or patients deemed to be not suitable for the study by the investigator for other reasons.

Treatment randomization

Eligible participants who met criteria were randomly assigned using central randomization software (SAS, version 9.4) 1:1:1 to the M group (receiving arthroscopic microfracture treatment and NS injections), MS group (receiving arthroscopic microfracture treatment and sodium hyaluronic acid injections) and MSR group (receiving arthroscopic microfracture treatment, sodium hyaluronic acid and Re-Join injections). Using computer-generated random numbers, Tang J generated the random allocation sequence and assigned participants to interventions. Whereas physicians were aware of the allocated arm, patients and outcome assessors were kept blinded to the allocation.

About Re-Join therapy & sodium hyaluronic acid

Re-Join is a product composed of *in vitro* expanded autologous MPCs derived from adipose tissue of patients combined with cell suspension solution (see Supplementary Method 1 for detailed information). Our preliminary experiment with dose escalation for the adipose MSCs has shown that the dosage of 5×10^7 haMSCs exhibited the highest improvement [41]. Sodium HA used in this study was Artz (Seikagaku Corporation, Japan; see Supplementary Method 3 for detailed information).

Treatment

All procedures were performed in the supine position under general anesthesia. A single orthopedic surgeon performed all procedures. The procedures were arthroscopic, and the principles of the technique introduced by Steadman *et al.* [10] were used. Articular cartilage lesions were measured with a calibrated arthroscopic probe. To obtain stable and healthy cartilage edges, debridement of all damaged and unstable cartilage was performed. Microfractures were performed at all defect areas (medial femoro-tibial condyles or trochlea and patella) with an arthroscopic awl. The holes were 3–4 mm apart from each other and deep enough to show the ‘fat-pearls’ from the subchondral bone. For patients with medial femoro-tibial condylar cartilage defect, all the defects in medial femoro-tibial compartment were microfractured. For trochlear-patellar cartilage defects, all the defects in trochlear-patellar compartment were microfractured. Cartilage defects in other areas were not treated. In patients who gave consent, a 2-mm-punch biopsy specimen was obtained from the center of the cartilage defect of the medial femoral condyle. The day on which the arthroscopic surgery was performed was marked as day 1. For the M group, subjects received NS injections on days 1, 8, 15 and 22. For the MS group, subjects received sodium hyaluronic acid injections on days 1, 8, 15 and 22. For the MSR group, subjects received Re-Join injections on days 1 and 22 and sodium hyaluronic acid injections on days 8 and 15. A study flowchart detailing this information is shown in Figure 1B. Postoperative rehabilitation program started from day 1. All patients were educated using crutches and avoiding weight bearing for 4 weeks. A stepwise increase in load bearing was introduced after that.

After completing the 24-month observation, all patients can receive follow-up treatment with Re-Join.

Second-look arthroscopy

Second-look arthroscopy 6 months post-treatment was conducted by two surgeons at our hospital. Cartilage defects were measured with a calibrated arthroscopic probe, and the healing status was visually inspected and scored as per the validated International Cartilage Repair Society (ICRS) [42] macroscopic score. In case of multiple cartilage defects, the size of each defect was summed up. The observation was confirmed only once a consensus was reached by both two surgeons. A 2-mm punch biopsy specimen was obtained from the adjacent area to the first biopsy site in patients who gave consent.

Outcome measures

The primary end points were the safety and the Western Ontario and McMaster Universities Arthritis Index (WOMAC) [43] total index (0–96 scale) at month 12 after treatment. The safety was systematically assessed with vital signs, physical examination, laboratory tests and adverse events at each visit throughout the study.

Secondary efficacy end points included: WOMAC physical function (0–68 scale), pain (0–20 scale) and stiffness (0–8 scale) subscales and quality of life measured by the short-form 36 (SF-36) questionnaire [44] (time frame: 0 day, 3 months, 6 months, 9 months, 12 months and 24 months). The size of the cartilage defect was measured using MRI by two independent blinded radiologist specialize in musculoskeletal reads [45]. For calculating size of the cartilage defect, the maximum diameter of the cartilage defect in each compartment was measured in the sagittal (anteroposterior) and coronal (mediolateral) images measured using imaging processing software (Mimics 15.0 Materialise Corporation, Leuven, Belgium). Size of the cartilage defect was calculated by multiplying the two diameters. In case of multiple cartilage defects, the size of each defect was summed up. This also applied to follow-ups. Changes in the cartilage volume and the defected area were measured using Mimics 15.0 (Materialise Corporation, Leuven, Belgium) by a single observer (ZQ) blinded to other results (time frame: 0 day and 6 months; see [Supplementary Method 2](#)). The defect size and healing status of degenerated cartilage was measured with a calibrated arthroscopic probe and the ICRS macroscopic score [46,47] during the second-look arthroscopy (time frame: 0 day and 6 months). For histological assessment, biopsy specimens were subject to hematoxylin–eosin (H&E) staining, safranin O/fast green (SOFG) staining, Masson's trichrome staining and immunohistochemistry for type I and II collagen. Hematoxylin staining was used as a counter-stain for immunohistochemistry. Thickness of regenerated cartilage was measured, and specimens were evaluated with ICRS II (time frame: 0 day and 6 months) [48]. Inflammation parameter was proved difficult to score in analyzed biopsy specimens in previous study thus not scored in our study.

Statistical analysis

Based on the result from previous studies, for each treatment group, 8 participants were required to achieve 85% power and an α level of 0.05 (one-way analysis of variance). Thus, 24 participants were required. Considering a compliance rate of 80%, 30 participants were planned to be randomly assigned to allow for dropouts.

Outcome measures were analyzed based on the intention-to-treat population. All values are expressed as number (%) of patients or mean $\pm$ standard deviation. The significance of differences between groups was assessed using one-way analysis of variance (one-way ANOVA) for parametric variables, or Kruskal–Wallis ANOVA test and a Dunn–Bonferroni test for *post hoc* comparisons for nonparametric variables. Changes from baseline to treatment visits were assessed with paired-samples t-test for parametric variables, or Mann–Whitney U test for nonparametric variables within the group. A value of $p < 0.05$ was considered statistically significant. All analyses were performed using IBM SPSS Statistics version 19.0 for Windows (IBM Corporation, NY, USA).

Results

Characteristics of patients

A total of 59 patients with medial femoro-tibial condylar cartilage defects were assessed for eligibility and 30 patients were enrolled. For patients with trochlear-patellar defect, 67 patients were screened and 30 were enrolled. Eligible participants were randomly assigned to treatment (for patients with medial femoro-tibial condylar defect, 10 in M, 10 in MS and 10 in MSR each group; for patients with trochlear-patellar defect, 10 in each group; Figure 1A). All treatment groups had similar baseline characteristics (Tables 1 & 2).

