



Original Article

Transplantation of autologous bone marrow-derived mesenchymal stem cells under arthroscopic surgery with microfracture versus microfracture alone for articular cartilage lesions in the knee: A multicenter prospective randomized control clinical trial

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ABSTRACT

Introduction: To investigate the efficacy of the transplantation of autologous bone marrow-derived mesenchymal stem cells (BMSCs) under arthroscopy with microfracture (MFX) compared with microfracture alone.

Methods: Eleven patients with a symptomatic articular cartilage defect of the knee were included in the study. They were randomized to receive BMSCs with MFX (cell-T group, n=7) or MFX alone (control group, n=4). Clinical results were evaluated using International Knee Documentation committee (IKDC) knee evaluation questionnaires and the Knee Injury and Osteoarthritis Outcome Score (KOOS) before and 48 weeks after surgery. Quantitative and qualitative assessments of repair tissue were carried out at 48 weeks by T2 mapping of magnetic resonance images (MRIs) and the magnetic resonance observation of cartilage repair tissue (MOCART) scoring system with follow-up MRI.

Results: No significant differences between preoperative and postoperative IKDC and KOOS were observed in the cell-T or control group. However, forty-eight weeks after surgery, the cell-T group showed a trend for a greater KOOS QOL score compared with the control group (79.4 vs. 39.1, respectively; P=0.07). The T2 value did not differ significantly between the two groups, but the mean MOCART score was significantly higher in the cell-T group than in the control group (P=0.02).

Abbreviations: BMSCs, bone marrow-derived mesenchymal stem cells; MFX, microfracture; IKDC, International Knee Documentation committee; KOOS, Knee Injury and Osteoarthritis Outcome Score; MRIs, magnetic resonance images; MOCART, magnetic resonance observation of cartilage repair tissue; QOL, quality of life; HA, hyaluronic acid; KL, Kellgren–Lawrence; RCT, randomized controlled trial; CPC, cell processing centers; GFP, green fluorescent protein.

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Conclusions: Compared with MFX alone, BMSC transplantation with MFX resulted in better postoperative healing of the cartilage and subchondral bone as determined by the MOCART score. Clinically, BMSC transplantation with MFX gave a higher KOOS QOL score after 48 weeks.

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1. Introduction

Articular cartilage lesions in the knee can cause pain and swelling [1], and increase the risk of osteoarthritis [2]. The healing potential of articular cartilage is very low with conservative treatment [3]. Currently, conventional surgical treatment for articular cartilage defects is bone marrow stimulation [4] in which the subchondral bone is perforated to facilitate cartilage repair by bone marrow-derived cells [5]. Although this is a simple procedure, repaired cartilage is fibrocartilage, which is biochemically and biomechanically different from normal hyaline cartilage [6]. Recently, osteochondral autograft transplantation and autologous chondrocyte implantation have been reported to have good results for cartilage defects [7,8]; however, these require the harvesting of normal cartilage tissues.

Bone marrow mesenchymal stem cells (BMSCs, a form of somatic stem cell, are most commonly used because they can be collected easily without causing tissue defects [9]. We previously used BMSCs as a cell source to assess new methods for enhancing cartilage regeneration. In 1994, we showed the effectiveness of BMSC transplantation for the repair of osteochondral defects using a rabbit model [10]. Autologous BMSCs are safe because their use does not cause either immunological reactions or disease transmission. We transplanted autologous BMSCs to repair articular cartilage in the first clinical trial of its kind worldwide [11]. Subsequently, we have performed this procedure in 41 patients [12–14]. To confirm the safety of this procedure, we investigated the records of all patients who together had received 45 transplantations. Neither tumors nor infections were observed between 5 and 137 months of follow-up, indicating that autologous BMSC transplantation is safe [15]. However, the method requires large skin incisions to be made, so less invasive therapy would be beneficial.

The intraarticular injection of BMSCs was reported to be effective for cartilaginous healing in animal experiments [16–18] and clinical case series [19–21]. However, no randomized control trial has been conducted for cartilage defects. We designed this study to compare the clinical and radiologic efficacy of BMSCs with microfracture (MFX versus MFX alone in patients with symptomatic knee cartilage defects under arthroscopic surgery with a smaller skin incision. The purpose of this study was to investigate the safety and clinical results of a novel, minimally invasive technique combining arthroscopic MFX with injections of bone marrow-derived BMSCs containing hyaluronic acid (HA. Our hypothesis was that the injected MSCs would improve the clinical and radiographic results at the 48-weeks follow up.

