Allogeneic Umbilical Cord Blood–Derived Mesenchymal Stem Cell Implantation Versus Microfracture for Large, Full-Thickness Cartilage Defects in Older Patients

A Multicenter Randomized Clinical Trial and Extended 5-Year Clinical Follow-up

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Investigation performed at 10 tertiary-care hospitals in the Republic of Korea

Background: There is currently no optimal method for cartilage restoration in large, full-thickness cartilage defects in older patients.

Purpose: To determine whether implantation of a composite of allogeneic umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate (UCB-MSC-HA) will result in reliable cartilage restoration in patients with large, full-thickness cartilage defects and whether any clinical improvements can be maintained up to 5 years postoperatively.

Study Design: Randomized controlled trial; Level of evidence, 1.

Methods: A randomized controlled phase 3 clinical trial was conducted for 48 weeks, and the participants then underwent extended 5-year observational follow-up. Enrolled were patients with large, full-thickness cartilage defects (International Cartilage Repair Society [ICRS] grade 4) in a single compartment of the knee joint, as confirmed by arthroscopy. The defect was treated either with UCB-MSC-HA implantation through mini-arthrotomy or with microfracture. The primary outcome was proportion of participants who improved by ≥1 grade on the ICRS Macroscopic Cartilage Repair Assessment (blinded evaluation) at 48-week arthroscopy. Secondary outcomes included histologic assessment; changes in pain visual analog scale (VAS) score, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and International Knee Documentation Committee (IKDC) score from baseline; and adverse events.

Results: Among 114 randomized participants (mean age, 55.9 years; 67% female; body mass index, 26.2 kg/m^2), 89 completed the phase 3 clinical trial and 73 were enrolled in the 5-year follow-up study. The mean defect size was 4.9 cm^2 in the UCB-MSC-HA group and 4.0 cm^2 in the microfracture group ($P = .051$). At 48 weeks, improvement by ≥1 ICRS grade was seen in 97.7% of the UCB-MSC-HA group versus 71.7% of the microfracture group ($P = .001$); the overall histologic assessment score was also superior in the UCB-MSC-HA group ($P = .036$). Improvement in VAS pain, WOMAC, and IKDC scores were not significantly different between the groups at 48 weeks, however the clinical results were significantly better in the UCB-MSC-HA group at 3- to 5-year follow-up ($P < .05$). There were no differences between the groups in adverse events.

Conclusion: In older patients with symptomatic, large, full-thickness cartilage defects with or without osteoarthritis, UCB-MSC-HA implantation resulted in improved cartilage grade at second-look arthroscopy and provided more improvement in pain and function up to 5 years compared with microfracture.

Registration: NCT01041001, NCT01626677 (ClinicalTrials.gov identifier).

Keywords: full-thickness cartilage defect; cartilage restoration; mesenchymal stem cells; umbilical cord blood; microfracture

Articular cartilage defects remain a challenging clinical problem. Currently available treatment options are generally more applicable to localized, focal defects in relatively young

patients rather than the large, full-thickness, often bipolar lesions typically found in the osteoarthritic joints of older patients.[25,29] Microfracture is a popular option for small cartilage defects, but the results are usually inferior for large chondral defects.[10,20,39] Moreover, microfracture generally leads to fibrous repair tissue with limited durability, just up to 1 or 2 years.[1,48] Autologous chondrocyte implantation (ACI) is typically recommended for large lesions, particularly in younger patients, but ACI procedures are less often indicated in the older population because of the diminished reparative potential of the autologous chondrocytes.[12] Therefore, neither microfracture nor ACI is generally recommended in large chondral defects in older patients. However, many older patients have an active lifestyle and are reluctant to undergo joint replacement, so an innovative regenerative treatment option for this population is needed.

Mesenchymal stem cells (MSCs) have recently been proposed as a potential option for cartilage restoration in elderly patients. MSCs are known to have unique biological characteristics, including immunomodulatory and anti-inflammatory properties and secretion of proregenerative cytokines and chemokines.[3,30] MSCs can be obtained from various tissues of the human body.[13,26] Umbilical cord blood–derived MSCs (UCB-MSCs) have advantages over other MSCs, including noninvasive cell collection, hypoimmunogenicity, and high expansion capacity.[8,19] Moreover, allogeneic MSC implantation has the advantages of 1-step surgery and better quality control of the cells compared with a 2-step procedure such as ACI or a 1-step procedure using autologous cell concentrate such as bone marrow aspirate concentrate or autologous adipose tissue–derived cell therapy, which have heterogeneous cell populations.[18,49]

Several preclinical studies have evaluated human UCB-MSCs combined with hyaluronic acid (HA) hydrogels for the restoration of full-thickness cartilage defects.[4,11,33,34,38] A phase 1/2, first-in-human clinical trial has suggested the safety and efficacy of UCB-MSCs when combined with 4% HA hydrogel (UCB-MSC-HA) for the cartilage repair of osteoarthritic defects in older patients; durable improvement was found up to 7 years after treatment.[36] The current study was a randomized controlled phase 3 clinical trial conducted to determine whether implantation of UCB-MSC-HA results in reliable cartilage restoration compared with microfracture (control) in patients with symptomatic, large, full-thickness cartilage defects. The study and control populations were observed for 5 years to determine whether any clinical improvements could be maintained for that duration.

METHODS

Study Design

This randomized, active controlled, phase 3, multicenter trial was conducted at 10 tertiary-care hospitals between February 2, 2009, and January 24, 2011. The trial evaluated surgical implantation of an allogeneic UCB-MSC-HA composite versus microfracture for treating full-thickness cartilage defect of the femoral condyle in patients who had knee pain. An observational, extended follow-up study (36, 48, and 60 months) was performed on participants of the phase 3 portion who consented to the follow-up portion.

The trials were conducted according to current Good Clinical Practices and principles of the Declaration of Helsinki (1989). Study protocols were approved by institutional

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review boards at each institution and the Ministry of Food and Drug Safety (MFDS) of South Korea (equivalent to US Food and Drug Administration) through the investigational new drug pathway.

Participants

Eligible participants were patients aged >18 years with a symptomatic, large, full-thickness femoral condyle or trochlear cartilage defect (2-9 cm^2), International Cartilage Repair Society (ICRS)[23] grade 4, in a single compartment of the knee joint as confirmed by arthroscopy, regardless of whether the defect was focal or osteoarthritic. For patients with multiple lesions, the defect in the most symptomatic compartment was considered. Major exclusion criteria included ligament instability of >5 mm or chronic inflammatory articular disease, Kellgren-Lawrence grade 4 osteoarthritis, or significant deformity (>10° varus/valgus). More details of the primary inclusion and exclusion criteria are provided in Appendix Table A1. Informed consent was obtained from all participants.

Microfracture is not the gold standard for large chondral defects, especially in older patients; however, the participants in this clinical trial had experienced failure of previous nonoperative treatment, and another year of placebo control during the trial was not considered ethical or practical by the investigators and the regulatory body (MFDS). Thus, after a discussion with the MFDS and a thorough review of previous reports that showed some clinical benefit of microfracture in similar disease states,[1,48] microfracture was determined as the active control. Patients were recommended not to take nonsteroidal anti-inflammatory drugs, pain relievers, or injections; however, these agents were allowed if needed by the patients, after consultation with their physicians.

After enrollment, participants were randomized 1:1 through use of a stratified, random, permuted block design with block size of 4 to 6. Participants who met the inclusion and exclusion criteria were assigned to either the UCB-MSC-HA group or the microfracture group, randomly as described above. All outcomes were assessed by blinded evaluators. Surgeons and participants could not be double-blinded because of the differences in surgical scars—arthrotomy for UCB-MSC-HA and arthroscopy for microfracture—however, the macroscopic and histologic evaluations were performed in a completely blinded manner by independent evaluators.