For patients who had medial femoro-tibial condylar cartilage defects, 28 patients underwent second-look arthroscopy 6 months post-treatment and 23 patients completed the 24-month follow-up. For patients who had trochlear-patellar cartilage defects, 28 underwent second-look arthroscopy after 6 months. 23 patients completed the 24-month follow-up. However, due to lack of consent for biopsy on second-look arthroscopy (only four patients gave consent), patients with trochlear-patellar cartilage defects were excluded from following analysis.

Adverse effects

AEs are summarized in Table 3. No serious AEs were observed in this study. No local or systemic safety concerns were indicated through vital signs, physical examinations or laboratory tests during each follow-up. Several minor AEs were potentially related to the procedures: slight joint swelling and pain in treated knees were reported by five patients, in which three patients it was resolved with intermittent acetaminophen and in two patients the swelling

Table 1. Baseline characteristics of patients with medial femoro-tibial cartilage defect.

Characteristic	M (n = 10)	MS (n = 10)	MSR (n = 10)	p-value
Age (year)	62.3 ± 5.27	59.7 ± 7.12	62.0 ± 8.33	0.667
Women	7 (70)	5 (50)	7 (70)	0.574
BMI (kg/m ²)	25.4 ± 3.16	26.4 ± 2.14	26.0 ± 2.86	0.705
Kellgren–Lawrence system				
– Grade III	10 (100)	10 (100)	10 (100)	1.000
– Global knee pain VAS (0–10 cm)	7.57 ± 1.42	7.44 ± 1.00	6.85 ± 1.04	0.355
WOMAC				
– Total index (0–96 scale)	40.2 ± 12.9	40.9 ± 6.26	45.8 ± 10.2	0.419
– Function subscale (0–68 scale)	30.3 ± 10.4	31.3 ± 5.81	33.7 ± 9.08	0.669
– Pain subscale (0–20 scale)	7.00 ± 2.16	6.80 ± 2.15	8.80 ± 2.70	0.131
– Stiffness subscale (0–8 scale)	2.90 ± 2.08	2.80 ± 1.99	3.30 ± 1.34	0.813
SF-36				
– Physical component	48.8 ± 5.47	51.1 ± 4.85	48.4 ± 5.84	0.500
– Mental component	53.0 ± 7.93	49.9 ± 8.75	54.0 ± 5.94	0.466

Data are number (%) of patients or mean ± standard deviation values. Differences in parameters between groups were analyzed using one-way ANOVA for continuous variables, or Kruskal–Wallis ANOVA test and a Dunn–Bonferroni test for *post hoc* comparisons for categorical variables.

BMI: Body mass index; M: Microfracture and saline; MS: Microfracture and sodium hyaluronic acid; MSR: Microfracture, sodium hyaluronic acid and Re-Join®; SF-36: Short-form 36 (quality of life); VAS: Visual analog scale; WOMAC: Western Ontario and McMaster University Osteoarthritis Index.

Table 2. Baseline characteristics of patients with trochlear-patellar cartilage defect.

Characteristic	M (n = 10)	MS (n = 10)	MSR (n = 10)	p-value
Age, year	62.0 ± 6.39	64.1 ± 7.62	60.2 ± 9.21	0.545
Women	7 (70)	7 (70)	6 (60)	0.865
BMI, kg/m ²	26.2 ± 3.80	24.7 ± 3.97	25.3 ± 4.13	0.694
Kellgren–Lawrence system				
– Grade III	10 (100)	10 (100)	10 (100)	1.000
– Global knee pain VAS (0–10 cm)	7.57 ± 1.42	7.44 ± 0.906	6.72 ± 1.71	0.350
WOMAC				
– Total index (0–96 scale)	42.8 ± 9.02	37.5 ± 8.24	38.7 ± 7.26	0.332
– Function subscale (0–68 scale)	32.7 ± 8.62	28.1 ± 8.18	28.8 ± 8.01	0.419
– Pain subscale (0–20 scale)	7.80 ± 3.29	6.70 ± 2.67	6.00 ± 2.58	0.380
– Stiffness subscale (0–8 scale)	2.30 ± 1.77	2.70 ± 1.34	3.90 ± 1.91	0.107
SF-36				
– Physical component	47.4 ± 4.05	51.5 ± 7.18	49.4 ± 9.83	0.484
– Mental component	51.3 ± 5.33	54.4 ± 7.44	53.0 ± 7.96	0.617

Data are number (%) of patients or mean ± standard deviation values. Differences in parameters between groups were analyzed using one-way ANOVA for continuous variables, or Kruskal–Wallis ANOVA test and a Dunn–Bonferroni test for *post hoc* comparisons for categorical variables.

BMI: Body mass index; M: Microfracture and saline; MS: Microfracture and sodium hyaluronic acid; MSR: Microfracture, sodium hyaluronic acid and Re-Join®; SF-36: Short-form 36 (quality of life); VAS: Visual analog scale; WOMAC: Western Ontario and McMaster University Osteoarthritis Index.

and pain relieved spontaneously; skin erythema around the treated knees was reported by two patients and was resolved without medication.

Complications not related to the procedure were also identified and recorded, including urinary tract, respiratory and dental infections, diarrhea, cardiac failure, urinary retention, confusional state and headache.

Changes in WOMAC Scores

Figure 2A, B and Table 4 show the WOMAC scores for patients with medial femoro-tibial condylar cartilage defects. Pretreatment WOMAC total scores were nearly identical, with a small nonsignificant difference observed among M, MS and MSR groups (40.2 ± 12.9 vs 40.9 ± 6.26 vs 45.8 ± 10.2 , respectively; $p = 0.419$) (Table 1). At 3 months after treatment, the WOMAC total scores in all groups improved, but the difference among three groups were nonsignificant ($p = 0.625$). At 6 months post-treatment, the differences in improvement in WOMAC

Figure 2. Changes in Western Ontario and McMaster University Osteoarthritis Index and Short-form 36 (quality of life) scores after treatment. Medial femoro-tibial condyle defect. **(A & B)** The WOMAC total index and WOMAC subscales. A tendency of improvement in WOMAC total index and all the subscales was shown in the first year in all groups. However, after 1 year, the scores in M group declined, while in the other two groups, the WOMAC scores continued to improve. The changes in WOMAC score among the treatment groups were similar at month 3 and became significant later. **(C)** SF-36 score. Improvement of SF-36 score was seen in the MS and MSR groups. In the M group, a similar tendency was seen during the first year, while SF-36 score declined at month 24. Differences were analyzed by the one-way analysis of variance (one-way ANOVA) test and a Dunn-Bonferroni test for *post hoc* comparisons. p-values were calculated only for comparisons between Δ values.

* $p < 0.05$ and ** $p < 0.001$ vs M group, # $p < 0.05$ and ## $p < 0.001$ vs MS group.

Δ : Change from baseline; M: Microfracture and saline; MS: Microfracture and sodium hyaluronic acid; MSR: Microfracture, sodium hyaluronic acid and Re-Join® injections; SF-36: Short-form 36 (quality of life); WOMAC: Western Ontario and McMaster University Osteoarthritis Index.