2. Materials and methods

2.1. Study population

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Japanese Ministry of Health, Labour and Welfare in accordance with the guidelines on clinical research using human stem cells and the institutional review board of Hiroshima University, Hyogo College of Medicine, Nara Medical University, Kindai University Faculty of

Medicine, and Osaka City University. It was registered with the University Hospital Medical Information Network (registration number: R000008607 and monitored by the Medical Center for Translational Research, Osaka University Hospital and Wellbe Inc. Written informed consent was obtained from each patient.

Patients with an articular cartilage lesion in the knee caused by trauma or osteochondritis dissecans of the knee were enrolled in this multicenter randomized control trial. Experienced senior knee surgeons at each participating center selected the patients according to the following inclusion criteria (Table 1: indication for bone marrow stimulation, International Cartilage Repair Society articular injury classification \geq Grade III, size of defect ≥ 2 cm², and aged 16–70 years. Exclusion criteria were previous surgical treatment for anterior and/or posterior cruciate ligament reconstruction within 2 months, cancer, pregnancy, osteoarthritis of \geq Grade 3 under the Kellgren–Lawrence (KL classification, infectious diseases, mental disorders, and contraindications to general anesthetic or inability to adhere to the trial. Eligible patients willing to have their treatment allocated by randomization entered the randomized controlled trial (RCT arm of the study. Allocations were made by the CapTool® randomization service (Mebix Cooperation, Tokyo, Japan, which was administered after patients entered the trial. Patients willing to enroll in the study but not to receive randomized intervention were withdrawn. The follow up period was 48 weeks after surgery.

2.2. Bone marrow mesenchymal stem cell preparation

BMSC preparation has been described previously [12]. Cell culture was performed at the cell processing centers (CPC of three of the hospitals, or at the CPC of Osaka University for hospitals without a CPC. CPC facilities maintained good manufacturing practice levels. The main part of the culture was performed under appropriate standard operative procedures. Heparinized bone marrow (30–40 ml, i.e. 10×3 –4 ml was aspirated from the superior posterior spine of the iliac crest and placed in 50 ml tubes. This was carried to the CPC on ice, where bone marrow blood was cultured in six T-500 plastic culture flasks with media changes three times/week. The culture medium was α Minimum Essential Medium (GIBCO, 41061–029, NY supplemented with 15% autologous serum. During the media change, non-adherent hematopoietic cells were removed, leaving only adherent cells in the dish.

Table 1
Study inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Indication for bone marrow stimulation	Operative history of ACL and/or PCL reconstruction the last 2 months
International Cartilage Repair Society (ICRS) articular cartilage injury classification	Malignancy
\geq Grade 3	
Size of defect ≥ 2 cm ²	Kellgren Lawrence OA grade ≥ 3
Aged between 16 and 70 yr	Pregnant woman
	Infectious diseases (HBV, HCV, ATLA, HIV)
	Mental disorders
	Contraindications to a general anesthesia
	Unable to adhere to trial.

After around 14 days, the number of adherent cells had reached several million, and cells were fibroblastic in appearance and positive for stromal cell markers such as CD44 and CD105 [22]. After collecting the cells by trypsinization and confirming that over 80% expressed CD44 and CD105, they were resuspended in 2.5 ml of HA (molecular weight 800,000, 1% and 2.5 ml of autologous serum. A portion of expanded BMSCs was used for contamination checks, which were performed at the beginning of culture and at the final medium change. Some of the media also underwent endotoxin analysis at the time of the first tests, while at the time of the final test we checked for mycoplasma contamination using a PCR mycoplasma detection kit (Takara Bio Inc., Otsu, Japan. All tests were performed at the CPCs. The cells were confirmed to be MSCs, and a total of $1\text{--}10 \times 10^7$ were prepared and transferred to the operating theater once they were shown to be contamination-free.

2.3. Surgical procedure

MFX was performed according to the technique published by Steadman et al. [23] as an arthroscopic procedure. This involved the accurate debridement of all unstable and damaged cartilage in the lesion, including the calcified layer down to the subchondral bone plate. All loose or marginally attached cartilage was debrided from the surrounding rim of the defect to form a stable perpendicular edge of healthy cartilage. An arthroscopic awl was then used to make multiple holes in the defect, 3–4 mm apart (Fig. 1a). Prepared MSCs were injected around the cartilage lesion under arthroscopy (Fig. 1b). HA was injected around the cartilage lesion under arthroscopy for the control group.

2.4. Postoperative rehabilitation

Participants in both groups underwent the same postoperative rehabilitation protocol. After the operation, the knee was immobilized for 1 day with a knee brace. A range of motion exercises were allowed 1 day after surgery, and partial weight bearing was initiated 3 weeks after surgery. Full weight bearing was permitted 6 weeks postoperatively.