## Cell Preparation and Characterization

Allogeneic UCB-MSCs were produced at a cell manufacturing facility operated by MEDIPOST Co Ltd, in full compliance with the Good Manufacturing Practice requirements of the MFDS as well as with donor screening, cell isolation and expansion, and quality control measures. In brief, donor umbilical cord blood was collected from full-term infants after informed maternal consent and stored in bags containing anticoagulant. The cord blood was processed within 24 hours of collection. Mononuclear cells in the low-density fraction were separated over Histopaque

(density 1.077 g/cm^3; Sigma-Aldrich) and then cultured according to a previously published method.[47] The ex vivo culture-expansion manufacturing process of the UCB-MSCs is a scaled adaptation of the technique described by Yang et al[47] and involves initial isolation steps to remove hematopoietic elements, followed by MSC expansion of the nucleated cells in culture medium (alpha-Minimal Essential Medium; Gibco BRL) supplemented with 10% fetal bovine serum. After multiple passage expansion, the UCB-MSCs are cryopreserved at –150°C or colder in the presence of 10% dimethyl sulfoxide. For use in the clinical trial, cryopreserved UCB-MSCs were carefully thawed and subjected to the final passage with harvested cells vialed at a concentration of 7.5×10^6 cells per 1.5 mL and released. Potency, sterility, mycoplasma, and endotoxin testing during the manufacturing process and upon final release were performed in compliance with requirements by the MFDS.

Interventions

For the study population, the UCB-MSC-HA composite was prepared at the time of surgery in the operating room (Appendix Figure A1) and implanted as previously reported in the phase 1/2 clinical trial.[35,36] After a standard arthroscopic examination to assess cartilage defects, during which arthroscopic procedures such as debridement of the cartilage flaps or meniscectomy were performed, a small longitudinal arthrotomy incision was made alongside the patellar tendon to expose the cartilage defect on the femoral condyle. Multiple drill holes (5 × 5 mm [diameter × depth]; approximately 2 mm apart) were made in the subchondral bone for insertion of the UCB-MSC-HA composite. In addition, multiple small drill holes (1.4 × 5 mm [diameter × depth]) were made between the 5-mm drill holes for better lateral integration between the repair tissues from the 5-mm drill holes, based on experience from a previous clinical trial.[36] The UCB-MSC-HA composite was carefully implanted into the 5-mm drill holes to completely fill in the holes (Figure 1).[36] The wound was then closed, and a splint was applied. In the active control group, the traditional microfracture technique was performed, which entailed making multiple awl holes arthroscopically at the subchondral bone.[41]

All participants complied with standardized posttreatment rehabilitation. Quadriceps sitting and straight leg–raising exercises were performed from the day of surgery. Participants were allowed to start active, passive, and active-assisted range of motion exercises from postoperative day 1, progressing as tolerated. Nonweightbearing walking with crutches or a walker was encouraged for the first 12 weeks for the UCB-MSC-HA group and the first 8 weeks for the microfracture group. After the nonweightbearing period had passed, partial weightbearing for 4 weeks followed. We educated participants about the rehabilitation protocol and emphasized the importance of compliance for successful outcome after cartilage repair surgery, as is routine practice for other standard cartilage repair procedures.
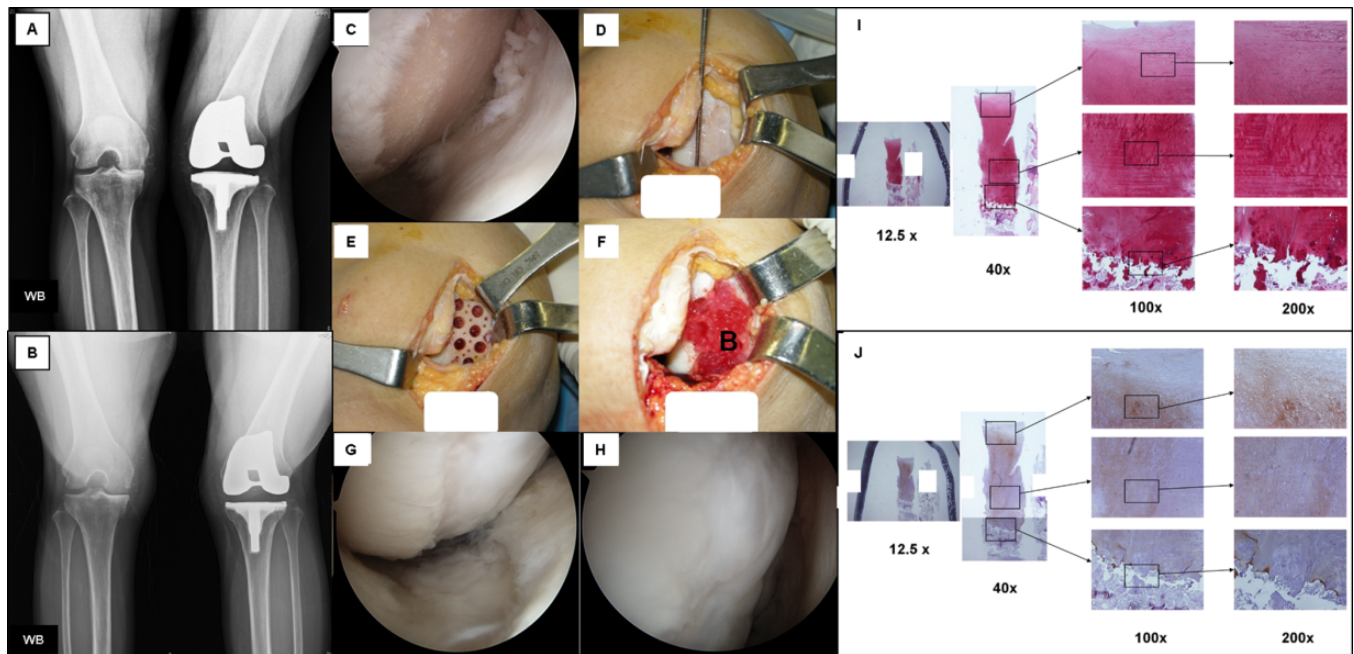
Figure 1. Surgical implantation procedure for the allogeneic human umbilical cord blood–derived mesenchymal stem cell and 4% hyaluronate (UCB-MSC-HA) composite. (A) Preoperative weightbearing (WB) posteroanterior radiograph (Rosenberg view) shows osteophyte formation of the right knee of a 54-year-old female patient. She had previously undergone a total knee arthroplasty for her left knee. (B) The same radiographic view taken at 5-year follow-up shows maintenance of restored medial joint space without significant deterioration. (C) Arthroscopic inspection and confirmation of International Cartilage Repair Society (ICRS) grade 4 cartilage defect at the initial examination. (D) Mini-arthrotomy and measurement of the size of the full-thickness cartilage defect in the medial femoral condyle of the patient's right knee. (E) Multiple drilling with a drill bit of 5-mm diameter, which is larger than the diameter of holes made during standard microfracture at the subchondral bone. The large drill holes were mainly made for containment of the UCB-MSC-HA composite. (F) Implantation of the UCB-MSC-HA composite in the 5-mm drill holes. (G, H) Restored articular cartilage at 48 weeks after implantation, which was assessed as ICRS grade 3 in this case. (I) Safranin O staining showing abundant presence of glycosaminoglycan in the restored cartilaginous tissue. (J) Type II collagen immunostaining showing abundant type II collagen in the restored cartilaginous tissue.
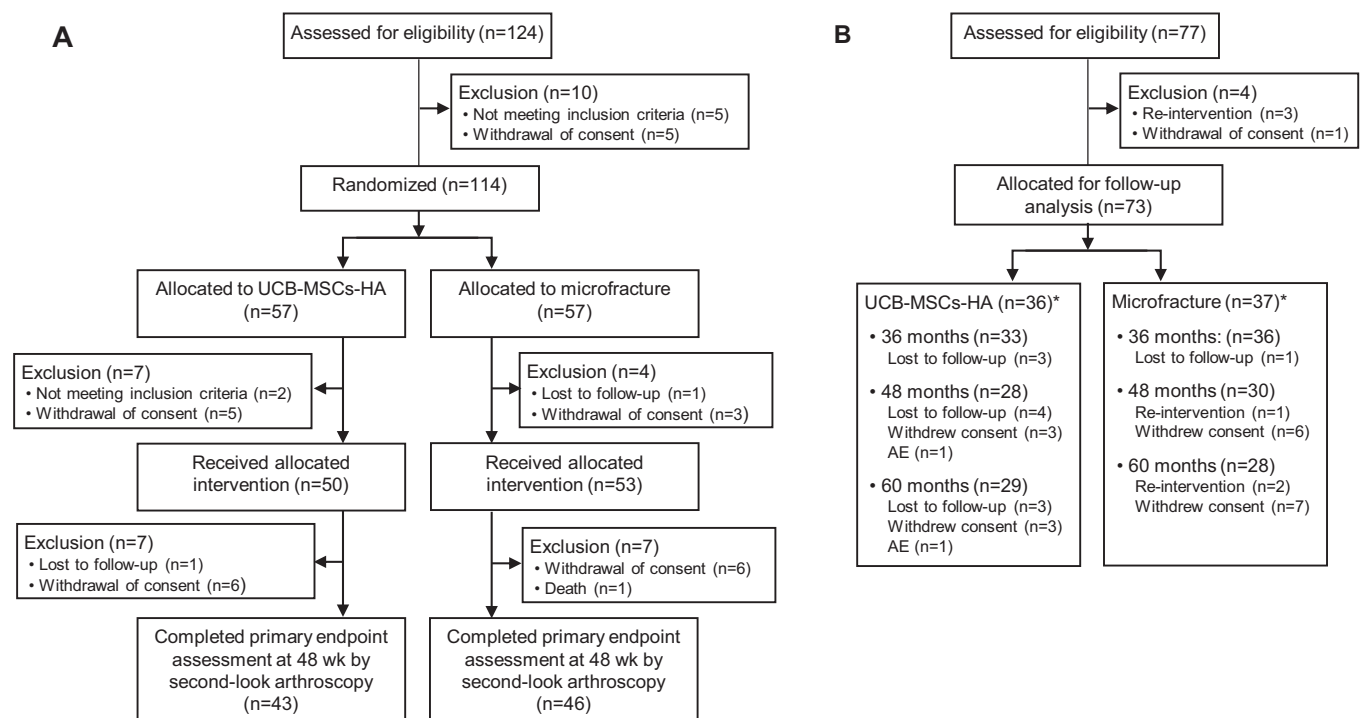
Outcome Measures