Table 3. Summary of adverse events during the clinical trial.

Treatment Group	0–6 Months			6–12 Months			After 12 Months		
	M (n = 9)	MS (n = 9)	MSR (n = 10)	M (n = 9)	MS (n = 8)	MSR (n = 9)	M (n = 7)	MS (n = 8)	MSR (n = 8)
AEs	10	11	9	6	5	3	4	4	1
Patients with AEs	4	4	5	3	4	3	3	3	1
Patients with serious AEs	–	–	–	–	–	–	–	–	–
Infections									
– Urinary tract infection	1	–	–	1	–	–	–	1	–
– Respiratory infection	2	–	1	1	–	1	1	–	1
– Dental infection	–	1	–	–	1	–	–	1	–
Musculoskeletal disorders									
– Joint effusion/swelling, treated knee	2	1	2	–	–	1	–	–	–
– Sciatic pain	1	2	2	1	–	–	–	–	–
– Low back pain	2	1	1	2	1	–	1	1	–
– Skin erythema around the treated knee	–	1	1	1	–	1	–	–	–
Gastrointestinal disorders									
– Diarrhea	–	1	–	–	1	–	1	–	–
Cardiovascular disorders									
– Pulmonary embolism	–	–	–	–	–	–	–	–	–
– Cardiac failure	–	1	–	–	–	–	–	–	–
– Myocardial infarction	–	–	–	–	–	–	–	–	–
– Acute cardiac arrhythmia	–	–	–	–	–	–	–	–	–
Urinary disorders									
– Urinary retention	1	2	1	–	–	–	–	–	–
Neurological disorders									
– Cerebrovascular accident	–	–	–	–	–	–	–	–	–
– Confusional state	1	–	–	–	1	–	–	1	–
– Headache	–	1	1	–	1	–	1	–	–

–: None; AE: Adverse event; M: Microfracture and saline; MS: Microfracture and sodium hyaluronic acid; MSR: Microfracture, sodium hyaluronic acid and Re-Join®.

total scores among M, MS and MSR groups were significant ($p = 0.016$, M vs MS, $p = 0.116$; MS vs MSR, $p = 1.000$; M vs MSR; $p = 0.017$) and the differences became more pronounced after 12 months of monitoring ($p < 0.001$; M vs MS, $p = 0.366$; MS vs MSR, $p < 0.001$; M vs MSR; $p < 0.001$). Significant differences in the WOMAC function subscales among the three groups were also observed at 9 months ($p = 0.034$), 12 months ($p < 0.001$) and 24 months ($p < 0.001$) after surgery. More importantly, the improvement in WOMAC scores in the MS group and MSR group maintained, while in the M group, the clinical effect nearly disappeared. The improvement in WOMAC stiffness was bigger in the MS and MSR group, but the difference among three groups were nonsignificant except at months 24 ($p = 0.044$). *Post hoc* comparisons showed that the difference was between M and MSR group (M vs MSR, -0.429 ± 1.13 vs -1.89 ± 1.05 ; $p = 0.048$).

Changes in SF-36 Scores

Figure 2C and Table 4 show the SF-36 scores for patients with medial femoro-tibial condylar cartilage defects. The differences in baseline physical scales ($p = 0.500$) and mental scales were nonsignificant ($p = 0.466$). After treatment, the physical scales in all groups increased compared with the pretreatment scores, and the improvement among the groups was significant ($p < 0.05$) at all the follow-ups except at month 6 ($p = 0.165$). *Post hoc* analysis showed that the improvement was significant better in MSR group, especially at month 12 and 24. Mental scales showed a similar tendency of improvement for all the treatment groups during the 2-year follow-up, but in the M group, mental scores were worse than baseline at month 12 and 24. The differences in SF-36 mental improvement were significantly at months 12 and 24 ($p < 0.001$) and were nonsignificant at months 6, 9 and 12 ($p > 0.05$). *Post hoc* analysis showed that the improvement was significantly better in MSR group at month 12 (M vs MS, -1.22 ± 0.833 vs 1.25 ± 1.49 , $p = 0.001$; M vs MSR, -1.22 ± 0.833 vs 1.44 ± 1.33 ; $p < 0.001$), and at month

Table 4. Clinical outcomes for patients with medial femoro-tibial condylar cartilage defect.