2.5. Clinical evaluation

We recorded results from the International Knee Documentation committee (IKDC knee evaluation questionnaires [24] and the Knee Injury and Osteoarthritis Outcome Score (KOOS [25] before

and 48 weeks after surgery. Scores were normalized and presented as between 0 and 100, with 100 being the best possible score.

2.6. Radiographical evaluations

Knee X-ray and magnetic resonance imaging (MRI including T2 mapping were undertaken before and 6, 24, and 48 weeks after surgery. Knee X-rays were obtained in the anteroposterior view and evaluated as Kellgren–Lawrence grade. Quantitative and qualitative assessments of the repair tissue were carried out 6, 24, and 48 weeks after surgery on follow-up MRI using T2 mapping and the magnetic resonance observation of cartilage repair tissue (MOCART scoring system [26].

2.7. Statistical methods

Sample size calculation was performed based on an independent samples t-test. The calculations assumed a difference of $p < 0.05$ in the IKDC subjective score based on a previous study [27]. Accepting a two-sided type I error rate of 5% and a dropout rate of 10%, we would achieve 90% power to detect a difference (effect size, 0.603 with 40 patients per arm. All analyses were conducted with significance defined as $p < 0.05$. A *t* test was used to compare continuous variables, and a χ^2 test and Fisher's exact test were used for demographic data. The Wilcoxon rank sum test was used to compare clinical scores between the two groups at 48 weeks after surgery. To establish whether there was a significant difference in MOCART score between patients with and without BMSC, a restricted maximum likelihood, mixed-model regression was used. The χ^2 test was used to detect differences in the MOCART subcategory score.

3. Results

Flow diagrams of the RCT and preference group arms are shown in Fig. 2. A total of 14 patients were recruited. Three in the control group were withdrawn before intervention because they did not undergo the procedure. Therefore, seven patients received BMSC transplantation with MFX as the treatment group (cell-T group, and four were treated with MFX alone as the control group. The mean age at the time of the operation was 44.1 years. Seven patients were male and four were female. Baseline patient characteristics are shown in Table 2, and there was no significant difference in the characteristics between the two groups.

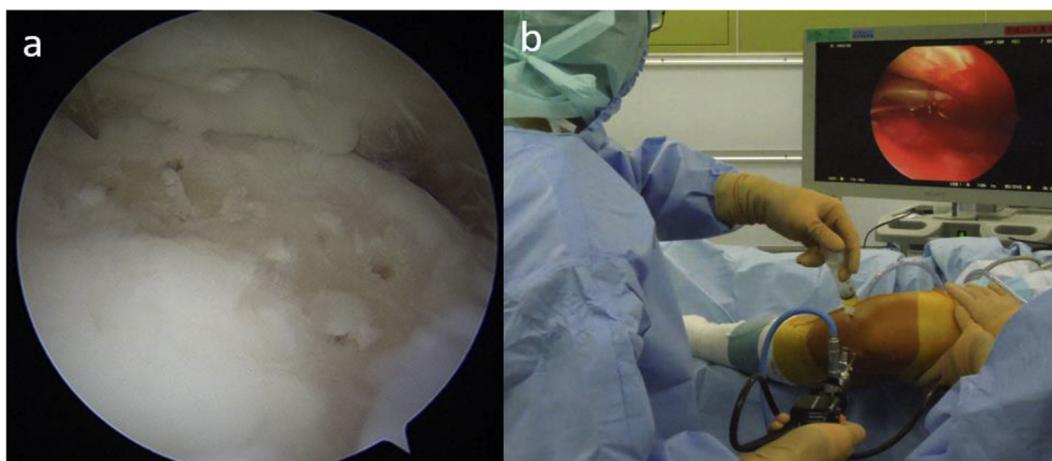


Fig. 1. Arthroscopic view and photograph of injection. a Microfracture for cartilage defect of lateral tibia plateau. b Injection of autologous BMSCs under arthroscopy.