The primary outcome of the phase 3 clinical trial was the proportion of participants with cartilage restoration equivalent to at least 1 grade improvement on the ICRS Macroscopic Cartilage Repair Assessment at 48-week arthroscopic evaluation (see Appendix Table A2 for scoring).[43] Secondary outcomes were the ICRS II Histological Evaluation System score from tissue biopsies[23,24] and changes in 100-mm visual analog scale (VAS) for pain, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC),[2] and International Knee Documentation Committee (IKDC) scores[15] from baseline to 48 weeks. Biopsies of the repair tissue taken from the center of the lesion site during the 48-week arthroscopic evaluation were used for the histologic assessment.

Owing to differences in the surgical scars, the ICRS Macroscopic Cartilage Repair Assessment was conducted in a blinded fashion as follows: Arthroscopic images were captured and videos were recorded during both the initial surgical procedure and the second-look arthroscopy for efficacy evaluation at 48 weeks. Ten investigators were divided into 2 groups (group 1: HC Lim, BK Lee, HJ Jeong, CH Choi, CW Ha; group 2: MK Kim, SI Bin, CH Choi, JD Yoo, JR Yoon), and each group assessed the arthroscopic images and videos of the other group without any information regarding the treatment assignment. The 5 investigators in each group initially assessed preoperative and postoperative images and videos independently using the ICRS Macroscopic Cartilage Repair Assessment. The evaluations were collected and compared to arrive at a final consensus. If agreement was reached by at least 3 of the 5 reviewers, the 3 matching results were selected as the final ICRS grade. For cases of agreement by <3 reviewers, the final grade was determined through an open discussion among the 5 investigators in each group.

The WOMAC is a well-validated, disease-specific measure of osteoarthritis of the hip or knee that includes pain, stiffness, and function subscales and addresses activities of daily living.[2] The IKDC subjective knee form[15] is designed to measure symptoms and limitations in function and sports activity for various knee conditions, including cartilage injuries, and has demonstrated strong psychometric characteristics. The IKDC has shown adequate internal consistency and no remarkable floor or ceiling effects.[5]

A

Assessed for eligibility (n=124)

Exclusion (n=10)
• Not meeting inclusion criteria (n=5)
• Withdrawal of consent (n=5)

Randomized (n=114)

Allocated to UCB-MSCs-HA
(n=57)

Exclusion (n=7)
• Not meeting inclusion criteria (n=2)
• Withdrawal of consent (n=5)

Received allocated
intervention (n=50)

Exclusion (n=7)
• Lost to follow-up (n=1)
• Withdrawal of consent (n=6)

Completed primary endpoint
assessment at 48 wk by
second-look arthroscopy
(n=43)

Allocated to microfracture
(n=57)

Exclusion (n=4)
• Lost to follow-up (n=1)
• Withdrawal of consent (n=3)

Received allocated
intervention (n=53)

Exclusion (n=7)
• Withdrawal of consent (n=6)
• Death (n=1)

Completed primary endpoint
assessment at 48 wk by
second-look arthroscopy
(n=46)

B

Assessed for eligibility (n=77)

Exclusion (n=4)
• Re-intervention (n=3)
• Withdrawal of consent (n=1)

Allocated for follow-up
analysis (n=73)

UCB-MSCs-HA (n=36)*
• 36 months (n=33)
   Lost to follow-up (n=3)
• 48 months (n=28)
   Lost to follow-up (n=4)
   Withdrew consent (n=3)
   AE (n=1)
• 60 months (n=29)
   Lost to follow-up (n=3)
   Withdrew consent (n=3)
   AE (n=1)

Microfracture (n=37)*
• 36 months: (n=36)
   Lost to follow-up (n=1)
• 48 months (n=30)
   Re-intervention (n=1)
   Withdrew consent (n=6)
• 60 months (n=28)
   Re-intervention (n=2)
   Withdrew consent (n=7)

Figure 2. CONSORT (Consolidated Standards of Reporting Trials) flow diagram of study participants. (A) Phase 3 clinical trial of 48 weeks. (B) Observational extended follow-up study of 60 months. *Some of the participants were lost to follow-up at specific follow-up time points only (eg, a participant who was "lost to follow-up" at the 36- and 48-month timepoints could have been included at the 60-month timepoint, and vice versa). AE, adverse event; UCB-MSCs-HA, umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate.

At 48 weeks, cylindrical biopsies (2-mm diameter) included both restored cartilage tissue and subchondral bone. The biopsies were fixed (4% paraformaldehyde) and embedded in paraffin. Then, 4 μm–thick sections were obtained and stained with hematoxylin and eosin for general morphologic features, Safranin O for glycosaminoglycan, and Masson trichrome for general collagen distribution. Immunohistochemical staining for collagen types I and II was also performed. Biopsies were assessed by 2 blinded, independent professional pathologists according to the ICRS II Histological Evaluation System.[24]

Safety was assessed by physical examinations, laboratory tests, adverse event (AE) monitoring, and 24-week ex vivo mixed lymphocyte reaction to allogeneic cells. All of the AEs were categorized using the World Health Organization Common Toxicity Criteria for Adverse Events.[42]

In the observational extended follow-up study of 60 months, longer term safety was assessed by any AEs incurred, and longer term efficacy was assessed by VAS pain, WOMAC,[2] and IKDC scores[15] as well as surgical reintervention rates.