	M group	MS group	MSR group	p-value
WOMAC score				
Total index				
Δ, 3 months	-11.6 ± 3.69 (n = 10)	-12.1 ± 1.79 (n = 10)	-12.8 ± 2.44 (n = 10)	0.625
Δ, 6 months	-9.00 ± 4.94 (n = 10)	-13.4 ± 4.43 (n = 10)	-15.1 ± 4.18 [†] (n = 10)	0.016
Δ, 9 months	-9.10 ± 4.20 (n = 10)	-13.2 ± 1.81 (n = 10)	-17.4 ± 9.03 [†] (n = 10)	0.014
Δ, 12 months	-8.33 ± 4.06 (n = 9)	-11.4 ± 2.20 (n = 8)	-21.0 ± 4.80 ^{†¶} (n = 9)	<0.001
Δ, 24 months	-2.86 ± 5.40 (n = 7)	-9.86 ± 2.34 [†] (n = 7)	-16.8 ± 4.97 ^{†§} (n = 9)	<0.001
Function subscale				
Δ, 3 months	-7.70 ± 3.16 (n = 10)	-8.70 ± 1.42 (n = 10)	-8.90 ± 1.59 (n = 10)	0.438
Δ, 6 months	-6.50 ± 4.33 (n = 10)	-9.60 ± 3.50 (n = 10)	-10.4 ± 2.99 (n = 10)	0.057
Δ, 9 months	-6.30 ± 3.59 (n = 10)	-9.90 ± 2.08 (n = 10)	-12.9 ± 8.25 [†] (n = 10)	0.034
Δ, 12 months	-5.67 ± 2.87 (n = 9)	-8.25 ± 1.91 (n = 8)	-15.4 ± 3.36 ^{†¶} (n = 9)	<0.001
Δ, 24 months	-1.86 ± 3.89 (n = 7)	-7.14 ± 3.18 (n = 7)	-12.8 ± 4.74 ^{†§} (n = 9)	<0.001
Pain subscale				
Δ, 3 months	-2.20 ± 0.632 (n = 10)	-1.80 ± 1.03 (n = 10)	-1.90 ± 1.10 (n = 10)	0.621
Δ, 6 months	-2.10 ± 0.738 (n = 10)	-2.60 ± 0.699 (n = 10)	-3.00 ± 1.70 (n = 10)	0.229
Δ, 9 months	-2.00 ± 1.15 (n = 10)	-1.80 ± 1.23 (n = 10)	-2.90 ± 1.59 (n = 10)	0.167
Δ, 12 months	-1.56 ± 1.51 (n = 9)	-1.88 ± 1.46 (n = 8)	-3.78 ± 1.56 ^{†§} (n = 9)	0.010
Δ, 24 months	-0.571 ± 1.13 (n = 7)	-1.14 ± 1.21 (n = 7)	-2.11 ± 1.54 (n = 9)	0.088
Stiffness subscale				
Δ, 3 months	-1.70 ± 1.49 (n = 10)	-1.60 ± 1.51 (n = 10)	-2.00 ± 0.943 (n = 10)	0.787
Δ, 6 months	-0.400 ± 1.35 (n = 10)	-1.20 ± 1.23 (n = 10)	-0.700 ± 0.949 (n = 10)	0.064
Δ, 9 months	-0.800 ± 1.03 (n = 10)	-1.50 ± 1.08 (n = 10)	-1.60 ± 1.07 (n = 10)	0.205
Δ, 12 months	-1.11 ± 1.05 (n = 9)	-1.25 ± 0.707 (n = 8)	-1.78 ± 1.09 (n = 9)	0.332
Δ, 24 months	-0.429 ± 1.13 (n = 7)	-1.57 ± 1.13 (n = 7)	-1.89 ± 1.05 [†] (n = 9)	0.044
SF-36 score				
Physical component				
Δ, 3 months	3.61 ± 0.381 (n = 10)	5.65 ± 2.50 [†] (n = 10)	6.44 ± 1.21 [†] (n = 10)	0.002
Δ, 6 months	6.12 ± 1.97 (n = 10)	6.62 ± 1.19 (n = 10)	7.83 ± 2.60 (n = 10)	0.165
Δ, 9 months	6.20 ± 1.59 (n = 10)	7.59 ± 2.10 (n = 10)	9.10 ± 2.09 [†] (n = 10)	0.009
Δ, 12 months	2.23 ± 2.66 (n = 9)	6.30 ± 3.42 (n = 8)	9.62 ± 5.15 [†] (n = 9)	0.002
Δ, 24 months	2.94 ± 0.824 (n = 7)	6.19 ± 1.26 [†] (n = 7)	9.57 ± 2.86 ^{†§} (n = 9)	<0.001
Mental component				
Δ, 3 months	1.70 ± 0.483 (n = 10)	1.70 ± 0.675 (n = 10)	1.30 ± 1.25 (n = 10)	0.501
Δ, 6 months	1.30 ± 4.32 (n = 10)	0.500 ± 2.46 (n = 10)	1.80 ± 3.49 (n = 10)	0.708
Δ, 9 months	0.900 ± 0.568 (n = 10)	1.30 ± 1.77 (n = 10)	0.800 ± 3.46 (n = 10)	0.873
Δ, 12 months	-1.22 ± 0.833 (n = 9)	1.25 ± 1.49 [†] (n = 8)	1.44 ± 1.33 [†] (n = 9)	<0.001
Δ, 24 months	-4.00 ± 1.83 (n = 7)	-0.286 ± 0.951 [†] (n = 7)	4.33 ± 2.12 ^{†¶} (n = 9)	<0.001

Data are expressed as mean ± standard deviation values. Differences in parameters between groups were analyzed by the one-way analysis of variance (one-way ANOVA) test and a Dunn-Bonferroni test for *post hoc* comparisons.

[†]p < 0.05.

[‡]p < 0.001 vs M group.

[§]p < 0.05.

[¶]p < 0.001 vs MS group.

Δ: Change from baseline; M: Microfracture and saline; MS: Microfracture and sodium hyaluronic acid injections; MSR: Microfracture, sodium hyaluronic acid, and Re-Join® injections; WOMAC: Western Ontario and McMaster University Osteoarthritis Index.

24 (M vs MS, -4.00 ± 1.83 vs -0.286 ± 0.951, p < 0.001; M vs MSR, -4.00 ± 1.83 vs 4.33 ± 2.12, p < 0.001; MS vs MSR, -0.286 ± 0.951 vs 4.33 ± 2.12; p < 0.001).

Table 5. Arthroscopic and radiological outcomes for patients with femoro-tibial condylar cartilage defect.

Arthroscopic and radiological parameters	M group	MS group	MSR group	p-value
Arthroscopic defect size (mm²)				
– Baseline	n = 10	n = 10	n = 10	
– Femoral condyle	652.4 ± 121.3	585.3 ± 102.1	564.6 ± 133.8	0.325
– Tibial condyle	372.3 ± 124.3	333.9 ± 136.5	333.7 ± 139.0	0.882
– Month 6	n = 9	n = 9	n = 10	
– Femoral condyle	671.7 ± 92.7	513.9 ± 127.0	406.8 ± 134.8	
– Tibial condyle	362.4 ± 123.1	270.0 ± 148.8	239.8 ± 136.9	
Δ, Month 6				
– Femoral condyle	19.2 ± 45.8	-71.4 ± 45.7 [†]	-157.8 ± 43.5 ^{‡§}	<0.001
– Tibial condyle	-9.9 ± 42.4	-63.9 ± 44.6 [†]	-93.9 ± 36.9 [‡]	0.001
MRI defect size (mm²)				
– Baseline	n = 10	n = 10	n = 10	
– Femoral condyle	424.6 ± 71.3	382.5 ± 63.6	391.6 ± 63.5	0.341
– Tibial condyle	232.2 ± 78.0	210.2 ± 85.4	225.3 ± 72.8	0.817
– Month 6	n = 10	n = 10	n = 10	
– Femoral condyle	405.3 ± 68.5	337.5 ± 67.3	285.9 ± 70.5	
– Tibial condyle	227.8 ± 72.5	180.7 ± 82.1	167.2 ± 81.9	
Δ, Month 6				
– Femoral condyle	-19.3 ± 29.9	-45.0 ± 19.4	-105.7 ± 38.3 ^{‡¶}	<0.001
– Tibial condyle	-4.4 ± 13.2	-29.5 ± 19.2 [†]	-58.1 ± 20.7 ^{‡§}	<0.001
Cartilage volume (mm³)				
– Baseline	n = 10	n = 10	n = 10	
– Femoral condyle	3550.7 ± 317.0	3686.1 ± 311.3	3486.9 ± 364.0	0.403
– Tibial condyle	1382.3 ± 147.1	1483.0 ± 181.3	1375.9 ± 121.5	0.228
– Month 6	n = 10	n = 10	n = 10	
– Femoral condyle	3593.9 ± 393.2	3856.4 ± 304.0	3841.7 ± 424.6	
– Tibial condyle	1468.5 ± 168.0	1550.7 ± 223.5	1560.9 ± 151.9	
Δ, Month 6				
– Femoral condyle	48.2 ± 141.6	170.3 ± 119.4	354.8 ± 110.3 ^{‡§}	<0.001
– Tibial condyle	86.2 ± 88.4	67.7 ± 71.2	185.0 ± 57.6 ^{†§}	0.003

Data are expressed as mean ± standard deviation values. Differences in parameters between groups were analyzed by the one-way analysis of variance (one-way ANOVA) test and a Dunn-Bonferroni test for *post hoc* comparisons. Mann-Whitney U test for nonparametric variables within the group.