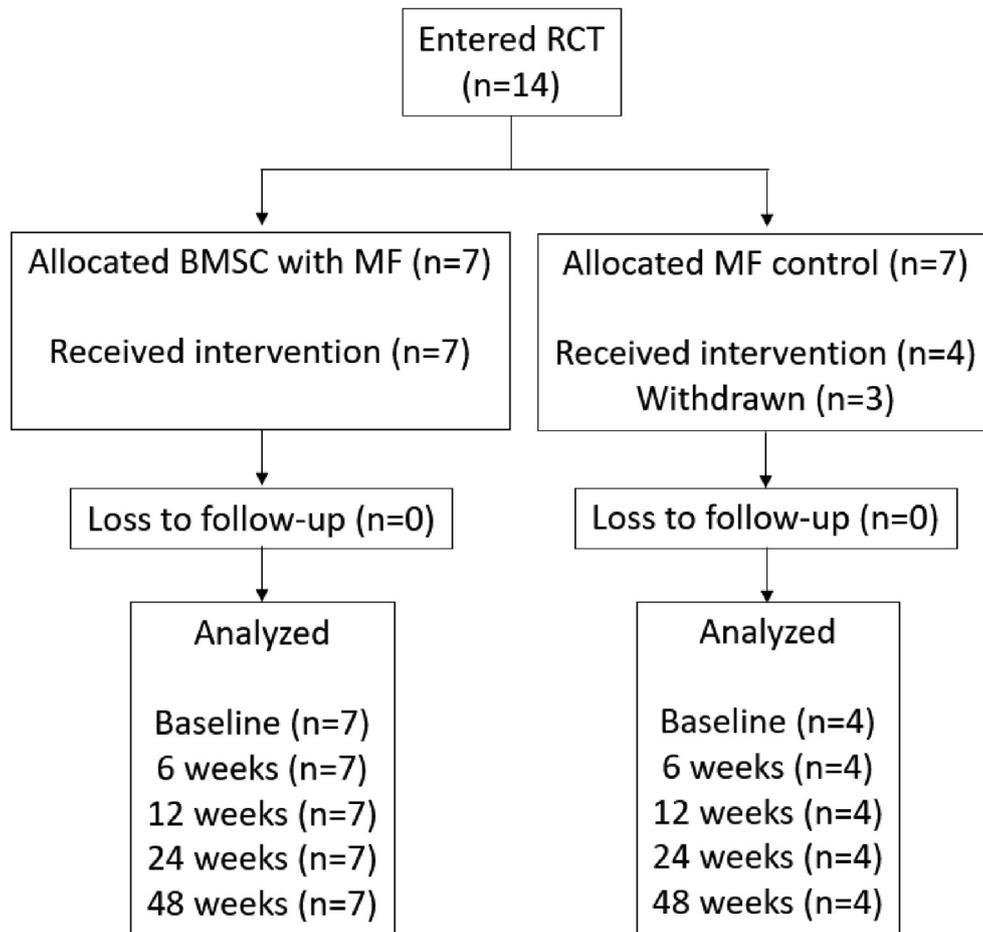


Fig. 2. Flowchart of patients through the randomized controlled trial (RCT. BMSC, autologous bone marrow-derived mesenchymal stem cells; MF, microfracture).

Table 2
Patient baseline demographics.

Variable	cell-T(n=7)	control(n=4)	P-value
Gender(Male/Female)	3/4	4/0	0.06
Age, mean \pm SD	42.6 \pm 15.7	46.3 \pm 9.4	0.67
Height, mean \pm SD(cm)	168.2 \pm 7.9	173.9 \pm 7.6	0.23
Weight, mean \pm SD(kg)	67.0 \pm 16.3	85.2 \pm 22.1	0.15
BMI,mean \pm SD(kg/m ²)	23.8 \pm 4.2	27.9 \pm 5.1	0.19
Size of defect, mean \pm SD(cm ²)	3.0 \pm 0.9	4.4 \pm 3.2	0.33
IKDC subjective score, median (IQR 25%, 75%)	47.1 (44.8, 50.6)	34.5 (24.1, 49.4)	0.34
KOOS, median (IQR 25%, 75%)			
Symptoms	42.9 (39.3, 64.3)	33.9 (28.6, 58.9)	0.65
Pain	63.9 (63.9, 66.7)	55.6 (37.5, 72.2)	0.6
Function, daily living	80.9 (70.6, 89.7)	80.2 (59.6, 88.3)	0.82
Sports/recreation	45.0 (30.0, 60.0)	25.0 (7.5, 47.5)	0.48
Quality of life	25.0 (25.0, 37.5)	15.7 (9.4, 31.3)	0.79
Total KOOS score	280.0 (264.0, 282.6)	217.4 (138.1, 288.7)	0.61

SD standard deviation. IQR Interquartile range, IKDC International Knee Documentation Committee, KOOS Knee injury and Osteoarthritis Outcome Score

3.1. Clinical outcomes

The clinical outcomes from the time of preoperative evaluation to the final follow-up of each group are summarized in Fig. 3. There were no significant differences between preoperative clinical score and postoperative clinical score in either group. At 48 weeks after surgery, the KOOS quality of life (QOL sub-score in the cell-T group tended to be higher than in the control group ($p = 0.07$). There were no clinically important trends in the physical examination, vital signs, or laboratory tests during the study

except for one patient in the control group who had a hematoma in the knee joint. No serious adverse events, infections, or complications deriving from the procedures or treatments were noted in either group.

