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Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis

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Abstract
Purpose In the present study, the clinical outcomes and second-look arthroscopic findings of intra-articular injection of stem cells with arthroscopic lavage for treatment of elderly patients with knee osteoarthritis (OA) were evaluated.
Methods Stem cell injections combined with arthroscopic lavage were administered to 30 elderly patients (≥65 years) with knee OA. Subcutaneous adipose tissue was harvested from both buttocks by liposuction. After stromal vascular fractions were isolated, a mean of 4.04×10^6 stem cells (9.7 % of 4.16×10^7 stromal vascular fraction cells) were prepared and injected in the selected knees of patients after arthroscopic lavage. Outcome measures included the Knee Injury and Osteoarthritis Outcome Scores, visual analog scale, and Lysholm score at preoperative and 3-, 12-, and 2-year follow-up visits. Sixteen patients underwent second-look arthroscopy.
Results Almost all patients showed significant improvement in all clinical outcomes at the final follow-up examination. All clinical results significantly improved at 2-year follow-up compared to 12-month follow-up ($P < 0.05$). Among elderly patients aged >65 years, only five patients demonstrated worsening of Kellgren–Lawrence grade. On second-look arthroscopy, 87.5 % of elderly patients (14/16) improved or maintained cartilage status at least 2 years postoperatively. Moreover, none of the patients underwent total knee arthroplasty during this 2-year period.
Conclusion Adipose-derived stem cell therapy for elderly patients with knee OA was effective in cartilage healing, reducing pain, and improving function. Therefore, adipose-derived stem cell treatment appears to be a good option for OA treatment in elderly patients.
Level of evidence Therapeutic case series study, Level IV.

Keywords Mesenchymal stem cell · Arthroscopic lavage · Knee osteoarthritis

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Introduction

Osteoarthritis (OA) is the most common musculoskeletal disorder [3]. Synovial inflammation, in particular, can affect joint homoeostasis [5] and is associated with pain and OA disease progression [31]. The current treatments for OA are not regenerative and have little impact on the progressive degeneration of joint tissues. Clinical interventions are primarily symptomatic and focus on pain reduction and inflammation control through nonsteroidal anti-inflammatory drugs and ultimately with total joint replacement [4]. Few options are currently available for elderly patients with moderate to severe arthritis. Most approaches are palliative and address symptoms rather than influencing the biochemical environment of the joint or disease process.

Because of their multilineage potential, immunosuppressive activity, limited immunogenicity, and relative ease of growth in culture, mesenchymal stem cells (MSCs) are an attractive option for clinical use. Therefore, MSCs have been suggested for use in the cell-based treatment of cartilage lesions. In our previous study, 25 patients affected by a knee degenerative condition were treated with infrapatellar

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fat pad-derived MSC therapy by intra-articular injections and assessed at a 16.3-month follow-up [17]. The results indicated that this procedure was safe and helps in reducing pain and improving function in patients with knee OA. In a subsequent study involving the use of stem cell therapy [18], we obtained good clinical and radiological results at 2 years of follow-up. However, changes in clinical and MRI scores were positively related to the number of cells injected, indicating that treatment efficacy improved with an increase in the number of cells injected. Therefore, in the present study, we used buttock subcutaneous fat tissue as the source for stem cells because a sufficient quantity of adipose tissue was available in this region. Moreover, there are wide variations in the amount of fat in the infrapatellar fat pad, but we were able to obtain a consistent volume of buttock subcutaneous fat tissue in all patients. Therefore, we obtained fat from the gluteus region rather than the infrapatellar fat pad in the present study.

Intra-articular insertion of adipose tissue-derived stem cells with arthroscopic lavage was believed to improve articular cartilage status and decrease pain for a long period in elderly patients with knee OA. Thus, in the present study, the potential treatment of OA symptoms with MSCs and arthroscopic lavage was evaluated using clinical results and second-look arthroscopic findings.

