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STEM CELLS

TRANSLATIONAL AND CLINICAL RESEARCH

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

ABSTRACT

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

INTRODUCTION

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

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

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

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

Materials and Methods

Study Design and Patients

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

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

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

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

MSC Preparation

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

Arthroscopy and Stem Cell Injection

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

Outcome Measures

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

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

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

Statistical Analysis

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

Results

Demographics of Patients

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

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

Safety

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

Clinical Outcomes

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

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

Radiological Outcomes

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

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

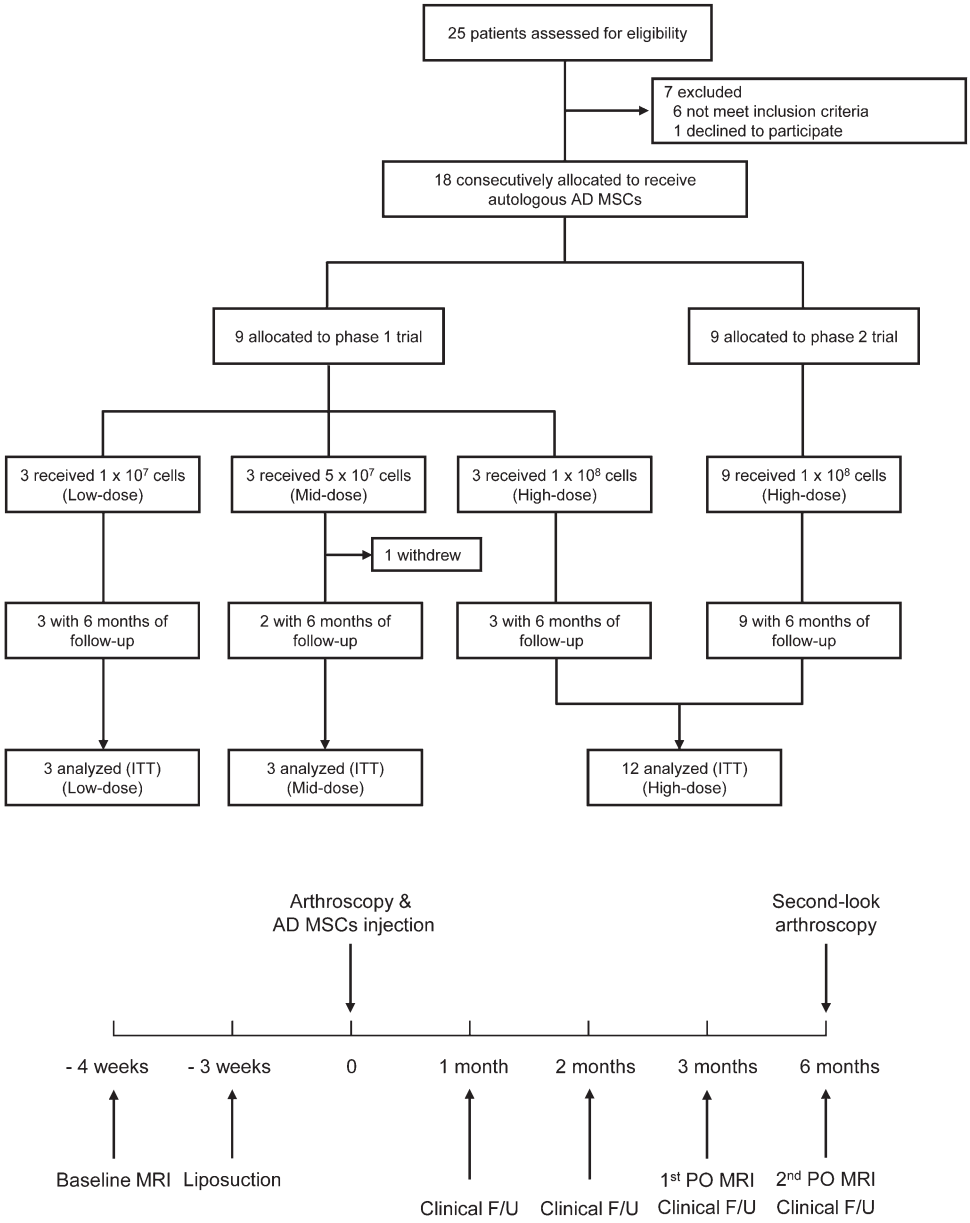

Figure 1. Study flow diagram. Abbreviations: AD MSCs, adipose-tissue derived mesenchymal stem cells; ITT, intention-to-treat; MRI, magnetic resonance imaging.

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

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

Second-Look Arthroscopy

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

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

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

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

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

Table 2. Summary of adverse events

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

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

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

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

Histological Outcomes

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

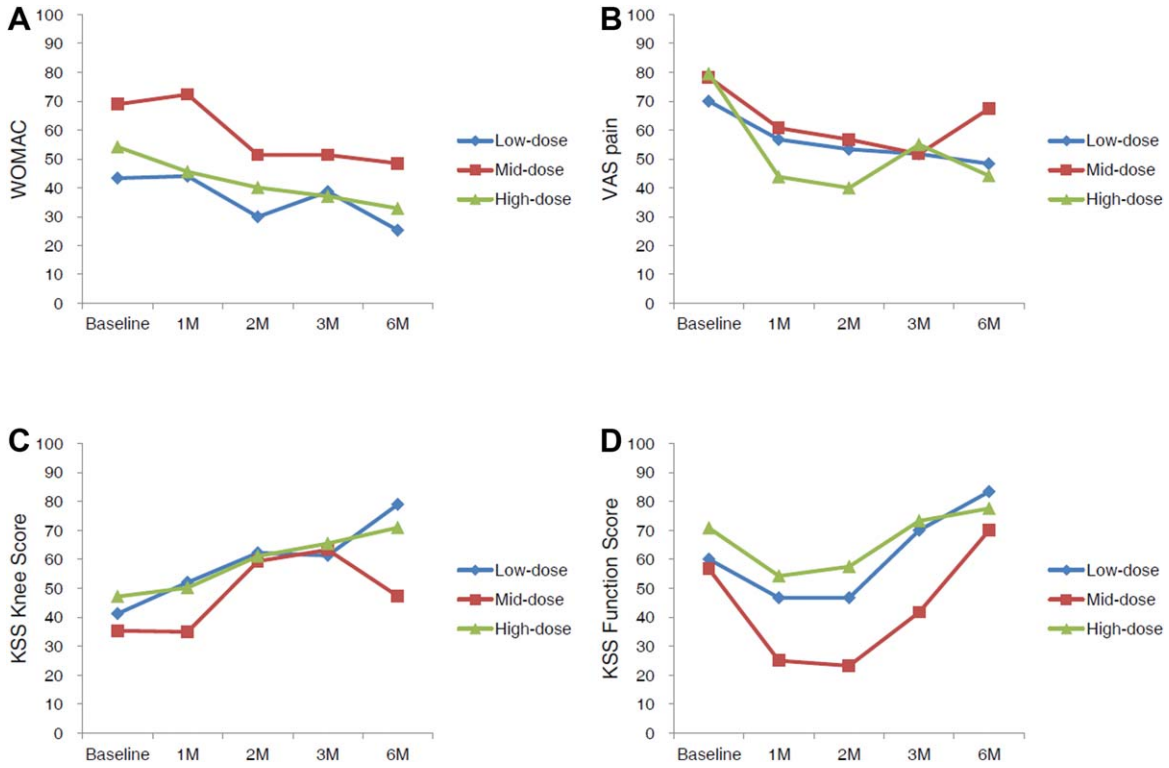

Figure 2. Changes of WOMAC, VAS for knee pain, and KSS knee and function score during 6 months after intra-articular injection of adipose derived mesenchymal stem cell (AD MSCs). **(A):** The WOMAC score. It showed a tendency of improvement in all dose groups over 6 months. However, the statistical significance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