3.2. Radiographic and MRI outcomes

One of seven patients in the cell-T group and one of four in the control group showed KL grade 1, while six patients in cell-T group and three in the control group showed KL grade 2 on the

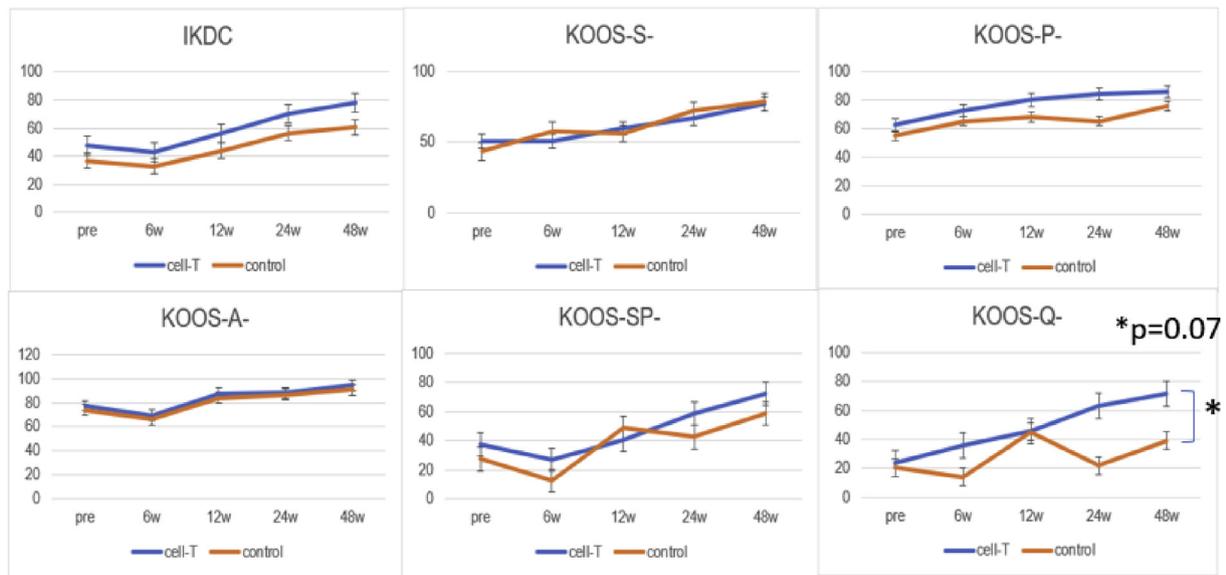


Fig. 3. Pre and postoperative clinical scores. At 48 weeks after surgery, the KOOS QOL sub-score tended to be higher in the cell-T group than the control group ($p = 0.07$).

preoperative X-ray. No patients had more than KL grade 2 on the preoperative X-ray. No patients showed KL grade progression on the AP X-ray at the time of follow-up. Of the 11 patients, three (two in the cell-T group and one in the control group) did not undergo MRI T2 mapping, so this was only assessed in eight patients (five in the cell-T group and three in the control group) at 6, 24, and 48 weeks after surgery. There was no significant difference between the two groups in preoperative, 6, 24, and 48 weeks after surgery (Fig. 4).

The mean MOCART score at 48 weeks was 80.7 in the cell-T group and 50.0 in the control group. Table 3 shows details of the MOCART score at 24 and 48 weeks after surgery. The mean MOCART score of the cell-T group was significantly greater than that of the control group (Fig. 5, $p = 0.02$). Fig. 6 shows a representative MRI taken preoperatively and 48 weeks after surgery. In the cell-T case, the cartilage was fully covered, integration to the border zone was complete, and the MOCART score was 100 at 48 weeks after surgery. In the control case, filling of the defect was incomplete, the surface of the repair tissue was damaged, the subchondral bone was not intact, and the MOCART score was 40 at 48 weeks after surgery.