Statistical Analysis

We assumed that approximately 40% of the microfracture-treated participants would have an improved ICRS grade based on previous reports,[10,28] and we sought to determine whether the ICRS grade would improve in 70% of UCB-MSC-HA implanted participants. A total of 84 participants (42 per group) would provide 80% power to detect a difference of 40% versus 70% in ICRS grade improvement with a 5%, 2-sided alpha level. With a possible 20% dropout rate, target enrollment was at least 104 participants (52 per group).

Between-group differences for the primary efficacy outcome and subgroup analyses (age and lesion size) were performed using the Fisher exact test. ICRS grade distribution at 48 weeks was compared between groups using the Wilcoxon rank-sum test. For secondary endpoint comparisons, the Wilcoxon rank-sum test or 2-sample t test was used for continuous variables, and the Fisher exact test was used for binary variables. The last observation carried forward was used for missing data at 36, 48, and 60 months. All analyses were performed using SAS 9.3 (SAS Institute), and all P values were 2-sided.

RESULTS

In the 48-week clinical trial, 124 participants were screened and 10 participants were excluded after screening; thus, 114 participants were enrolled and randomized to UCB-MSC-HA (n = 57) or microfracture (n = 57) (Figure 2A). Of these participants, 11 did not receive intervention, leaving 103 participants (50 in the UCB-MSC-HA group and 53 in the

TABLE 1
Demographics and Baseline Characteristics
of Study Participants[a]

	UCB-MSC-HA (n = 43)	Microfracture (n = 46)	P Value[b]
Age, y	55.3 ± 8.9	54.4 ± 10.8	.682
Sex			>.999
Male	15 (34.9)	16 (34.8)	
Female	28 (65.1)	30 (65.2)	
Body mass index, kg/m^2	25.7 ± 2.8	26.7 ± 3.9	.148
Diagnosis			
Osteoarthritis, K-L grade	41 (95.4)	42 (91.3)	
1	1	0	
2	18	21	
3	22	21	
4	0	0	
Osteochondritis dissecans	1 (2.3)	0 (0.0)	
Focal chondral defect	1 (2.3)	4 (8.7)	
VAS pain score[c]	44.0 ± 12.5	44.6 ± 12.9	.833
WOMAC score[d]	37.4 ± 15.1	40.4 ± 14.8	.360
IKDC score	42.7 ± 13.9	41.8 ± 13.4	.748
ICRS grade			
Grade 1, 2, or 3	0 (0.0)	0 (0.0)	
Grade 4	43 (100.0)	46 (100.0)	
Lesion size, cm^2	4.9 ± 2.0	4.0 ± 1.8	.051
Location			
Medial femoral condyle	34 (79.1)	40 (87.0)	
Lateral femoral condyle	5 (11.6)	4 (8.7)	
Trochlea	4 (9.3)	2 (4.3)	
Bipolar lesion	35 (81.4)	38 (82.6)	>.999
Compartment			
Unicompartmental osteoarthritis	26 (60.5)	26 (56.5)	.830
Multicompartmental osteoarthritis	17 (39.5)	20 (43.5)	
Concomitant procedures[c]	28 (65.1)	33 (71.7)	.648
Meniscectomy	26 (60.5)	27 (58.7)	>.999
Treated compartment	25 (58.1)	24 (52.2)	.671
Untreated compartment	2 (4.7)	7 (15.2)	.159
Other procedure[d]	10 (23.3)	15 (32.6)	.355

[a]Data are reported as n (%) or mean ± SD. ICRS, International Cartilage Repair Society; IKDC, International Knee Documentation Committee; K-L, Kellgren-Lawrence; UCB-MSC-HA, umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.
[b]Two-sample *t* test for continuous variables and chi-square test for binary variables.
[c]Study participant may have received >1 concomitant procedure.
[d]Includes plica excision, notchplasty, meniscal repair, cartilaginous loose body removal, osteophyte excision, ganglion cyst excision, synovectomy, and anterior cruciate ligament reconstruction or debridement.

TABLE 2
Primary Outcome of Phase 3 Clinical Trial (48 weeks)[a]

Variable	UCB-MSC-HA (n = 43)	Microfracture (n = 46)	P Value
Assessment of Cartilage Restoration			
Improved (at least 1 ICRS grade)	42 (97.7)	33 (71.7)	.001[b]
Difference between groups (95% CI)	25.94 (12.17-39.70)		
Odds ratio (95% CI)	16.55 (2.06-133.03)		
Not improved	1 (2.3)	13 (28.3)	
ICRS Grade Distribution			
ICRS grade			.008[c]
Grade 1	3 (7.0)	1 (2.2)	
Grade 2	25 (58.1)	20 (43.5)	
Grade 3	14 (32.6)	12 (26.1)	
Grade 4	1 (2.3)	13 (28.2)	
Subgroup Analysis: Participants With Improvement of at Least 1 ICRS Grade			
Age			
<50 y	9/9 (100.0)	11/11 (100.0)	NA
50 to 59 y	21/22 (95.5)	13/21 (61.9)	.009
≥60 y	12/12 (100.0)	9/14 (64.3)	.043
Initial cartilage defect size			
2.0 to 3.0 cm^2	8/8 (100.0)	15/15 (100.0)	NA
>3.0 to <6.0 cm^2	20/20 (100.0)	14/22 (63.6)	.004
≥6.0 cm^2	14/15 (93.3)	4/9 (44.4)	.015

[a]Data are reported as n (%) unless otherwise indicated. 9/9 means that 9 patients showed minimum grade 1 improvement in ICRS grade among 9 patients less than 50 years old. ICRS, International Cartilage Repair Society Macroscopic Cartilage Repair Assessment; NA, not applicable; UCB-MSC-HA, umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate.
[b]Pearson chi-square test.
[c]Wilcoxon rank-sum test.

(84.5%) had biopsy samples taken (tissue acquisition failed in 2 participants).

Demographics, lesion characteristics, and baseline scores were comparable between the 2 groups (Table 1). The mean age of participants was 55.3 years in the UCB-MSC-HA group and 54.4 years in the microfracture group; mean body mass index was 52.7 (UCB-MSC-HA) and 26.7 (microfracture); the male-to-female ratio was similar in both groups; and osteoarthritis was the most prevalent diagnosis in both groups: 95.4% in the UCB-MSC-HA group and 91.3% in the microfracture group (Table 1). The mean lesion size was 4.9 cm^2 in the UCB-MSC-HA group and 4.0 cm^2 in the microfracture group (P = .051). All treated defects were on the femur, mostly on the medial femoral condyle (83.1%).