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

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

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

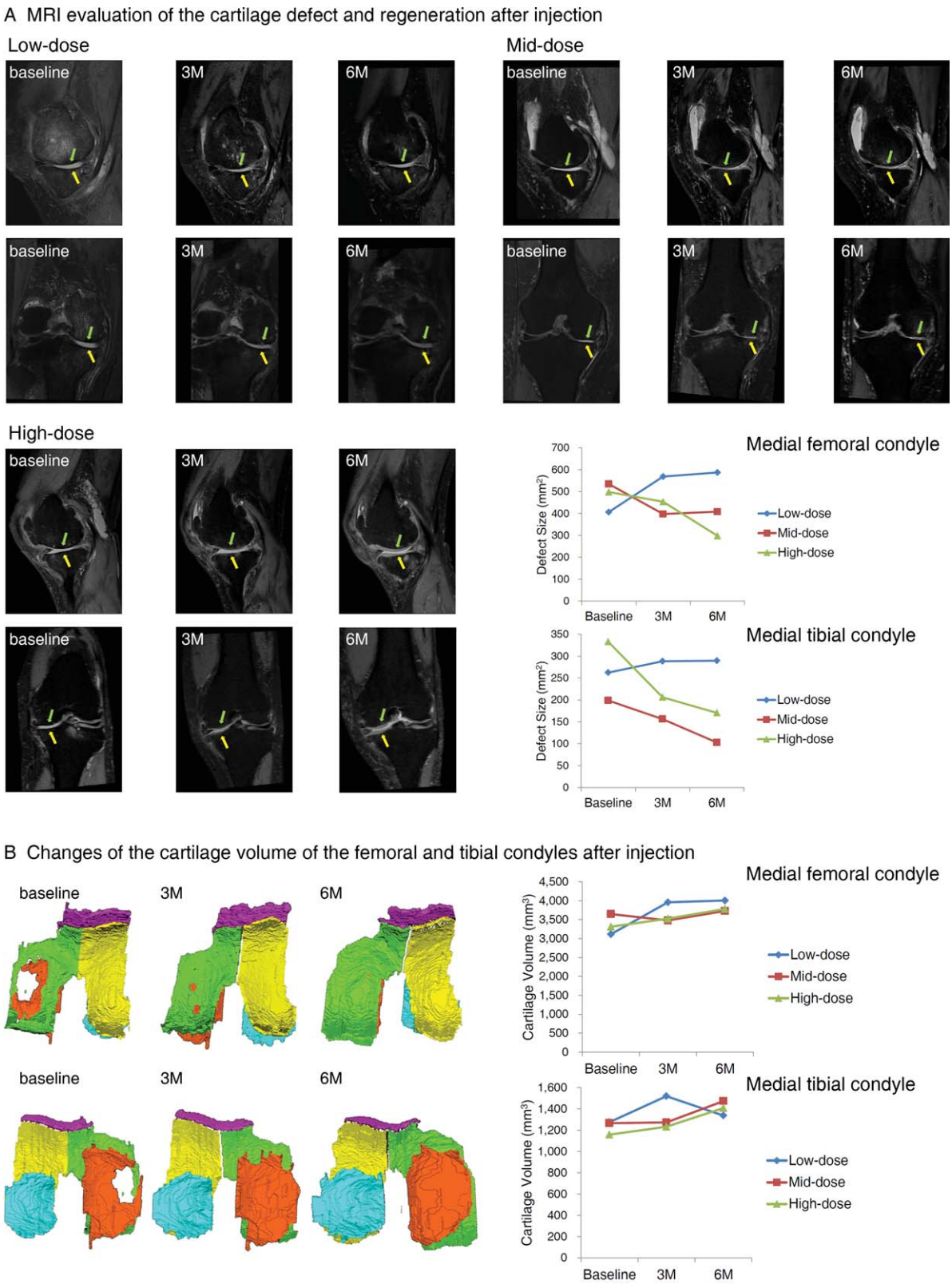

Figure 3.