[†]p < 0.05.

[‡]p < 0.001 vs M group.

[§]p < 0.05.

[¶]p < 0.001 vs MS group.

Δ: Change from baseline; M: Microfracture and saline; MRI: Magnetic resonance imaging; MS: Microfracture and sodium hyaluronic acid; MSR: Microfracture, sodium hyaluronic acid and Re-Join[®] injections; WOMAC: Western Ontario and McMaster University Osteoarthritis Index.

Arthroscopic evaluation of the cartilage defect

All the patients who participated in the clinical trial agreed to undergo a second-look arthroscopy at enrollment. However, at month 6 only 28 out of 30 patients underwent second-look arthroscopy. Representative arthroscopic findings at baseline and 6 months after treatment are shown in Figure 3, and the detailed information is shown in Table 5.

In medial femoral condyles, large areas of cartilage defects could be seen in all treatment groups at baseline. At second-look arthroscopy, only small cartilage islands formed in the M group. In the MS group, the defect area was covered with white cartilage, but the defect was not fully filled and the edge between normal cartilage and regenerated cartilage could be clearly seen. In the MSR group, the defect at baseline was fully filled with smooth, white hyaline-like cartilage, which integrated well with normal cartilage. The regenerated cartilage felt firm with a probe and no loose body, hypertrophy, or abnormal calcification was identified. The size of the cartilage defect is shown in the upper right graph in Figure 4B. The reduction in the cartilage defect in the M group was

Figure 3. Arthroscopic evaluation of cartilage regeneration. Medial femoro-tibial condyle defect. (A) At baseline, large cartilage defects in medial femoral condyles can be seen in all treatment groups. In the M group, while small cartilage islands formed, most cartilage defects are not covered 6 months after treatment. In the MS group, at 6 months, newly formed white cartilage is visible, while the defect is not fully filled and the edge can be seen clearly. In the MSR group, most of the defect area is covered with thick, smooth, hyaline-like cartilage 6 months after treatment. **(B)** In the MSR group, the ICRS grade of the articular cartilage significantly improved (femoral, $p = 0.016$; Tibial, $p = 0.011$), while no significant change was found in the M (femoral, $p = 1.000$; tibial, $p = 1.000$) and MS (femoral, $p = 0.211$; tibial, $p = 0.087$) groups.

* $p < 0.05$ and ** $p < 0.001$ vs M group, # $p < 0.05$ and ## $p < 0.001$ vs MS group. Differences in parameters between groups were analyzed by the one-way analysis of variance (one-way ANOVA) test and a Dunn-Bonferroni test for *post hoc* comparisons. The change in ICRS grade in each group were analyzed by the Mann-Whitney U test. Δ : Change from baseline; ICRS: International Cartilage Repair Society; M: Microfracture and saline; MS: Microfracture and sodium hyaluronate; MSR: Microfracture, sodium hyaluronate and Re-Join® injections.

Table 6. International Cartilage Repair Society histologic score (ICRS II) of biopsy specimens from the medial femoral condyle of the knee.

Histological parameter	M (n = 9)			MS (n = 9)			MSR (n = 10)		
	Baseline	Month 6	p-value	Baseline	Month 6	p-value	Baseline	Month 6	p-value
Tissue morphology	33.2 ± 19.2	37.6 ± 16.9	0.618	33.9 ± 17.6	43.3 ± 20.8	0.313	35.9 ± 18.3	48.7 ± 23.2	0.129
Matrix staining	27.0 ± 21.2	31.1 ± 23.2	0.700	32.6 ± 18.5	40.8 ± 18.1	0.355	28.3 ± 14.0	38.7 ± 14.5	0.083
Cell morphology	50.0 ± 23.5	50.3 ± 22.0	0.976	47.0 ± 27.1	46.2 ± 26.5	0.952	55.8 ± 21.7	57.6 ± 21.4	0.861
Chondrocyte clustering	46.3 ± 22.9	48.2 ± 25.4	0.870	49.4 ± 21.2	57.7 ± 20.8	0.419	50.7 ± 22.1	66.9 ± 16.2	0.070
Surface architecture	28.2 ± 17.1	28.2 ± 16.0	1.000	30.3 ± 11.0	51.9 ± 12.8	0.001	25.3 ± 12.0	68.4 ± 16.4	<0.001
Basal integration	55.8 ± 20.6	54.0 ± 23.4	0.867	52.8 ± 30.8	63.2 ± 26.3	0.450	56.4 ± 14.0	60.4 ± 13.9	0.563
Formation of the tidemark	30.6 ± 21.6	33.1 ± 24.9	0.819	33.7 ± 18.8	31.8 ± 17.7	0.829	37.8 ± 10.9	41.8 ± 15.0	0.919
Subchondral bone/marrow fibrosis	40.6 ± 21.2	40.7 ± 22.3	0.991	41.1 ± 24.7	43.9 ± 25.6	0.818	42.3 ± 15.3	35.3 ± 18.7	0.393
Inflammation	–	–	–	–	–	–	–	–	–
Abnormal calcification/ossification	56.2 ± 25.8	55.1 ± 27.7	0.931	58.1 ± 14.7	70.8 ± 14.1	0.081	60.1 ± 24.2	68.2 ± 23.7	0.392
Vascularization	80.8 ± 9.5	78.1 ± 11.1	0.593	75.2 ± 17.1	72.9 ± 15.6	0.767	82.3 ± 11.9	89.8 ± 6.0	0.108
Surface/superficial assessment	24.3 ± 14.7	26.3 ± 17.7	0.797	29.9 ± 18.6	45.3 ± 20.4	0.113	35.8 ± 23.9	68.8 ± 20.7	0.007
Mid/deep zone assessment	29.1 ± 16.2	31.2 ± 17.4	0.794	25.0 ± 13.5	38.0 ± 13.5	0.058	21.7 ± 8.0	32.4 ± 11.5	0.054
Overall assessment	28.1 ± 19.1	27.4 ± 19.7	0.943	27.7 ± 16.4	43.2 ± 14.5	0.049	32.0 ± 21.6	55.9 ± 23.2	0.030

Data are presented as mean ± standard deviation values. ICRS II is a histological scoring scheme designed to evaluate the quality of cartilage repair tissue in patients. Differences in parameters between groups were analyzed by the paired-samples t-test.

M: Microfracture and saline; MS: Microfracture and sodium hyaluronic acid; MSR: Microfracture, sodium hyaluronic acid and Re-Join® injections.