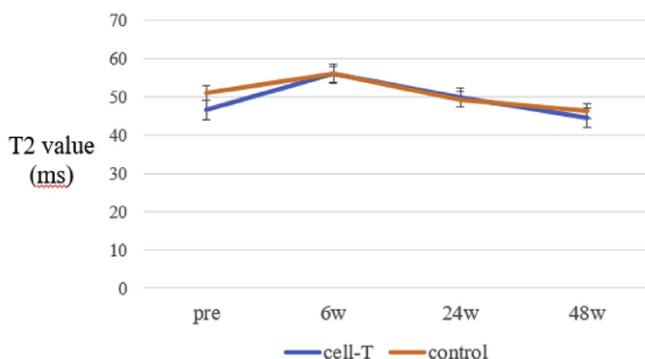


Fig. 4. Pre and postoperative T2 values of repaired cartilage. There was no significant difference between the two groups in preoperative, 6, 24, and 48 weeks after surgery.

4. Discussion

Our data indicate that BMSCs with MFX resulted in a significantly higher quality of articular surface and QOL of KOOS than those obtained using MFX alone in the treatment of symptomatic knee cartilage defects. At 24 and 48 weeks' follow-up, patients in the BMSCs with MFX group had superior MOCART scores and an improvement in the KOOS QOL sub-score of greater than 30 points compared with those in the MFX alone group.

The current standard of care for knee cartilage defects is MFX [28], which uses deliberate penetration of the subchondral bone below the cartilage lesion to elicit a bleeding response. This bone marrow stimulation initiates a repair response that essentially follows the traditional wound healing sequence [29]. Although MFX alone provides minor success in filling lesions, the results are not consistent, which is likely because of the formation of fibrocartilage tissue that lacks the HA structure [28]. Unfortunately, this poor-quality tissue and highly variable outcomes are frequently observed in clinical practice [28,30]. In this context, the critical component for bone marrow-derived cartilage repair is the quality of the initial blood clot that develops in the cartilage lesion, with more adherent and voluminous clots providing higher quality repair [29].

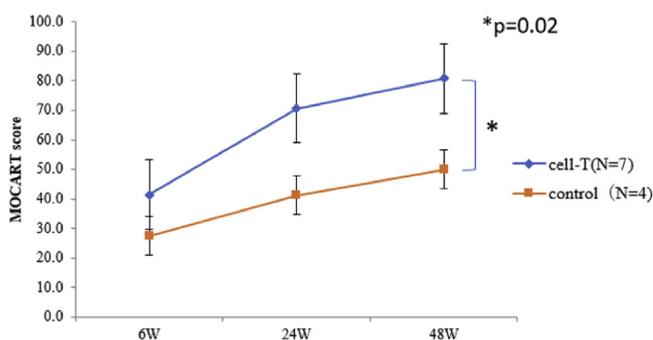
MSC-based therapy through injection or implantation is a promising treatment for traumatic chondral and osteochondral defects. However, although intra-articular injection of MSCs has been reported to effectively reduce pain while promoting cartilage regeneration in patients with knee osteoarthritis [16,17], no RCT has yet focused on cartilage regeneration using MSC injection for cartilage defects of the knee. In this present RCT, greater structural repair (based on MRI assessments) was observed in patients treated with BMSCs with MFX compared with those who underwent MFX alone. Therefore, on the basis of these findings, it appears that the injection of stem cells may facilitate greater cartilage remodeling, and achieve a significant improvement in the KOOS QOL clinical score in the short-term follow-up.

MSC injection offers the advantage of minimal invasiveness, but the dispersion of injected MSCs and lack of focus of these cells into defects make this method less appealing than direct implantation

Table 3
MOCART score in repaired cartilage.