Materials and methods

Between November 2010 and January 2011, 30 stem cell injections combined with arthroscopic lavage were administered to elderly patients (≥65 years) with knee OA. Thirty patients [5 men and 25 women; mean age, 70.3 (range 65–80) years], in whom previous nonsurgical treatment (undergoing nonoperative management options such as physical therapy and nonsteroidal anti-inflammatory drugs, for a minimum of 3 months) had failed and who had refused to undergo prosthetic replacement, underwent stem cell therapy as a salvage procedure, which was performed one senior surgeon (Y.G.K.) at the authors' institute. Eligibility requirements were age ≥65 years and diagnosis of idiopathic or secondary knee OA [Kellgren–Lawrence [12] (K–L) grade 2 or 3 OA in multiple compartments, including the medial or lateral tibiofemoral joint compartments or the patellofemoral compartment]. Patients were excluded if they met at least one of the following criteria: diagnosis with K–L [12] grade 4 OA or inflammatory or postinfectious arthritis, previous arthroscopic treatment for knee OA, previous major knee trauma, intra-articular hyaluronic acid or corticosteroid injection in the preceding 3 months, mechanical pain caused by meniscal tears (including flap tears, bucket-handle tears, and complex tears), and inability to provide informed consent. Three doctors, who were blinded to the grading results of the other examiners, performed K–L grading in all the patients. The knee joint is typically evaluated using an extended knee radiograph, which is a bilateral anteroposterior image acquired while the patient is in a weight-bearing condition, with both the knees completely extended.

Collection of subcutaneous adipose tissue

Subcutaneous adipose tissues were harvested from the patients' buttocks by liposuction, as described previously [13, 15]. One day before the arthroscopic surgery, we harvested the adipose tissue through liposuction using a tumescent solution. The patient was placed in the prone position under intravenous sedation. After surgical preparation, a hollow blunt-tipped cannula was introduced into the subcutaneous space through a small incision, and subcutaneous adipose tissue was infiltrated with a tumescent solution to minimize blood loss and tissue contamination by peripheral blood cells prior to aspiration, which consisted of 0.9 % saline solution (500 mL) supplemented with 2 % lidocaine (10 mL), 8.4 % sodium hydrogen carbonate (4 mL), and 0.1 % epinephrine (0.7 mL). The liposuction material was aspirated by gentle suction. We aimed to routinely collect 140 cc of liposuctioned adipose tissue, of which 120 cc was used for the injection, and 20 cc was subjected to laboratory analysis to examine the plastic-adherent cells that form colony forming units-fibroblast (CFU-F) and confirm the multilineage differentiation of adipose-derived stem cells.

Isolation of stromal vascular fraction and MSCs from subcutaneous adipose tissue

In the operating room, adipose tissue (120 cc) was suspended in phosphate-buffered saline (PBS), placed in a sterile box, and transported to a laboratory. Mature adipocytes and connective tissues were separated from the stromal vascular fraction by centrifugation, as reported by Zuk et al. [35]. The volume of the stromal vascular fraction is usually less than 0.1 cc. Prior to insertion, bacteriologic tests were performed to ensure the absence of contamination in the samples, and the viability of cells was assessed using the methylene blue dye exclusion test. The remaining 20 cc of adipose tissue was processed by the same method and used for cell analysis.

Assessment of plastic-adherent cells that form CFU-F and immunophenotyping of adipose-derived stem cells

To evaluate the frequency of mesenchymal-like progenitors in patient stromal vascular fraction, cells were cultured

in T-25 flasks at a final concentration of 16 cells/cm^2. Colonies consisting of ≥50-cell aggregates were scored under an optical microscope, and the immunophenotype of adipose-derived stem cells was analysed by flow cytometry (FACS). MSC marker phenotyping was performed as previously described [20].

Confirmation of multilineage differentiation of adipose-derived stem cells

Adipose-derived stem cells were plated at 2×10^3 cells/cm^2 in DMEM containing 10 % FBS and allowed to adhere for 24 h. The culture medium was then replaced with specific inductive media to determine the adipogenic, osteogenic, and chondrogenic differentiation potential, as previously reported [20].