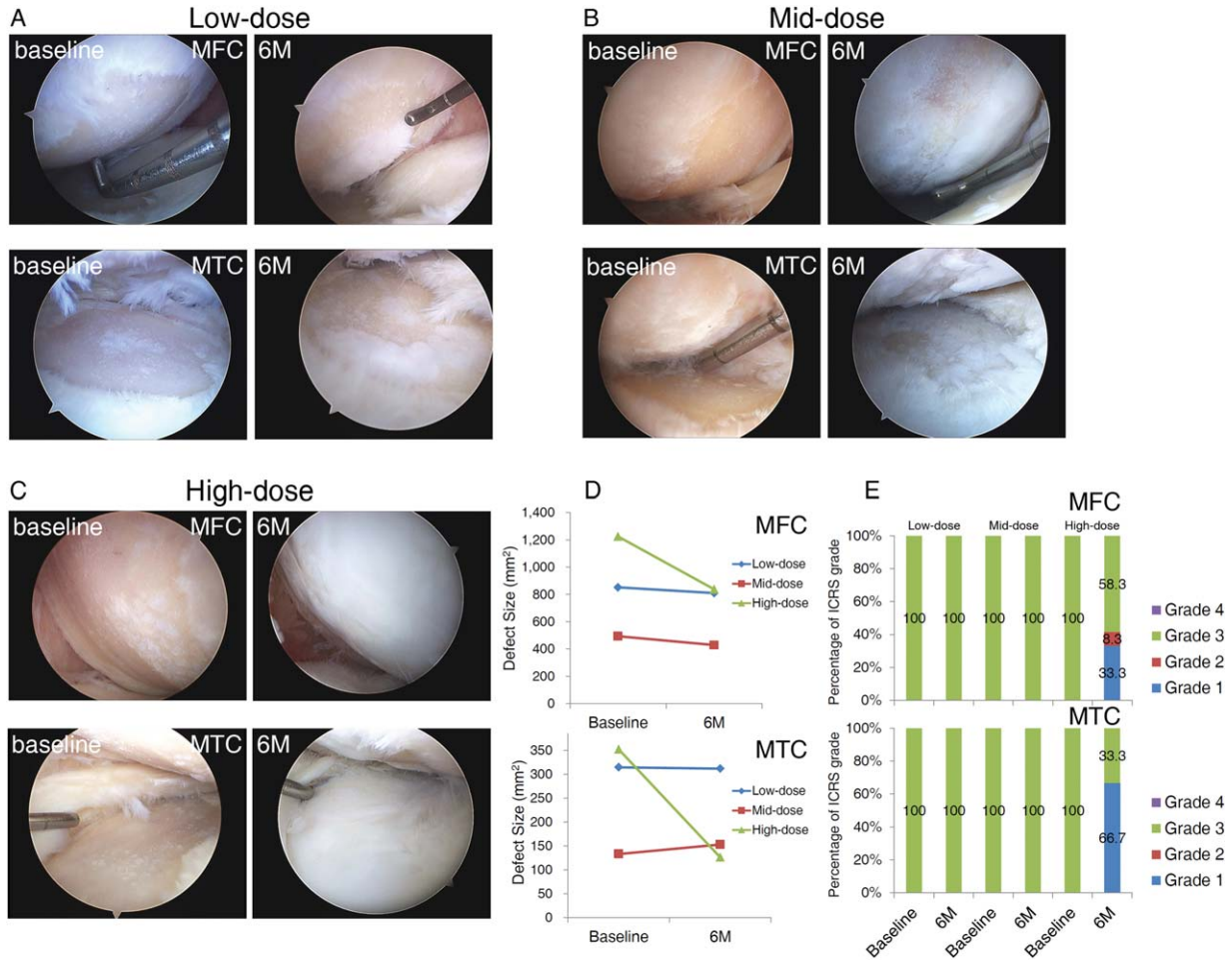

Figure 4. Arthroscopic evaluation of articular cartilage regeneration in the medial and femoral condyles after intra-articular injection of adipose derived mesenchymal stem cells (AD MSCs). **(A):** Arthroscopic finding shows large denuded medial femoral and tibial condyles (international cartilage repair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