Variables	Score	24 weeks			48 weeks		
		cell-T, n (%)	control, n (%)	P Value	cell-T, n (%)	control, n (%)	P Value
1 Degree of defect repair and filling of defect				0.043			0.086
Complete	20	6 (85.7)	0		6 (85.7)	1 (25.0)	
Hypertrophy	15	0	1 (25.0)		0	0	
Incomplete							
>50% of adjacent cartilage	10	1 (14.3)	2 (50.0)		1 (14.3)	2 (50.0)	
<50% of adjacent cartilage	5	0	1 (25.0)		0	1 (25.0)	
subchondral bone exposed	0	0	0		0	0	
2 Integration to border zone				0.184			0.201
Complete	15	5 (71.4)	2 (50.0)		6 (85.7)	2 (50.0)	
Incomplete							
Demarcating border visible	10	1 (14.3)	0		0	0	
Defect visible							
<50% of length of repair tissue	5	1 (14.3)	2 (50.0)		1 (14.3)	2 (50.0)	
>50% of length of repair tissue	0	0	0		0	0	
3 Surface of repair tissue				0.125			0.058
Surface intact	10	3 (42.9)	0		4 (57.1)	0	
Surface damaged							
<50% of repair tissue depth	5	4 (57.1)	4 (100.0)		3 (42.9)	4 (100.0)	
>50% of repair tissue depth	0	0	0		0	0	
4. Structure of repair tissue				0.125			0.044
Homogeneous	5	3 (42.9)	0		6 (85.7)	1 (25.0)	
Inhomogeneous or defect formation	0	4 (57.1)	4 (100.0)		1 (14.3)	3 (75.0)	
5 Signal intensity of repair tissue				0.047			0.171
Normal (identical to adjacent cartilage)	30	1 (14.3)	0		3 (42.9)	0	
Nearly normal (slight areas of signal alteration)	15	6 (85.7)	2 (50.0)		4 (57.1)	3 (75.0)	
Abnormal (large areas of signal alteration)	0	0	2 (50.0)		0	1 (25.0)	
6 Subchondral lamina				0.200			0.428
Intact	5	6 (85.7)	2 (50.0)		6 (85.7)	4 (100.0)	
Not intact	0	1 (14.3)	2 (50.0)		1 (14.3)	0	
7. Subchondral bone				0.125			0.058
Intact	5	3 (42.9)	0		4 (57.1)	0	
Not intact	0	4 (57.1)	4 (100.0)		3 (42.9)	4 (100.0)	
8 Adhesions				1.000			1.000
No	5	7 (100.0)	4 (100.0)		7 (100.0)	4 (100.0)	
Yes	0	0	0		0	0	
9 Effusion				0.898			0.303
No	5	2 (28.6)	1 (25.0)		4 (57.1)	1 (25.0)	
Yes	0	8 (71.4)	3 (75.0)		3 (42.9)	3 (75.0)	
Mean±SD		70.7±15.4	41.25±6.3	0.009	80.7±18.4	50.0±11.6	0.018

*Statistically significant (P<.05).

**Fig. 5.** The mean MOCART score was significantly higher in the cell-T group than the control group ($p = 0.02$).

techniques. To date, several pre-clinical studies have been performed, but only one group has assessed MSC injection clinically [31]. The current literature supports performing MFX or subchondral drilling in conjunction with weekly injections of MSCs and HA over the course of multiple weeks [31–33]. This protocol presumably increases the likelihood of defect seeding with MSCs from both injection and subchondral marrow sources.

Recently, MSCs derived from adipose tissue, synovial tissue, and autologous peripheral blood stem cells have been used for cartilage regeneration. In a recent equine study using an osteochondral fragment with bone and cartilage debris to induce osteoarthritis (rather than relying on joint instability to create secondary osteoarthritis), there was a significant reduction in synovial fluid prostaglandin E2 levels in response to treatment with BMSCs injected intra-articularly [34]. However, this effect was not seen with adipose-derived stem cells. That study also showed a negative response through an increase in synovial fluid tumor necrosis factor concentrations in response to the intraarticular injection of adipose-derived cells. Positive effects on cartilage repair could therefore be gained by inhibiting catabolism as well as promoting anabolism through a cytokine mediator.

The homing ability of injected MSCs has been further demonstrated by pre-clinical studies [16,17,35]. In a porcine model, the *in vivo* tracing of green fluorescent protein (GFP)-labeled MSCs showed that these cells localized and formed neo-cartilage at the site of a surgically created full-thickness chondral defect. In a rat model, Nishimori et al. [17] reported that intra-articular injections of GFP+ MSCs together with a bone marrow stimulation procedure was more effective for repairing a chronic osteochondral lesion than the bone marrow-stimulating procedure alone. Importantly, GFP cells were present in the specimens up to 4 weeks after treatment and were localized to the site of the osteochondral