Arthroscopic lavage and implantation of MSCs

Patients received arthroscopic lavage under spinal anaesthesia with the use of a tourniquet. The orthopaedic surgeon evaluated the medial, lateral, and patellofemoral joint compartments; graded the articular lesions according to the International Cartilage Repair Society (ICRS) Cartilage Injury Evaluation Package; and irrigated the compartment with at least 1 L of saline. While performing arthroscopic lavage on the 30 patients, we noted that the cartilage status in the medial compartment was grade II in two patients, grade III in 15 patients, and grade IV in 13 patients. Further, the cartilage status in the lateral compartment was grade II in ten patients, grade III in 11 patients, and grade IV in nine patients. In addition, the cartilage status in the patellofemoral compartment was grade II in eight patients, grade III in 14 patients, and grade IV in eight patients. The following treatments were not performed: synovectomy; excision of degenerative tears of the menisci or osteophytes that prevented full extension, and abrasion or microfracture of chondral defects. Because we excluded patients who experienced mechanical pain caused by a meniscal tear, only 16 patients exhibiting degenerative meniscal tears that did not cause mechanical knee pain were included in the study. After arthroscopic lavage, a mean of 4.2×10^7 stromal vascular fraction cells were prepared with approximately 3.0 mL of platelet-rich plasma (PRP). The stromal vascular fraction cells were injected into the most severe cartilage defect area in the selected knees of patients under arthroscopic guidance. Immediately after arthroscopic lavage, the affected knee was placed in a cylinder splint for 24 h. No analgesics, anti-inflammatory drugs, or immunosuppressive drugs were administered or permitted after the procedure for 3 months.

For PRP preparation, a 30-mL venous blood sample (collected in a bag containing 4 mL of sodium citrate) was collected for every lesion that was treated. The complete peripheral blood count was determined using the first blood sample collected. Thereafter, the samples were centrifuged twice (at 1,800 rpm for 15 min to separate the erythrocytes, and then at 3,500 rpm for 10 min to concentrate the platelets) to yield 6 mL of PRP. The total number of platelets per microlitre in the PRP was a mean of 500 % times greater than that in the whole blood, and an average of 1,280,000/μL platelets were administered at the lesion sites during every injection. Prior to injection in all cases, calcium chloride was added to the PRP unit to activate the platelets. All the procedures were performed in the same laboratory setting, and all open procedures were performed in an A-class sterile hood.

Clinical assessment

Clinical outcome was evaluated using the Lysholm score [16], the Knee injury and Osteoarthritis Outcome Score (KOOS) [30], and visual analog pain score (VAS) on a 10-point scale (0–10) for pain (0 = no pain; 10 = worst possible pain). Patients were evaluated preoperatively as well as postoperatively at 3-, 12-month, and 2-year follow-up visits. At the 2-year follow-up, patients also completed a questionnaire intended to assess their satisfaction with the treatment. Radiographic evaluation included the standing weight-bearing anteroposterior view, lateral view, skyline view, and full-length anteroposterior view.

Second-look arthroscopy

Among the 30 patients who received stem cell therapy, 16 underwent second-look arthroscopy by one surgeon at our hospital. The indications for second-look arthroscopy were as follows: (1) asymptomatic patients, to evaluate the healing status of degenerative cartilage, and (2) patients who complained of knee pain at follow-up. The healing status of degenerative cartilage was classified as very positive, positive, neutral, or negative in the most severe cartilage defect area of the knee. We noted the presence of severe cartilage lesions at the medial compartment in 9, at the lateral compartment in 4, and at the patello-femoral compartment in three patients. "Very positive" was considered when a remarkable change was noted throughout the degenerative cartilage with good integration to adjacent normal articular surface and normal gross appearance. "Positive" was considered when newly forming cartilage tissue was found to partially cover the degenerative cartilage compared to that noted preoperatively. "Neutral" was considered when an uncertain change was noted over 2 years compared to the preoperative status. "Negative" was considered when progression of degenerative cartilage was noted compared to preoperative status. The

examinations were performed during second-look arthroscopy by all members of the surgical team (Y.G.K., S.K.K., and Y.J.C.). The observation was confirmed only once a consensus was reached among all the three surgeons.

This study was approved by the Research Ethics Board of Yonsei Sarang Hospital (registration number 10-R03-05), and written informed consent was obtained from all participants.