Figure 4. Magnetic resonance imaging evaluation of cartilage defect after treatment. Medial femoro-tibial condyle defect. (A) Sagittal and coronal MRIs of the medial femoral and tibial condyles before and 6 months after treatment. Cartilage defects in the medial femoral condyle and in the medial tibial condyle were identified as signal voids between the two condyles. In the M group, no substantial change was identified after treatment at months 6. In the MS group, thin regenerated cartilage with small signal voids can be observed. In the MSR group, smoother and thicker regenerated cartilage was observed in the former defect areas. **(B)** Line chart showing cartilage defect size at baseline and month 6.

* $p < 0.05$ and ** $p < 0.001$ vs M group, # $p < 0.05$ and ## $p < 0.001$ vs MS group. Differences in parameters between groups were analyzed by the one-way analysis of variance (oneway ANOVA) test and a Dunn-Bonferroni test for *post hoc* comparisons.

M: Microfracture and saline; MRI: Magnetic resonance imaging; MS: Microfracture and sodium hyaluronic acid; MSR: Microfracture, sodium hyaluronic acid and Re-Join® injections.

Table 7. Cartilage thickness before and after treatment for patients with femoro-tibial condylar cartilage defect.

	M group	MS group	MSR group	p-value
Cartilage thickness (mm)				
– Baseline	0.52 ± 0.28	0.45 ± 0.22	0.57 ± 0.30	0.645
– Month 6	0.59 ± 0.34	0.85 ± 0.37	1.50 ± 0.47	
– Δ, Month 6	0.07 ± 0.37	0.39 ± 0.28	0.93 ± 0.55	0.001

Δ: Change from baseline; M: Microfracture and saline; MS: Microfracture and sodium hyaluronic acid; MSR: Microfracture, sodium hyaluronic acid and Re-Join® injections.

–19.9 ± 45.8 mm² in the medial femoral condyle and 9.9 ± 42.4 mm² in the medial tibial condyle; in the MS group, 71.4 ± 45.7 mm² in the medial femoral condyle and 63.9 ± 44.6 mm² in the medial tibial condyle; and in the MSR group, 157.8 ± 43.5 mm² in the medial femoral condyle and 93.9 ± 36.9 mm² in the medial tibial condyle. The difference between groups was significant ($p < 0.05$). *Post hoc* analysis showed that the decrease in cartilage defect in MS and MSR groups was significantly larger than M group (femoral, M vs MSR, 19.2 ± 45.8 vs –157.8 ± 43.5, $p < 0.001$; M vs MS, 19.2 ± 45.8 vs –71.4 ± 45.7, $p = 0.001$; MS vs MSR, –71.4 ± 45.7 vs –157.8 ± 43.5, $p = 0.001$; tibial, M vs MSR, –9.9 ± 42.4 vs –93.9 ± 36.9, $p < 0.001$; M vs MS, –9.9 ± 42.4 vs –63.9 ± 44.6, $p = 0.031$; MS vs MSR, –63.9 ± 44.6 vs –93.9 ± 36.9, $p = 0.378$). The detailed information is shown in Table 7. The ICRS grade of the cartilage defect significantly improved in the MSR group (femoral, $p = 0.016$; tibial, $p = 0.011$), while no significant change was observed in the M and MS ($p > 0.05$) groups (Figure 3B).

MRI evaluation of the cartilage defect

Representative MRI images of the cartilage defect before and 6 months after treatment are shown in Figure 4, and the change of the cartilage defect measured with MRI was shown in Table 5.

The reduction in cartilage defect in the M group was 19.3 ± 29.9 mm² in the medial femoral condyle and 4.4 ± 13.2 mm² in the medial tibial condyle; in the MS group, 45.0 ± 19.4 mm² in the medial femoral condyle and 29.5 ± 19.2 mm² in the medial tibial condyle; and in the MSR group, 105.7 ± 38.3 mm² in the medial femoral condyle and 58.1 ± 20.7 mm² in the medial tibial condyle. The difference between groups was significant ($p < 0.001$) and *post hoc* analysis show that compared with M and MS group, MSR group had a significantly larger reduction of cartilage defect ($p < 0.05$ for all analysis). For patients with trochlear-patellar cartilage defects, the reduction of the cartilage defect in the M group was 10.9 ± 26.0 mm² in the trochlea and –4.5 ± 19.8 mm² in the patella; in the MS group, 46.3 ± 21.9 mm² in the trochlea and 55.0 ± 20.7 mm² in the patella; and in the MSR group, 85.8 ± 23.4 mm² in the trochlea and 117.9 ± 24.8 mm² in the patella. The difference between groups was significant ($p < 0.001$). *Post hoc* analysis show that the reduction in cartilage defect of MSR group was significantly larger than the other two groups ($p < 0.05$ for all analysis).

Changes in the cartilage volume

Representative 3D images of the cartilage at baseline and month 6 are shown in Figure 5. Detailed information is shown in Table 5.

For patients with cartilage defects in medial femoral and tibial condyles, the increase in the cartilage volume in the M group was 48.2 ± 141.6 mm³ in the medial femoral condyle and 86.2 ± 88.4 mm³ in the medial tibial condyle; in the MS group, 170.3 ± 119.4 mm³ in the medial femoral condyle and 67.7 ± 71.2 mm³ in the medial tibial condyle; and in the MSR group, 304.8 ± 64.0 mm³ in the medial femoral condyle and 185.0 ± 57.6 mm³ in the medial tibial condyle. The difference between groups was significant ($p < 0.001$ for the femoral condyle and $p = 0.003$ for the tibial condyle). *Post hoc* analysis showed that the increase in cartilage volume of MSR group was significantly larger than the other two groups (femoral, M vs MS, $p = 0.069$; M vs MSR, $p < 0.001$; MS vs MSR, $p = 0.039$; tibial, M vs MS, $p = 1.000$; M vs MSR, $p = 0.017$; MS vs MSR, $p = 0.004$).

Histological outcomes

Overall, the cartilage repair effect of the MSR group outperformed the other two groups. Representative histological images of the cartilage at baseline and month 6 are shown in Figure 6. Generally, biopsy specimens from the medial femoral condyles had only thin and rough articular cartilage before treatment (Figure 6). In the M group, a combination of thin fibrocartilage and fibrotic tissue with a severely degraded surface is formed and integrated with the subchondral bone. Safranin O is stained mostly in the middle zones. Masson's trichrome and

Figure 5. Changes in articular cartilage volume after treatment. Changes of the cartilage volume after treatment. **(A)** Changes in articular cartilage volume 6 months after treatment in the medial femoral condyle (blue) and in the medial tibial condyle (red) in the treatment groups. For each column, left is viewed from below, right is viewed from above, and the covered areas are shown in the middle. In the M group, the void seen at baseline exhibited little change at 6 months in the medial femoral and tibial condyles; while in the MS group, the void became smaller; and in the MSR group, the void was filled. **(B)** Line chart showing cartilage volume at baseline and month 6.

* $p < 0.05$ and ** $p < 0.001$ vs M group, # $p < 0.05$ and ## $p < 0.001$ vs MS group. Differences in parameters between groups were analyzed by the one-way analysis of variance (one-way ANOVA) test and a Dunn-Bonferroni test for *post hoc* comparisons.