Among the 89 participants who completed the initial 48-week clinical trial, 73 were enrolled in the extended observational 60-month follow-up study (Figure 2B). Detailed information on participants of the extended follow-up study is provided in Appendix Table A3. There were no significant

microfracture group). Ultimately, 89 participants (86.4%; 43 in the UCB-MSC-HA group and 46 in the microfracture group) completed the 48-week primary endpoint assessment by second-look arthroscopy (Figure 2A), and 87 participants

TABLE 3
Summary of Macroscopic and Histologic Evaluation
of Phase 3 Clinical Trial (48 weeks)[a]

	UCB-MSC-HA (n = 43)	Microfracture (n = 46)	P Value
ICRS Macroscopic Cartilage Repair Assessment score			
Overall	8.4 ± 2.3	6.4 ± 3.5	.017
Degree of defect repair	3.1 ± 0.7	2.5 ± 1.3	.045
Integration to border zone	2.6 ± 0.9	1.9 ± 1.2	.005
Macroscopic appearance	2.7 ± 0.9	2.1 ± 1.2	.015
ICRS II Histological Evaluation System score			
Overall	60.2 ± 16.8	51.1 ± 23.0	.036
Tissue morphology	53.2 ± 20.5	51.2 ± 22.4	.689
Matrix staining	54.9 ± 21.3	55.5 ± 20.2	.902
Cell morphology	49.3 ± 22.7	51.4 ± 21.8	.664
Chondrocyte clustering	76.0 ± 14.2	70.9 ± 19.1	.092
Surface architecture	66.0 ± 23.7	67.6 ± 27.2	.917
Basal integration	76.0 ± 14.2	72.6 ± 18.7	.220
Tidemark formation	18.4 ± 23.4	25.9 ± 27.3	.169
Subchondral bone abnormalities	77.4 ± 14.2	70.0 ± 19.4	.043
Inflammation	96.1 ± 7.3	96.0 ± 5.9	.975
Abnormal calcification	88.4 ± 13.3	84.4 ± 17.2	.237
Vascularization	87.2 ± 25.4	94.0 ± 7.8	.109
Surface assessment	69.5 ± 22.1	58.7 ± 33.4	.077
Mid/deep zone assessment	76.7 ± 12.3	66.3 ± 24.0	.012

[a]Data are reported as mean ± SD. ICRS, International Cartilage Repair Society; UCB-MSC-HA, umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate.

differences in baseline characteristics between the groups at 60-month follow-up.

Efficacy Outcomes

Phase 3 Clinical Trial at 48 Weeks

The primary outcome analysis revealed that the proportion of participants showing improvement of ≥1 ICRS grade at 48 weeks was 97.7% (42/43) in the UCB-MSC-HA group and 71.7% (33/46) in the microfracture group (odds ratio, 16.55; 95% CI, 2.06-133.03; $P = .001$) (Table 2). The proportion of participants showing the restored cartilage status as ICRS Macroscopic Cartilage Repair Assessment grade 1 or 2 was 65.1% in the UCB-MSC-HA group and 45.7% in the microfracture group (Table 2). The distribution of ICRS repair assessment grade for UCB-MSC-HA versus microfracture was significantly different ($P = .008$). Subgroup analyses according to age (<50, 50-59, and ≥60 years) demonstrated that the improvement in ICRS grade was affected by age in the microfracture group (100%, 61.9%, and 64.3%, respectively) but not in the UCB-MSC-HA group (100%, 95.5%, and 100%, respectively) (Table 2). Subgroup analyses according to size of cartilage defect (2.0 to 3.0, >3.0 to <6.0, and ≥6.0 cm[2]) also demonstrated that the efficacy of microfracture decreased as the lesion size increased (100%, 63.6%, and 44.4%, respectively), but that tendency was not

noticeable in the UCB-MSC-HA group (100%, 100%, and 93.3%, respectively) (Table 2).

Scores on the ICRS Macroscopic Cartilage Repair Assessment were significantly higher in the UCB-MSC-HA group, both overall and for every subcategory (degree of defect repair, integration to border zone, and macroscopic appearance) (Table 3). Histologic assessment according to the ICRS II Histological Evaluation System also revealed that the UCB-MSC-HA group had better histologic restoration in terms of the overall score, the subchondral bone assessment, and the mid-/deep zone assessment (Table 3). Both groups had significantly improved VAS pain, WOMAC, and IKDC scores at 48 weeks versus baseline ($P < .05$). No significant difference was seen between the 2 groups regarding these clinical parameters at 48 weeks (Figure 3).

### Observational Extended Follow-up Study of 60 Months

From 36 to 60 months after intervention, the significant improvements from baseline regarding VAS pain, WOMAC, and IKDC scores were maintained in the UCB-MSC-HA group, whereas the improvements in VAS pain and WOMAC deteriorated in the microfracture group (Figure 3). The VAS pain score was significantly better in the UCB-MSC-HA group compared with the microfracture group at the 60-month follow-up (Figure 3A). The WOMAC and IKDC scores were significantly better in the UCB-MSC-HA group than in the microfracture group at the 36- and 60-month follow-up (Figure 3, B and C). In addition, improvement in VAS pain, WOMAC, and IKDC scores at 36- and 60-month follow-up in the UCB-MSC-HA group was greater than the minimal clinically important difference (MCID; VAS 13.7,[14] IKDC 11.5,[16] and WOMAC 11.5[9]), but this was not the case in the microfracture group (Appendix Table A4).

By the 60-month follow-up, 2 total knee replacements and 1 osteotomy had been performed in the UCB-MSC-HA group, whereas 3 total knee replacements, 1 osteotomy, and 1 meniscectomy had been performed in the microfracture group ($P = .481$) (Table 4).

Safety

No significant differences were observed between the UCB-MSC-HA group and the microfracture group with regard to overall or specific treatment-emergent AEs in the initial 48-week clinical trial (Appendix Table A5) or the 60-month follow-up study (Appendix Table A6). No participant was withdrawn from the study because of AEs.

Three serious AEs (SAEs) occurred in 3 participants in the UCB-MSC-HA group, whereas 2 SAEs occurred in 1 participant in the microfracture group within the initial 48 weeks (Table 4). Surgical site pain in the UCB-MSC-HA group was the only SAE considered by the investigator as "probably related" to treatment (due to arthrotomy). In the 60-month follow-up, 8 SAEs occurred in 7 participants in the UCB-MSC-HA group and 7 SAEs in 5 participants in the microfracture group (Table 4). None of the SAEs were considered treatment related by the investigators. The
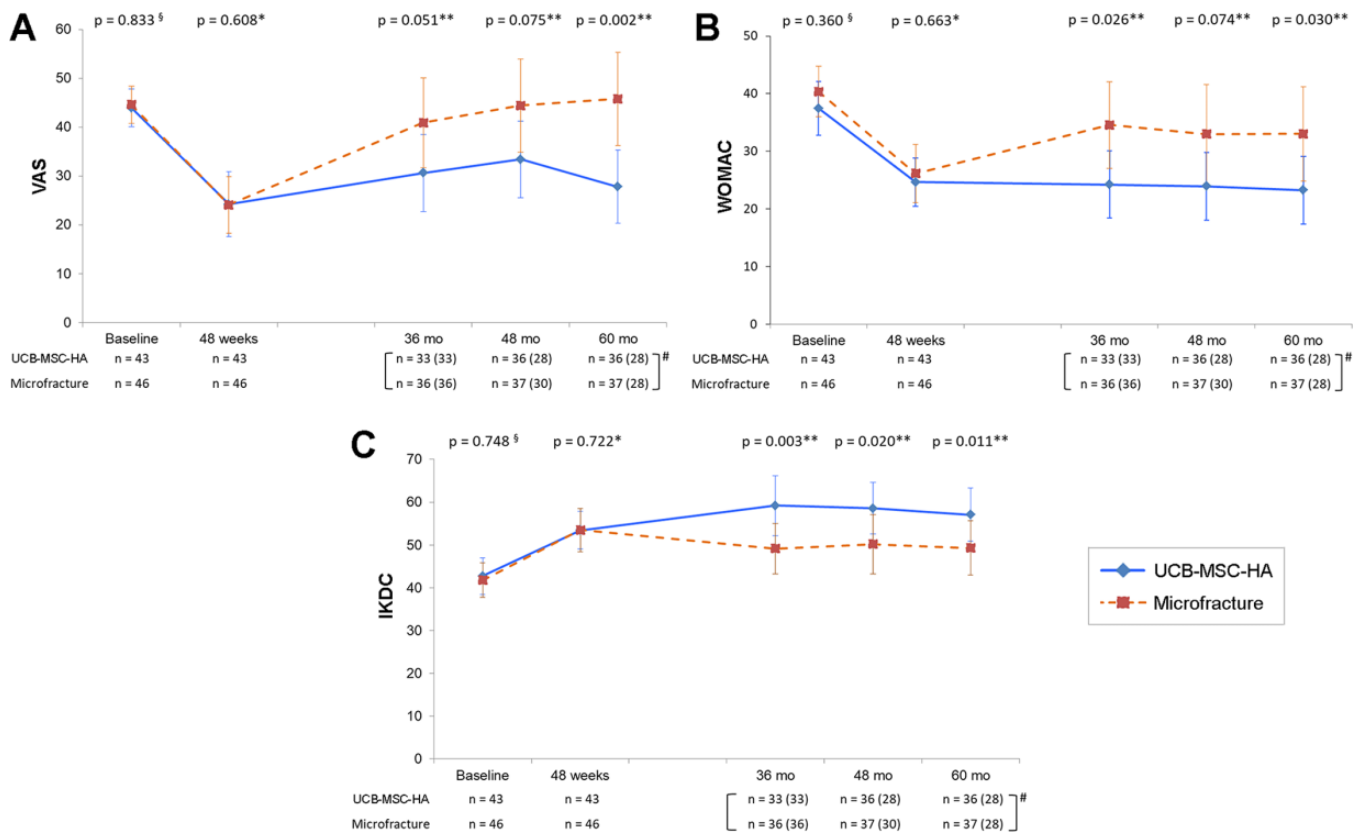
Figure 3. Clinical outcomes from baseline to 60-month follow-up in the umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate (UCB-MSC-HA) versus microfracture groups. Vertical bars indicate 95% CIs for the mean scores. (A) Visual analog scale (VAS) for pain; (B) Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC); and (C) International Knee Documentation Committee (IKDC) score. §2-sample *t* test. *Wilcoxon rank-sum test on score changes from baseline to the time point. **2-sample *t* test on score changes from baseline to the time point. #Sample sizes for follow-up study (36, 48, and 60 months) include missing data replacements using last observation carried forward (LOCF). The numbers in parentheses indicate the participants assessed at each time point.