Statistical analysis

Statistical analysis was performed using SPSS software version 12.0.1 (SPSS Inc., Chicago, Illinois), with significance defined as $P < 0.05$. Descriptive statistics were calculated as mean ± standard deviation. The normality of distribution was checked using the Shapiro–Wilk test. Our data followed normal distribution because the probability of the Shapiro–Wilk test was $P > 0.05$ and the number of patients was 30. The principal dependent variables of clinical outcomes were KOOS, Lysholm score, and VAS at the last follow-up. The paired *t* test was conducted to evaluate changes in preoperative and serial follow-up values. We analysed the association of factors—patient characteristics and radiological grade of OA—with clinical outcomes. Mean values were used as standard values for dividing patients according to age and K–L grade. Differences between groups were analysed using the independent *t*-test.

Results

Cell isolation and characterization of adipose-derived stem cells

We evaluated the capacity of human subcutaneous adipose tissue to generate mesenchymal progenitors using the CFU-F. Thus, after isolation, adipose-derived stem cells represented a mean of 9.7 % of stromal vascular fraction cells (range 6.8–12.4 % of stromal vascular fraction cells). After the stromal vascular fractions were isolated, a mean of 4.0×10^6 stem cells (9.7 % of 4.2×10^7 stromal vascular fraction cells) were prepared. FACS characterization indicated positive expression of the surface markers CD90 (99.8 %) and CD105 (88.9 %) and negative expression of CD34 (12.0 %) and CD14 (1.2 %), as shown previously (Fig. 1a) [35]. Adipose-derived stem cells treated with conditioned media demonstrated characteristics of adipogenic, osteogenic, and chondrogenic differentiation after staining (Fig. 1b).

Clinical outcomes at follow-up

The mean Lysholm score significantly increased from 54.3 ± 15.4 to 74.2 ± 13.4 ($P < 0.05$). The mean VAS decreased from 4.7 ± 1.6 preoperatively to 1.7 ± 1.4 at 2-year follow-up ($P < 0.05$). The median KOOS from preoperative to 2-year follow-up assessments is summarized in Fig. 2. Moreover, all clinical results significantly improved at 2-year follow-up compared to those at 1-year follow-up ($P < 0.05$). With regard to overall patient satisfaction with the operation, 16 patients reported their satisfaction as excellent (53 %), 7 as good (23 %), 4 as fair (13 %), and 3 as poor (10 %). At 2-year follow-up, the K–L grade in five patients increased by one grade. The K–L grade in two patients increased from grade 2 to 3, whereas the K–L grade in three patients increased from grade 3 to 4. However, no patients underwent a second operation such as total knee arthroplasty. No major complications associated with arthroscopic lavage and liposuction, either intraoperatively or postoperatively, were observed in this series. In three patients, slight knee pain was experienced in the first week after the stem cell injection, which resolved spontaneously in two patients in 1 week with no medication and resolved after 2 weeks in the other patient with anti-inflammatory drug medication.

Associations between patient characteristics and outcomes

A statistically significant association was observed between patients' age and mean improvement from baseline in all KOOS subscales to 2-year follow-up ($P < 0.05$; Fig. 3), and a statistically significant association was observed between K–L grade 2 and higher Lysholm score improvement ($P = 0.002$; Table 1). No other parameters showed a statistically significant association.

Second-look arthroscopy

At a minimum follow-up of 24 months (median 25.0 months; range 24–26 months), 16 patients treated with MSC therapy underwent second-look arthroscopy, including 12 who were asymptomatic to evaluate the cartilage status, and 4 subsequent symptomatic patients with recurrent knee joint pain to plan further treatment. We explained the purpose of second-look arthroscopy to patients before surgery and received written consent. On second-look arthroscopy, 3 knees (all were asymptomatic) were rated "very positive" and 7 were rated "positive" (1 was symptomatic and 6 were asymptomatic). Four knees were rated "neutral" (2 each were symptomatic and asymptomatic), and the other 2 patients experienced failed healing (1 each was symptomatic and asymptomatic; Table 2). The differences between parameters of four groups were not significant.

The findings of a 67-year old woman during the first and second arthroscopy procedure showed marked changes in cartilage defects of the medial femoral condyle (Fig. 4).