M: Microfracture and saline; MS: Microfracture and sodium hyaluronic acid; MSR: Microfracture, sodium hyaluronic acid and Re-Join® injections.

immunohistochemistry for type I and II collagen stain show that the regenerated tissue is fibrocartilage, composed of type II collagen and minimally type I collagen. Zonal structure or tide mark is not definite. In the MS group, 6 months after treatment, the cartilage defects were mostly completely filled with a fibrocartilaginous tissue. The surface is relatively smooth with little signs of degradation. Safranin O is stained mostly in the lower half of the middle and the deep zones. Regenerated cartilage shows more positive Safranin O and collagen II staining. In the MSR group, a thick, hyaline-like cartilage with a smooth surface is regenerated and integrated with the subchondral bone 6 months after treatment. Safranin O and type II collagen is stained mostly in the lower half of the middle and the deep zones. Masson's trichrome staining and immunohistochemistry staining for type I collagen are less positive. Type II collagen staining is more positive. Zonal structure and tide mark are formed. Chondrocytes are flattened in the superficial zone and round in the middle and deep zones.

In the M group, the ICRS II scores did not change significantly after treatment. In the MS group, surface architecture and overall assessments improved significantly. While in the MSR group, after treatment the ICRS II scores improved significantly in four parameters: surface architecture, and surface, mid, and overall assessments (Table 6).

Figure 6. Histological evaluation of articular cartilage of biopsy from the medial femoral condyle after treatment. Medial femoro-tibial condyle defect. **(A)** A typical biopsy sample from the medial femoral condyle of a patient in the M group at the baseline and 6 months after microfracture stained with H&E, safranin O, Masson's trichrome and anti-type I and II collagen antibodies. Thin and rough articular cartilage is seen at the baseline. At month 6, a combination of thin fibrocartilage and fibrotic tissue is formed and integrated with the subchondral bone. The surface of the repair tissue is severely degraded. Safranin O is stained mostly in the middle zones. Masson's trichrome and immunohistochemistry for type I and II collagen stain show that the regenerated cartilage is fibrocartilaginous tissue, composed of type II collagen and minimally type I collagen. Zonal structure or tide mark is not definite. **(B)** A typical biopsy sample of a patient in the MS group. At baseline the articular cartilage is similar to **(A)**. 6 months after treatment, the cartilage defects were mostly completely filled with a fibrocartilaginous tissue. The surface is relatively smooth with little signs of degradation. Safranin O is stained mostly in the lower half of the middle and the deep zones. Regenerated cartilage shows more positive Safranin O and collagen II staining. **(C)** A typical biopsy sample of a patient in the MSR group. At month 6, a thick, hyaline-like cartilage with a smooth surface is regenerated and integrated with the subchondral bone. Safranin O and type II collagen is stained mostly in the lower half of the middle and the deep zones. Masson's trichrome staining and immunohistochemistry staining for type I collagen are less positive. Type II collagen staining is more positive. Zonal structure and tide mark are formed. Chondrocytes are flattened in the superficial zone and round in the middle and deep zones.

H&E: Hematoxylin–eosin; M: Microfracture and saline; MS: Microfracture and sodium hyaluronic acid; MSR: Microfracture, sodium hyaluronic acid and Re-Join® injections; SOFG: Safranin O/fast green.

In the M group, the mean thickness of articular cartilage increased from 0.52 ± 0.28 mm to 0.59 ± 0.34 after treatment; in the MS group, the mean thickness of cartilage was 0.45 ± 0.22 at baseline and 0.85 ± 0.37 after treatment; and in the MSR group, 0.57 ± 0.30 mm³ before treatment and 1.50 ± 0.47 mm³ at month 6. The increase in cartilage thickness was statistically different between groups ($p < 0.001$). *Post hoc* analysis showed that the increase in cartilage thickness of MSR group was significantly larger than the other two groups (M vs MS, $p = 0.362$; M vs MSR, $p < 0.001$; MS vs MSR, $p = 0.030$; Table 7).

Discussion

We conducted a Phase IIa proof-of-concept randomized, single-blind clinical trial to evaluate the safety and efficacy of Re-Join in combination with microfracture and HA in patients with knee cartilage defects. The findings support the hypothesis that microfracture in combination with HA and haMPCs will provide clinical improvement for patients with knee cartilage defects.

Baseline characteristics were well-matched, and study retention was adequate. Patients in MS and MSR group showed a significant response to treatment, for clinically evaluation with WOMAC scores and SF-36 scores, and for quantitative assessment of defect size and cartilage volume with MRI and arthroscopy. During the first 9 months post-treatment, the differences between groups were mostly nonsignificant. After 12 months, clinical improvement in the MSR group was significantly better than M and MS group in most scales. In the M group, clinical improvement was also observed, but only within 9 months follow-up, after which the benefits of microfracture were gradually lost, supporting the findings in published studies [49,50] that arthroscopic microfracture reduce pain and improve knee function after the procedure, but these benefits only last for about 1 year. In the MS and MSR groups, clinical improvement also seemed to diminish after 1 year. But these outcome measures were still slightly improved at 24 months follow-up compared with baseline.

Published studies have reported significant relief of symptoms and improved joint function for knee OA after microfracture [6–10]. The mechanism is that penetration of the subchondral bone plate in defect areas lead to clot formation in the defect. This clot contains pluripotent, marrow-derived mesenchymal stem cells, which produce a fibrocartilage repair with varying amounts of type-II collagen content [51–58]. However, study groups in these studies were younger and with lower grade OA. In the present study, the patient population were 50–75 with moderate to severe (KL Grade 3) OA. The Ethics Committee required that patients in all treatment groups microfractured, therefore there were no cell-only group.

The WOMAC score decreased in all treatment groups shortly after treatment. We believe that in our scenario, older patients with cartilage defects in KL grade 3 knees can also benefit from microfracture, though the efficacy was not as much as in younger patients with lower grade OA. The differences in the improvement in WOMAC scores between groups were mostly nonsignificant during the first 3 months post-treatment. After 6 months, the difference in clinical improvement between treatment groups was significantly. Presumably HA and Re-Join did not add significantly to pain relief shortly following the procedure, and the difference in later follow-ups results in different durability of regenerative tissues of these treatments. These findings are consistent with a systematic review and meta-analysis which indicated that the efficacy of microfracture is only short term [50].