patients who underwent total knee arthroplasty and high tibial osteotomy were considered to have required the procedure as a natural course of osteoarthritis and not because of implantation of the UCB-MSC-HA composite or microfracture. One death due to myocardial infarction (at 41 months postintervention) was reported in the UCB-MSC-HA group. No immunological reactions were observed in any of the 43 participants treated with UCB-MSC-HA, according to the mixed lymphocyte reaction test.

DISCUSSION

The results of the present study demonstrated that implantation of UCB-MSC-HA provides superior cartilage restoration compared with microfracture at 48 weeks after intervention, meeting the a priori primary endpoint in patients with symptomatic, large, full-thickness cartilage defects. The UCB-MSC-HA group demonstrated consistent improvement in ICRS grade of cartilage repair, even in the patients and those with larger lesions, whereas microfracture resulted in unreliable improvement in these groups. Of

note, baseline IKDC score and cartilage restoration with microfracture in this study were inferior to those of at least 1 other study,[40] likely due to older patient age (mean age, 56 vs 34 years) and inclusion of large, full-thickness lesions in osteoarthritic knees in the present study versus focal chondral defects treated in the other study. A reliable regenerative treatment option is not currently available for large cartilage defects in older patients with osteoarthritis, and our study results indicate that this novel application of UCB-MSC-HA may be applicable to these patients. UCB-MSC-HA may be an alternative treatment option to unicompartmental knee arthroplasty for those who desire joint preservation and the maintenance of an active lifestyle.

Despite the superior structural restoration after implantation of UCB-MSC-HA as assessed macroscopically and histologically, clinical outcomes such as pain and function at 48 weeks were not significantly superior to those experienced by patients who underwent microfracture. That both groups showed significant improvement in clinical outcomes at 48 weeks compared with baseline seemed to

TABLE 4
Serious Adverse Events of Phase 3 Clinical Trial (48 weeks)
and Extended Follow-up Study (60 months)[a]

Serious Adverse Events	UCB-MSC-HA	Microfracture
Phase 3 clinical trial	(n = 50)	(n = 53)
Hepatitis B	0 (0.0)	1 (1.9)
Implant site pain	1 (2.0)	0 (0.0)
Pneumonia	1 (2.0)	1 (1.9)
Renal cancer	1 (2.0)	0 (0.0)
Extended follow-up study	(n = 36)	(n = 37)
Acute myocardial infarction	1 (2.8)	0 (0.0)
Bone operation	1 (2.8)	0 (0.0)
Contusion	0 (0)	1 (2.7)
Femur fracture	1 (2.8)	0 (0.0)
Hemorrhoid operation	1 (2.8)	0 (0.0)
Implant site pain	0 (0)	1 (2.7)
Liver abscess	1 (2.8)	0 (0.0)
Meniscectomy (intervention knee)	0 (0.0)	1 (2.7)
Osteotomy (intervention knee)	1 (2.8)	1 (2.7)
Total knee arthroplasty (intervention knee)	2 (5.6)	3 (8.1)
Rotator cuff syndrome	0 (0)	1 (2.7)
Spinal laminectomy	0 (0.0)	1 (2.7)
Spondylolisthesis	0 (0.0)	1 (2.7)
Uterine leiomyoma	1 (2.8)	0 (0)
Wheezing	1 (2.8)	0 (0)

[a]Data are reported as n (%). UCB-MSC-HA, umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate.

justify our decision to use microfracture as an active control rather than placebo.

No reliable gold standard is available to be used as the active control in studies of large, full-thickness cartilage defects; however, the results of the current study support previous findings that microfracture can provide clinical benefit for this condition for at least 1 year. In this study, the microfracture group showed deterioration in clinical outcomes after 3 years, which is compatible with many previous reports.[21,27,28] In the UCB-MSC-HA group, the improvements in pain and knee function were maintained without significant deterioration until 5 years after implantation. However, improvements in IKDC score were relatively low, and VAS pain scores of 20 to 30 were found at final follow-up. Although it is difficult to pinpoint the reason for the relatively low clinical outcomes, the status of knee osteoarthritis in the older patients in this study population could have affected the results.

In this study, clinical outcome improvements were greater than the MCID only in the UCB-MSC-HA group. Moreover, most of the clinical outcomes that were significantly superior in the UCB-MSC-HA group occurred in the 3 to 5 years after the intervention. We believe the difference may stem from the better quality of the restored cartilage tissue in the UCB-MSC-HA group compared with the microfracture group, as demonstrated by histologic testing at 48 weeks.

To date, few cartilage repair methods have been clinically successful in large, full-thickness cartilage defects,

which are typical in patients with osteoarthritic knees.[6] In this regard, a novel regenerative strategy is needed for the treatment of such defects, especially in elderly patients. The UCB-MSC-HA in this study has the advantage of being available off-the-shelf and requiring only a single-stage procedure. The previous phase 1/2, single-arm study of UCB-MSC-HA implantation demonstrated that the improved clinical outcomes had not significantly deteriorated >7 years after treatment.[36] The results of the present study confirmed that cartilage restoration by UCB-MSC-HA leads to sustained clinical improvement until 5 years postintervention, which is encouraging for joint preservation. As osteoarthritic cartilage defects were present in about 90% of the study participants (see Table 1), we believe that UCB-MSC-HA is a viable treatment option for the regenerative treatment of large, full-thickness cartilage defects, even in osteoarthritic conditions.

Cartilage restoration using culture-expanded autologous MSCs has previously been attempted; however, the studies are limited to case reports or case series with small numbers of patients.[17,22,31,32,37,45] Two randomized clinical trials (RCTs) examined the efficacy of culture-expanded, autologous bone marrow–derived MSCs embedded in a collagen gel[45] or injected intra-articularly with hyaluronate[46] for the treatment of osteoarthritis. However, we believe that the clinical outcomes in those studies were confounded by concomitant high tibial osteotomy. In a double-blind RCT (N = 55) of intra-articular allogeneic, culture-expanded bone marrow MSCs versus hyaluronate after partial medial meniscectomy in patients with osteoarthritic changes, the authors reported no evidence of structural cartilage restoration at 1 year, which was the primary endpoint of the trial.[44] Our phase 3, 48-week study seems to be the first RCT of allogeneic, culture-expanded UCB-MSC implantation, and the results indicate cartilage restoration as well as improved pain and function until 5 years postintervention.