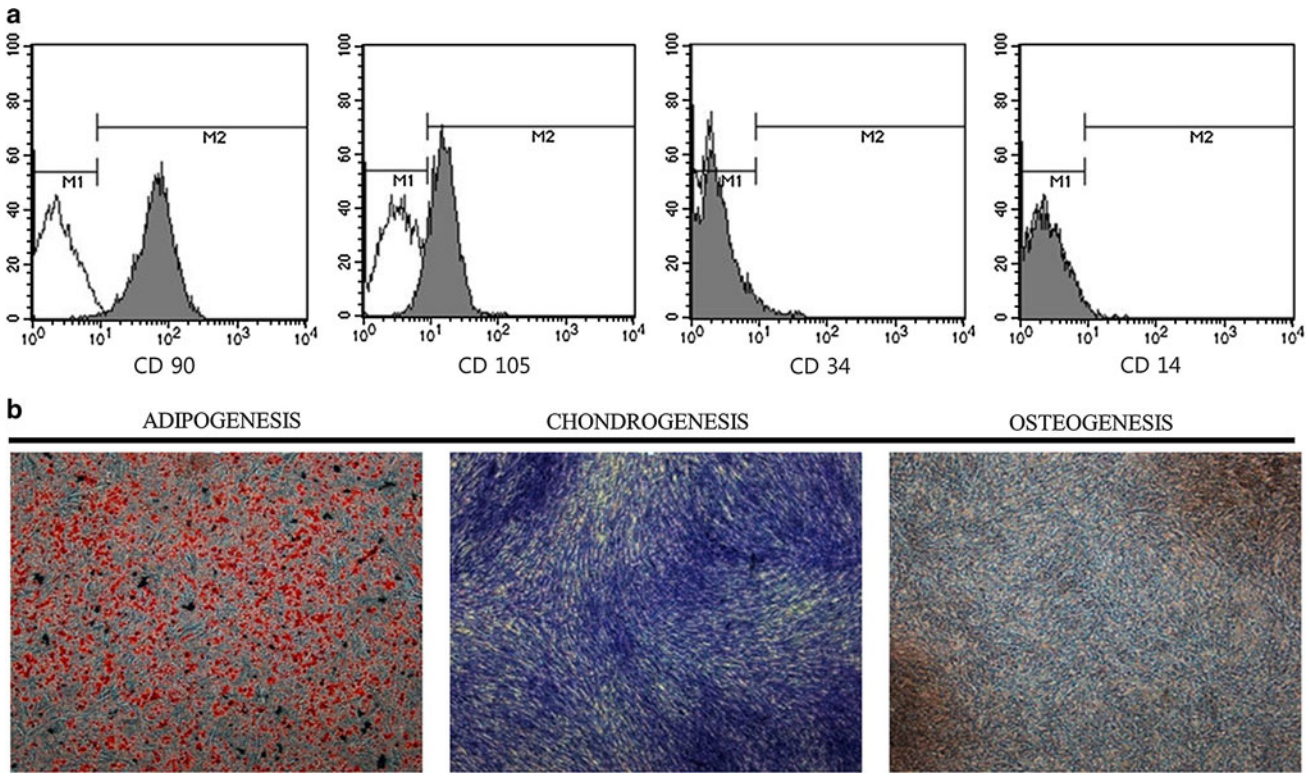

Fig. 1 Phenotypic characterization and differentiation potential of adipose-derived stem cells. **a** adipose-derived stem cells were isolated from stromal vascular fraction and then tested for mesenchymal surface markers (CD105 and CD90) and hematopoietic and endothelial markers (CD34 and CD14) by flow cytometry. **b** The differentiation potential of adipose-derived stem cells toward the adipogenic, chondrogenic, and osteogenic lineage was confirmed by Oil Red O, toluidine blue, and Von Kossa's method. Cells were cultured in normal medium for 2 weeks and then histochemically stained