Compared with historical studies, the efficacy of Re-Join combined with HA and microfracture surgery in knee cartilage defects seems encouraging. For example, in a prospective open-label dose-escalating trial on injection of autologous ADSCs in a limited number of patients with knee OA, the WOMAC score in the high-dose group decreased by 21.7 ± 15.4 (0–96 scale) at month 6 [59]. In the present study, between baseline and 6 months, the WOMAC score decreased by 15.1 ± 4.18 for patients with medial femoro-tibial condyle cartilage defects, and 16.2 ± 4.29 for patients with trochlear-patellar defects. In a similar dose-escalating trial using ADSCs in patients with knee OA conducted by Jo *et al.*, in the high-dose group in which the highest efficacy was found, the mean reduction in WOMAC score from baseline to 6 months was 54.2 ± 5.2 to 32.8 ± 6.3 [45]. Clinical results in the MSR group in our study are comparable to their results, and improvements were observed at later follow-ups.

The efficacy of microfracture and HA injection in knee OA in our study were also comparable with previous studies. In a randomized, double-blind, controlled trial using intra-articular HA for treating symptomatic knee OA, at 6 months, the WOMAC score decreased by 15.5 ± 2.6 [60]. In another randomized, double-blind, multicenter study comparing Artz to Durolane (nonanimal source HA) in treating knee OA, the WOMAC score decreased by 19.7 in the Artz group and 20.59 in the Durolane group at 18 weeks. At 26 weeks, the WOMAC score decreased by 20.43 in the Artz group and 21.26 in the Durolane group [61]. In our study, the WOMAC score in the MSR group decreased by 15.1 ± 4.18 at 24 weeks and further decreased by 17.4 ± 9.08 at 36 weeks after treatment.

Treatments in this trial were only associated with several minor AEs, which were resolved spontaneously or with nonsteroidal anti-inflammatory drugs, and several AEs were not related to the procedures. These findings demonstrated that microfracture in combination with intra-articular injections of haMPCs and HA is a safe and effective procedure for improving clinical function in patients with knee cartilage defects.

The histological outcomes of the present study show that the treatment in patients with knee cartilage defects results in a fibrocartilage (M and MS groups) or a combination of fibrocartilage and hyaline-like cartilage (MSR group). The thickness of cartilage increased after treatment and cartilage-specific stains of the repair tissue were more intense than baseline, especially in MS and MSR groups. However, the regenerated tissues in the trial were not hyaline cartilage, but fibrocartilage or a combination of fibrocartilage and hyaline-like cartilage. The repair tissue had a better appearance than the cartilage at baseline, but did not reach the quality of normal hyaline. Also, for ethical reasons, we could only take biopsies with diameters of about 2 mm, which do not allow judging the integration of the repair tissue with the adjacent articular cartilage, an important parameter for the long-term performance of the repair tissue. Given the above, the efficacy of MSR in cartilage regeneration cannot be proved, especially when we had histology data from a limited number of patients.

We recognize that there are limitations of the study. First, there was relatively small number of cases in this study. The limited number of patients included may explain why statistical significance was not reached for several scales. However, this study is designed to provide an impetus for further evaluation, it serves as a preliminary research to evaluate the potential role of intra-articular injection of haMPCs in knee cartilage defect, rather than provide definitive conclusions. Second, haMPCs were administered in combination with HA and microfracture. Due to ethical reasons, there were no cell-only group, therefore it was not able to fully evaluate the efficacy of haMPCs, and no specific conclusions can be drawn with regard to the effects of haMPCs. Third, although the WOMAC questionnaire is a validated instrument specifically designed to evaluate the condition of patients with OA of the knee and hip, it might not be specific enough for patients after arthroscopic microfracture and intra-articular injection of sodium HA and haMPCs. Researchers may need a specifically designed questionnaire for these patients.

Conclusion

In conclusion, this study confirms that intra-articular injections of haMPCs in conjunction with HA and microfracture improved clinical function in knee cartilage defect patients without inducing adverse events. Evaluation with WOMAC and SF-36 measures demonstrated the efficacy of the treatment. Radiological, arthroscopic and histological evaluation consistently confirmed that the treatment promoted cartilage defect decrease and cartilage regeneration, though cartilage regeneration efficacy of the treatment cannot be proved so far due to lack of evidence. These data demonstrated that intra-articular injections of haMPCs in conjunction with HA and microfracture are safe and effective for improving clinical functions for KL grade 3 patients with knee cartilage defects. The results are promising to encourage larger scales of randomized clinical trials to further verify the efficacy of haMPCs in conjunction with HA and microfracture in human knee cartilage defects.

Translational perspective

This study confirms the safety and clinical functional efficacy of haMPCs. HaMPCs are easy to collect for clinical application with high isolation yields and are not affected by the patients' age, sex and physiological status. There have been many reports and attempts on the clinical application of HaMPCs in regenerative medicine perspective. In China and worldwide, there are also many important enterprises and research institutes, hospitals and governments concerned about the clinical application of HaMPCs. These will promote the clinical translation of HaMPCs related technologies reported in this paper. We suggest that in the future, larger scales of randomized clinical trials should be conducted to further verify the efficacy of haMPCs in human cartilage defects, and this technique should be extended to the repair of articular cartilage defects in shoulder, hip and ankle.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/rme-2019-0068

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Ethical conduct of research

This study was approved by the local ethics committees of both institutions (No.22, 2014; Scientific Research Projects Approval Determination of Independent Ethics Committee of Shanghai Ninth People's Hospital affiliated to Shanghai Jiao Tong University School of Medicine and Scientific Research Projects Approval Determination of Independent Ethics Committee of Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine) and registered at clinicaltrials.gov with identifier NCT02855073. All procedures were conducted in accordance with the Declaration of Helsinki. The methods were carried out in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all patients after the physicians illustrated detailed relevant information, such as the purpose and overall procedure of the study, potential risks and benefits.

Data sharing statement

The authors certify that this manuscript reports original clinical trial data. Data reported in this manuscript are available within the article or posted publicly at www.clinicaltrials.gov, according to the required timelines. Individual de-identified participant data which underlie results reported in this article will be available from 9 months and ending 24 months following article publication. The study protocol will also be available. Material will be accessible to investigators whose proposed use of data has been approved by an independent review committee identified for this purpose and for data meta-analysis. Requests are to be directed to the corresponding author of the article up to 24 months following article publication.

Summary points

- Intra-articular injections of human adipose-derived mesenchymal progenitor cell (haMPCs) correlated with a significant reduction of the Western Ontario and McMaster Universities Osteoarthritis index and improvement in the short form 36 score, when combined with microfracture surgery.
- Intra-articular injections of haMPCs in combination with hyaluronic acid and microfracture did not correlate with treatment-related serious adverse events.
- Intra-articular injections of haMPCs were associated with a significantly reduction of the articular cartilage defect and improvement in International Cartilage Repair Society grade under arthroscopy when combined with microfracture surgery.
- Intra-articular injections of haMPCs were associated with a significantly reduction of the articular cartilage defect area under magnetic resonance imaging and increased cartilage volume when combined with microfracture surgery.
- Intra-articular injections of haMPCs were safe and improved the clinical function, reduced cartilage defect and increased cartilage volume of the knee joint, encouraging larger scales of randomized clinical trials to further verify the efficacy of haMPCs, when combined with microfracture surgery.

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