The rate of additional surgical intervention such as high tibial osteotomy and total knee arthroplasty was relatively low and was similar in both groups (6.0% in the UCB-MSC-HA group vs 7.6% in the microfracture group) during the extended follow-up period. Most of the study patients were middle-aged and physically active, and patients often consider knee replacement or osteotomy as a last resort. Therefore, we believe that only a few patients with severe deterioration during the extended follow-up period underwent replacement or osteotomy. Further long-term follow-up may reveal the need for surgical reintervention after treatment with UCB-MSC-HA composite compared with microfracture.

This study has certain limitations. First, this study was open-label to the patients and surgeons. However, it could not be double-blinded because arthrotomy was performed on the UCB-MSC-HA group and arthroscopy on the microfracture group. Sham surgery or microfracture using open arthrotomy could have been the best control group, but the regulatory authority and investigators agreed that such measures would have been unethical or too aggressive. We minimized bias by having blinded professionals assess the primary outcome (ICRS Macroscopic Cartilage Repair

Assessment score) as well as the secondary outcome (ICRS II Histological Evaluation System score). Second, microfracture is not generally considered the standard care option for the restoration of large, full-thickness cartilage defects, especially in elderly patients. However, a placebo control was not ethical or practical for our study patients, as discussed in the Methods. Implantation of HA only without MSCs was also considered as a control intervention. However, the results of previous animal studies showed no meaningful cartilage restoration with such a method.[7,38] A third limitation was that the participants received the intervention for only the femoral cartilage defect in the "most symptomatic" compartment. Thus, the effect of any untreated lesion in the intervention joint could not be determined. However, the results of the present study reveal the clinical benefit of treating only the most symptomatic lesion. Further research on the treatment of multiple lesions is warranted.

CONCLUSION

Implantation of UCB-MSC-HA resulted in improved cartilage grade at second-look arthroscopy at 48 weeks and provided more durable improvement of pain and function compared with microfracture in patients with symptomatic, large, full-thickness cartilage defects. UCB-MSC-HA appears to be a viable regenerative treatment option for large, full-thickness cartilage defects of the knee in older patients with osteoarthritis.

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APPENDIX TABLE A1
Inclusion and Exclusion Criteria for the Phase 3 (48-week) Study[a]

Inclusion Criteria	Exclusion Criteria
Knee joint cartilage defect of International Cartilage Repair Society grade 4, confirmed by arthroscopy (at screening, patients diagnosed from magnetic resonance image may be included)	Current or past history of autoimmune disease
	Infectious disease requiring antibiotics
Male or female patients aged 18 years or older	Current or past myocardial infarction, ischemic heart failure, other serious heart conditions, or uncontrolled hypertension
Lesion size of 2-9 cm^2 in 1 compartment (the compartment with the most prominent symptoms)	Serious medical comorbidity
Joint swelling, tenderness, and limitation of active range of motion of grade ≤ 2	Current pregnancy or nursing
	Current or past psychotic diseases or epilepsy
Pain ≤ 60 mm on a 100-mm visual analog scale in the affected joint	Alcohol abuse
Adequate blood coagulation activity: prothrombin time (international normalized ratio) <1.5, activated partial thromboplastin time $<1.5 \times$ control	Heavy smoking
	Chronic inflammatory articular disease such as rheumatoid arthritis
	Current enrollment in any other clinical trial
Adequate renal function: creatinine ≤ 2.0 mg/dL; proteinuria measured with dipstick of trace or less	Immunosuppressant use within the past 6 weeks
	Ligament instability of grade $\geq 2^a$ on physical examination
Adequate hepatic function: bilirubin ≤ 2.0 mg/dL; aspartate aminotransferase/alanine aminotransferase ≤ 100 IU/L	Known history of hypersensitivity or allergy to gentamicin
No surgery or radiation therapy in the affected joint within the past 6 weeks, and recovery from past treatments	Any other condition for which the principal investigator considers the patient inappropriate for participation in the trial (such as Kellgren-Lawrence grade 4 osteoarthritis, significant deformity, or previous osteotomy)
For women of childbearing potential, agreement to practice adequate birth control during the study	
Ligament instability grade $<2^a$ on physical examination	
Agreement to participate and signed informed consent	

[a]Ligamentous instability scale grades: grade 0, none; grade 1, 0-5 mm; grade 2, 5-10 mm; grade 3, >10 mm.
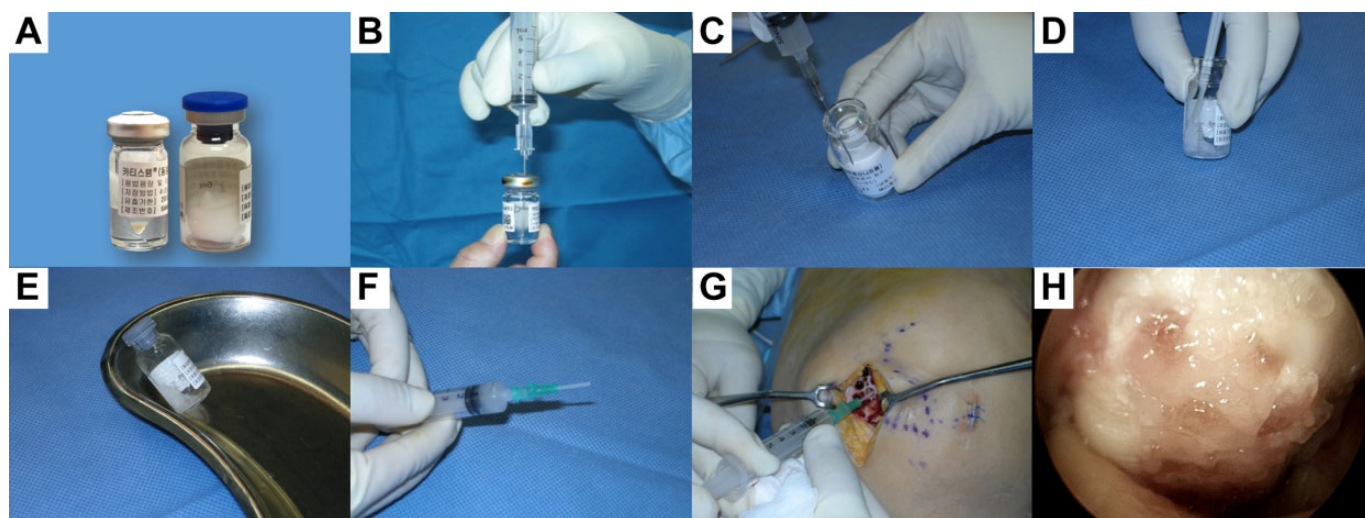
Appendix Figure A1. Preparation of umbilical cord blood–derived mesenchymal stem cell and 4% hyaluronate (UCB-MSC-HA) composite. (A) UCB-MSC and the HA hydrogel were transported in a portable refrigerator to the hospital on the day of surgery. (B) Cells were aspirated from the cell-containing vial before mixture. (C) Aspirated cells were transferred to the bottle containing the HA sponge, and (D) the cells and HA sponge were mixed gently. (E) Prepared UCB-MSC-HA composite formed a gel. (F) UCB-MSC-HA composite was transferred into a syringe for implantation. (G) UCB-MSC-HA composite was implanted into the drill holes. (H) The hydrogel of UCB-MSC-HA composite could be kept in the drill holes without additional paste material. Bone bleeding within the drill holes could permeate into the hydrogel that formed clots intermingled with the hydrogel.