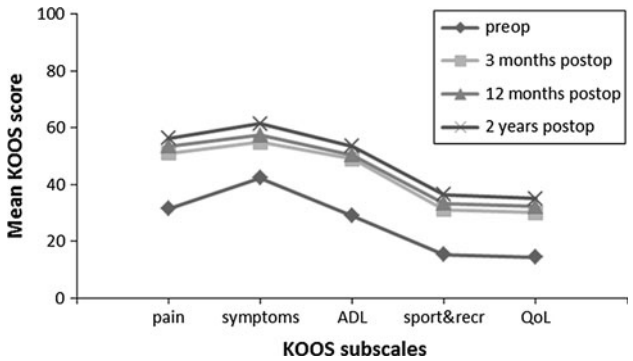

Fig. 2 KOOS profiles prior to and up to 2 years after stem cell therapy. Mean KOOS scores ($n = 30$) at the preoperative, 3-, 12-month and 2-year assessments after MSC therapy. At all follow-up point, differences in the values were statistically significant ($P < 0.05$) compared with the preoperative status. *ADL* activities of daily living, *sports/recr* sports and recreation, *QoL* quality of life

Discussion

The most important finding of the present study was that adipose-derived stem cell therapy was effective in cartilage healing, reducing pain, and improving function in elderly patients with knee OA. Additionally, among elderly patients aged >65 years, only 5 patients demonstrated worsening of the K–L grade. On second-look arthroscopy, 87.5 % of elderly patients (14/16) improved or maintained cartilage status at least 2 years postoperatively. Moreover, no patient underwent total knee arthroplasty during this 2-year period. Therefore, stem cell injection appears to be a good option for OA treatment in elderly patients. The results of stem cell injection with arthroscopic lavage were excellent at the final follow-up and showed improvement compared to those at 3 and 12 months. This finding indicates that even if the effect of arthroscopic lavage is eliminated, good results are achieved over medium-term follow-up. We acknowledge that arthroscopic lavage could be at least partly responsible for the improved clinical outcomes. However, arthroscopic lavage has only very short-term clinical effects in patients with advanced knee OA [26]; in the present study, clinical improvements persisted for more than 2 years (until at least the final follow-up at 24 months), and the second-look arthroscopy findings in these patients indicated that 87.5 % of elderly patients (14/16) improved or maintained cartilage healing status at 2 years postoperatively. Because the cartilage of OA