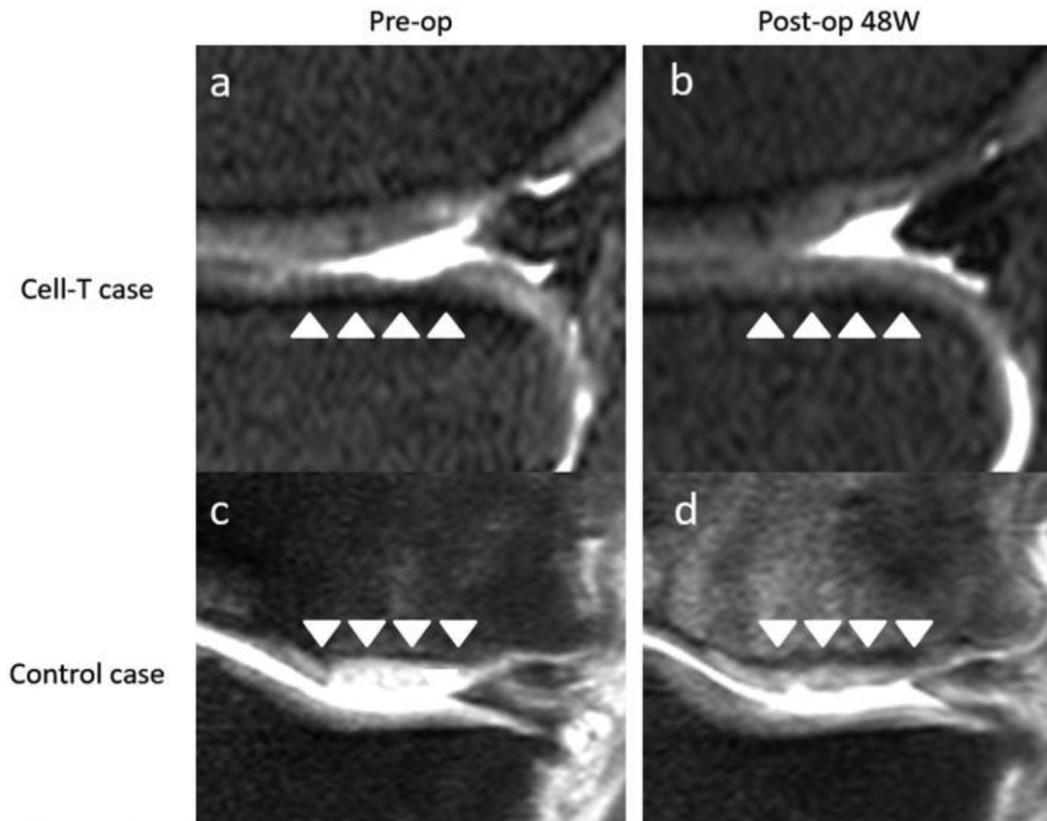


Fig. 6. Pre and postoperative MRI. a The cartilage defect was seen at the lateral tibia plateau in the preoperative MRI of the cell-T group. b The cartilage was full covered, integration to the border zone was complete, and the MOCART score was 100 at 48 weeks after surgery. c The cartilage defect was seen at the medial femoral condyle in the preoperative MRI of the control group. d The filling of the defect was incomplete, the repair tissue surface was damaged, the subchondral bone was not intact, and the MOCART score was 40 at 48 weeks after surgery.

defect, indicating that the injected MSCs “home in” to the site of injury. The authors hypothesized that growth factors were induced from the bone marrow, which could be attributed to the injected BMSCs adhering to the defect, preventing them from escaping, and aiding in their differentiation to chondrocytes.

The concept of MSC intra-articular injection promoting healing confirms the multiple potential ways in which these cells could influence repair in addition to their ability to differentiate into a target cell and synthesize new tissue. MSCs have previously been shown to secrete a variety of cytokines and growth factors with both paracrine and autocrine effects, including suppression of the local immune system, the inhibition of fibrosis and apoptosis, the stimulation of mitosis, and the differentiation of stem cells [36]. These have been referred to as trophic effects and are distinct from the direct differentiation of MSCs into repair tissue. It was also suggested that endogenous mesenchymal stromal cells could be augmented by these paracrine effects of MSCs themselves [37].

The present study has some limitations. First, it had a smaller sample size than anticipated, which may have increased the risk of obtaining anomalous results. Additionally, some baseline imbalances, such as more women in the BMSCs group, may have contributed to differences in outcomes between groups. Second, some patients had to be withdrawn from the control group, which may have resulted in the bias of psychogenic factors. Third, the follow-up period was relatively short, which may have complicated our assessment of the clinical outcomes. Finally, not of all the patients were examined by quantitative MRI using T2 mapping, with some receiving T1 rho mapping. Although we excluded those patients who underwent T1 rho mapping in our T2 value analysis, the

MOCART score was used as a semi-quantitative evaluation instead of T2 mapping, resulting in superiorities for the cell-T group over the control group with respect to the extent of defect repair and filling of the defect.

5. Conclusion

Compared with MFX alone, BMSCs with MFX provided a better quality of articular surface and KOOS QOL sub-score improvements in treatment of symptomatic cartilage defects of the knee. No remarkable adverse events or safety issues were noted in this heterogeneous patient population. However, a larger sample size and long-term studies are needed to confirm our findings.

Conflicts of interest

There are no conflicts of interest.

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