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

Discussion

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

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

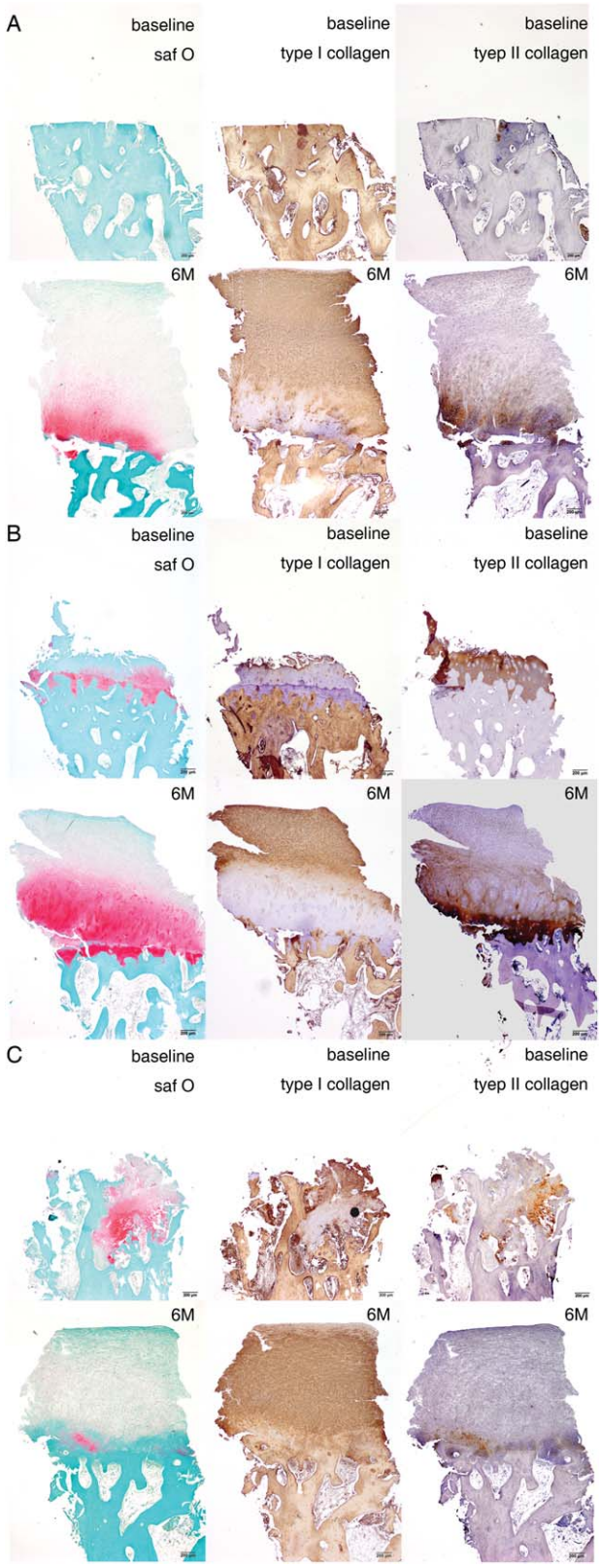

Figure 5. Histological evaluation of regenerated articular cartilage of biopsy from the medial femoral condyle after intra-articular injection of adipose derived mesenchymal stem cells (AD MSCs). **(A):** A typical biopsy sample from the medial femoral condyle of a patient with international cartilage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

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

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

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

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

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

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

Conclusions

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

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Author Contributions

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

Disclosure of Potential Conflicts of Interest

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

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