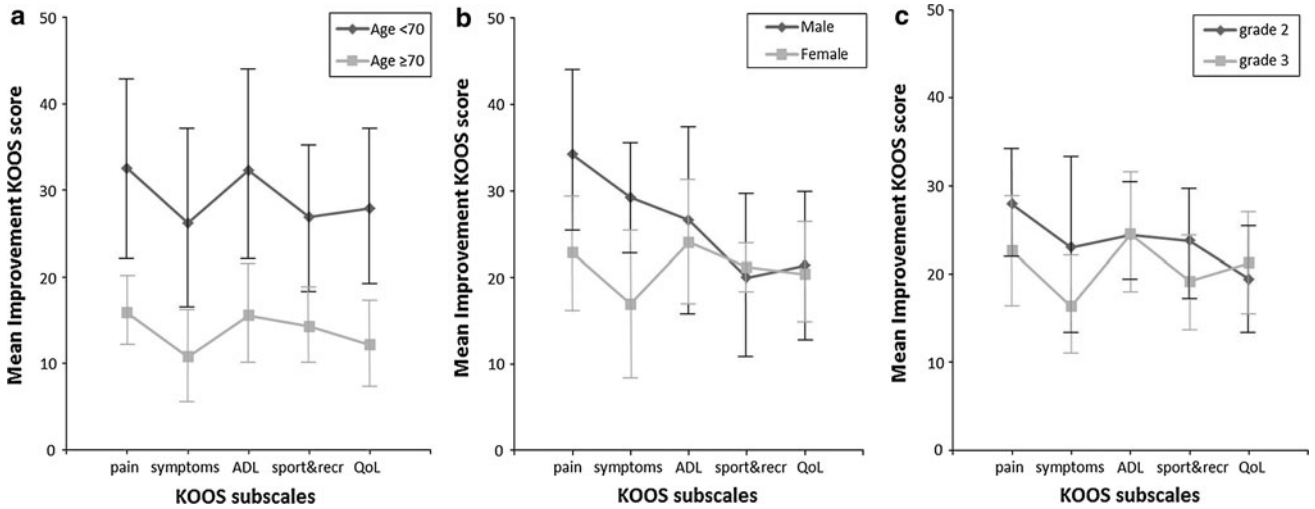

Fig. 3 Associations between patient characteristics and mean improvement from baseline in Knee injury and Osteoarthritis Outcome Score (KOOS) subscales to 2-year follow-up: **a** age (<70 vs >70), **b** sex (male vs female), **c** Kellgren–Lawrence grade (2 vs 3). *ADL* activities of daily living, *sports/recr* sports and recreation, *QoL* quality of life. A statistically significant association was only observed between patients' age and mean improvement from baseline in all KOOS subscales to 2-year follow-up

Table 1 Associations between patient characteristics and mean improvement of clinical outcomes, preoperatively to 2-year follow-up

Parameters	Age (years)		Sex		K–L[a]	
	<70	≥70	Male	Female	2	3
Lysholm score (SD)	23.8 (15.5)	15.5 (10.6)	9.2 (10.0)	22.1 (13.7)	29.0 (12.7)[†]	13.9 (11.4)
VAS (SD)	−3.3 (2.1)	−2.7 (1.2)	−3.2 (1.6)	−3.0 (1.8)	−3.1 (1.7)	−2.9 (1.8)

[†] Significant difference between both groups ($P < 0.05$)

[a] Radiological findings of osteoarthritis described by Kellgren and Lawrence

Table 2 Second-look patient demographics and general findings

Cartilage healing status (patient number, %)	Very positive (3, 18.7 %)	Positive (7, 43.8 %)	Neutral (4, 25.0 %)	Negative (2, 12.5 %)
Age, years (± SD)	70.3 ± 7.6	69.0 ± 2.4	76.0 ± 7.3	72.0 ± 2.8
Gender (M/F)	0/3	2/5	1/3	0/2
K–L[a] at 2nd look (II/III/IV)	1/2/0	2/5/0	1/2/1	0/1/1
Reason for 2nd look (evaluation/pain)	3/0	6/1	2/2	1/1
Follow-up period, months (± SD)	25.0 ± 0	24.9 ± 0.4	25.3 ± 0.96	24.5 ± 0.71

The differences between parameters of four groups were not significant ($P > 0.05$)

[a] Radiological findings of osteoarthritis described by Kellgren and Lawrence

patients has diffuse degenerative lesions, the grading system of severe lesions used by certain classifications such as Outerbridge's classification [24] or the ICRS grade does not seem to be appropriate to describe the change in the cartilage status in OA patients. Therefore, we believe that the identification of the change in cartilage status is difficult using Outerbridge's classification [24] or the ICRS grade. Thus, a different method for the classification of regeneration, which was used in the present study, is essential to classify the change in the cartilage status.

Previous studies have shown that the outcomes of chondrocyte transplantation in patients aged >40 years were inferior compared to those previously noted in younger populations, and the failure rate at medium-term follow-up was also comparatively higher [19]. However, in the present study, the combination of MSC therapy and PRP was found to be effective in elderly patients with knee OA. We believe that although the chondrocytes of older patients have lower activity, the adipose-derived stem cells in elderly patients have sufficient stem cell activity, as noted during our