APPENDIX TABLE A2
ICRS Macroscopic Cartilage Repair Assessment[a]

Cartilage Repair Assessment	Points
Degree of defect repair	
Level with surrounding cartilage	4
75% repair of defect depth	3
50% repair of defect depth	2
25% repair of defect depth	1
0% repair of defect depth	0
Integration to border zone	
Complete integration with surrounding cartilage	4
Demarcating border <1 mm	3
3/4 of graft integrated, 1/4 with a border >1 mm	2
1/2 of graft integrated with surrounding cartilage, 1/2 with a border >1 mm	1
From no contact to 1/4 of graft integrated with surrounding cartilage	0
Macroscopic appearance	
Intact, smooth surface	4
Fibrillated surface	3
Small, scattered fissures or cracks	2
Several small fissures or few but large fissures	1
Total degeneration of grafted area	0
Overall repair assessment	
Grade 1: normal	12
Grade 2: nearly normal	11-8
Grade 3: abnormal	7-4
Grade 4: severely abnormal	3-1

[a]ICRS, International Cartilage Repair Society.

APPENDIX TABLE A3
Baseline Characteristics of Participants Screened and Allocated for the 60-Month Follow-up Study[a]

	UCB-MSC-HA (n = 38)	Microfracture (n = 39)	P Value
Age, y	59.0 ± 7.7	59.0 ± 9.7	.970[b]
Sex			.935[c]
Male	13 (34.2)	13 (33.3)	
Female	25 (65.8)	26 (66.7)	
Body mass index, kg/m^2	26.3 ± 3.0	26.8 ± 3.5	.523[b]
Time since treatment, mo	38.3 ± 2.6	37.6 ± 2.6	.256[b]
Cartilage defect size, cm^2	4.6 ± 1.8	4.2 ± 1.7	.297[d]

[a]Data are reported as mean ± SD or n (%). UCB-MSC-HA, umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate.)
[b]2-sample t test.
[c]Pearson chi-square test.
[d]Wilcoxon rank-sum test.

APPENDIX TABLE A4
Details of Clinical Outcomes During Follow-up[a]

(A) Phase 3 study (48 weeks)

Variable	UCB-MSC (n = 43)	Microfracture (n = 46)	P Value
Pain on 100-mm VAS[b]	24.2 (17.5 to 31.0)	24.1 (18.3 to 29.9)	
Improvement, baseline to 48 wk	−19.7 (−11.6 to −27.9)	−20.5 (−14.6 to −26.3)	.887
WOMAC score[c]	24.7 (20.5 to 28.9)	26.2 (21.1 to 31.2)	
Improvement, baseline to 48 wk	−12.8 (−8.3 to −17.2)	−14.2 (−9.3 to −19.1)	.661
IKDC score	53.4 (49.0 to 57.8)	53.5 (48.5 to 58.5)	
Improvement, baseline to 48 wk	10.7 (7.0 to 14.4)	11.7 (7.6 to 15.7)	.720

(B) Follow-up study (36, 48, and 60 months)

Variable	UCB-MSC (n = 36)	Microfracture (n = 37)	P Value
Pain on 100-mm VAS			
36 mo	30.9 (23.6 to 38.2)	41.1 (32.2 to 50.0)	
Improvement, baseline to 36 mo	−14.6 (−22.3 to −6.9)	−3.6 (−12.6 to 5.4)	.064
48 mo	35.7 (29.2 to 42.3)	43.3 (34.7 to 51,8)	
Improvement, baseline to 48 mo	−9.8 (−17.1 to −2.5)	−1.4 (−10.1 to 7.2)	.140
60 mo	29.1 (22.4 to 35.8)	43.5 (35.3 to 51.6)	
Improvement, baseline to 60 mo	−16.4 (−23.5 to −9.2)	−1.2 (−8.1 to 5.6)	.003
WOMAC score			
36 mo	25.4 (19.9 to 31.0)	34.5 (27.2 to 41.8)	
Improvement, baseline to 36 mo	−14.7 (−20.3 to −9.2)	−5.4 (−12.4 to 1.6)	.036
48 mo	28.6 (22.4 to 34.9)	35.8 (27.6 to 44.1)	
Improvement, baseline to 48 mo	−11.5 (−17.3 to −5.7)	−4.1 (−11.6 to 3.4)	.119
60 mo	26.9 (20.4 to 33.5)	36.2 (28.6 to 43.8)	
Improvement, baseline to 60 mo	−13.2 (−18.7 to −7.8)	−3.8 (−9.7 to 2.2)	.020
IKDC score			
36 mo	57.4 (50.8 to 64.1)	49.0 (43.3 to 54.7)	
Improvement, baseline to 36 mo	17.3 (11.3 to 23.4)	7.0 (2.7 to 11.2)	.006
48 mo	53.7 (48.2 to 59.3)	48.9 (42.1 to 55.7)	
Improvement, baseline to 48 mo	13.6 (8.3 to 19.0)	6.8 (0.6 to 13.1)	.098
60 mo	54.7 (48.7 to 60.7)	47.1 (41.1 to 53.2)	
Improvement, baseline to 60 mo	14.6 (9.4 to 19.7)	5.1 (0.5 to 9.6)	.007

[a]Data are reported as mean (95% CI). IKDC, International Knee Documentation Committee; UCB-MSC-HA, umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

APPENDIX TABLE A5
Most Frequently Reported (≥5%) Treatment-Emergent
Adverse Events From the Phase 3 Study (48 weeks)[a]

Treatment-Emergent Adverse Event	UCB-MSC-HA (n = 50)	Microfracture (n = 53)
Nausea	15 (30.0)	12 (22.6)
Constipation	11 (22.0)	8 (15.1)
Headache	7 (14.0)	4 (7.6)
Dyspepsia	6 (12.0)	4 (7.6)
Dysuria	5 (10.0)	7 (13.2)
Pruritus	5 (10.0)	3 (5.7)
Implant site pruritus	5 (10.0)	1 (1.9)
Vomiting	4 (8.0)	1 (1.9)
Insomnia	4 (8.0)	1 (1.9)
Nasopharyngitis	3 (6.0)	4 (7.6)
Abdominal discomfort	3 (6.0)	2 (3.8)
Crepitations	3 (6.0)	2 (3.8)
Arthralgia	3 (6.0)	2 (3.8)
Diarrhea	3 (6.0)	1 (1.9)
Hypoesthesia	3 (6.0)	1 (1.9)
Micturition disorder	3 (6.0)	0 (0)
Dizziness	2 (4.0)	6 (11.3)
Cough	1 (2.0)	6 (11.3)
Hypertension	0 (0)	4 (7.6)

[a]Data are reported as n (%). UCB-MSC-HA, umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate.

APPENDIX TABLE A6
Most Frequently Reported (≥5%) Treatment-Emergent
Adverse Events From the Follow-up Study (60 months)[a]

Treatment-Emergent Adverse Event	UCB-MSC-HA (n = 36)	Microfracture (n = 37)
Back pain	2 (5.6)	2 (5.4)
Toothache	2 (5.6)	1 (2.7)
Upper respiratory tract infection	2 (5.6)	1 (2.7)
Urinary tract infection	2 (5.6)	0 (0)
Nausea	2 (5.6)	0 (0)
Vomiting	2 (5.6)	0 (0)
Headache	2 (5.6)	0 (0)
Dysuria	2 (5.6)	0 (0)
Hematuria	2 (5.6)	0 (0)
Stress urinary incontinence	2 (5.6)	0 (0)
Allergic rhinitis	2 (5.6)	0 (0)
Arthralgia	1 (2.8)	3 (8.1)
Arthritis	0 (0)	2 (5.4)
Arthropathy	0 (0)	2 (5.4)
Hemorrhoids	0 (0)	2 (5.4)
Meniscal lesion	0 (0)	2 (5.4)
Blood glucose increase	0 (0)	2 (5.4)

[a]Data are reported as n (%). UCB-MSC-HA, umbilical cord blood–derived mesenchymal stem cells and 4% hyaluronate.