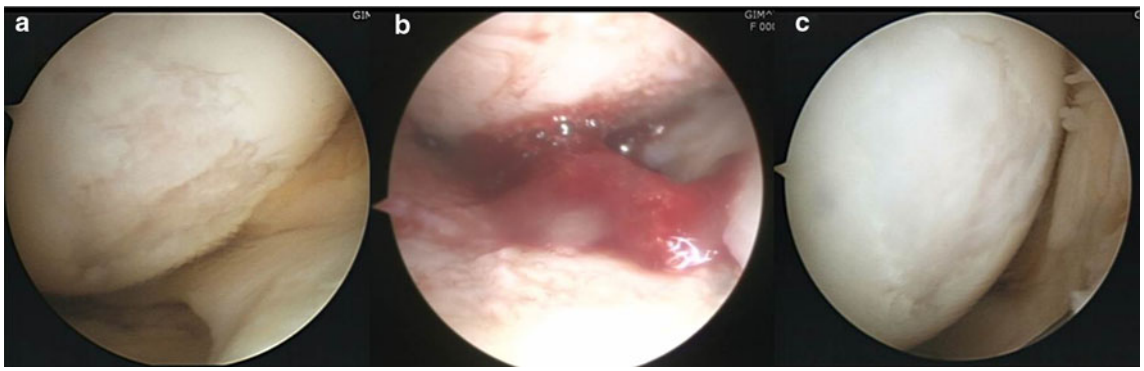

Fig. 4 **a** Intraoperative arthroscopic finding showing a cartilage defect in the medial femoral condyle (MFC). **b** Intraoperative arthroscopic finding showing insertion of stem cells with PRP. **c** Second-look arthroscopy revealed that the cartilage defect was completely covered with smooth tissues, which was considered to be cartilage. This finding of a remarkable change throughout the degenerative cartilage with good integration with the adjacent normal articular surface and normal gross appearance was defined as "very positive"

characterization of adipose-derived stem cells. Moreover, although chondrocytes are generally believed to develop only through tissue-specific differentiation, stem cells are believed to develop through tissue-specific differentiation as well as a powerful paracrine effect.

We previously reported that infrapatellar fat pad-derived MSC therapy with intra-articular injections is safe and aids in reducing pain and improving function in patients with knee OA [17]. Over a long-term follow-up period [18], both clinical assessments and MRIs indicated that MSC therapy involving the intra-articular injection of MSCs into the knee is effective for reducing pain and improving function in patients with knee OA; furthermore, 2-year follow-up results were better than short-term results. Because further analysis indicated that clinical and radiological results improved as the number of injected cells increased, we changed our source of stem cells to obtain a greater number. Therefore, in the present study, a larger number of cells were obtained by using subcutaneous adipose tissue from the buttock (mean number of stem cells, 4.0×10^6), compared with that from the infrapatellar fat pad, from which MSCs were derived and a mean number of 1.2×10^6 stem cells were obtained. Moreover, although there are wide variations (range 6.4–13.1 g) in the amount of fat in the infrapatellar fat pad, 120 cc of buttock subcutaneous fat tissue could be consistently obtained in all the patients.

A recent study demonstrated that adipose tissue contains multipotent stem cells [25], or adipose-derived stem cells, which can be easily purified after digestion of fat and selection by adhesion onto plastic from the very heterogeneous crude stromal fraction. Adipose tissue is the subject of great interest as a therapeutic cell source because the cells are obtained from adults, thereby avoiding ethical concerns, and from tissue that is abundant and easy to obtain, even compared with bone marrow where sampling requires general anaesthesia. Additionally, because the frequency of adipose-derived stem cells in adipose tissue is much higher than that of MSCs in bone marrow, many cells can be obtained without a large number of passages, thus greatly decreasing the risk of culture-induced chromosomal abnormality or senescence [33].

Adipose tissue is composed of two main cell populations: mature adipocytes and the stromal vascular fraction. The latter is a heterogeneous fraction including preadipocytes, endothelial cells, smooth muscle cells, pericytes, macrophages, fibroblasts, and adipose-derived stem cells, which share several characteristics with bone marrow stem cells [29, 32]. Adipose-derived stem cells are promising candidates in a broad range of innovative therapies, ranging from regenerative medicine to tissue engineering, in autoimmune pathologies. Moreover, the use of stromal vascular fraction or adipose-derived stem cells has been proposed in several chronic pathologies such as Crohn's disease [10], autoimmune pathologies (e.g., multiple sclerosis) [27], and allergic pathologies. Their effectiveness against these pathologies can be explained by the immunoregulatory and anti-inflammatory activities of adipose-derived stem cells or nonexpanded stromal vascular fraction cells [27]. Unfortunately, since the majority of scientific studies have focused on in vitro-expanded adipose-derived cells, relatively little is known about the potential clinical effects of the whole lipoaspirate, which contains numerous cell populations besides MSCs. Recently, adipose-derived stem cells have been identified as a new option for the treatment of osteochondral lesions, and the injection of MSCs with marrow stimulation treatment has been proposed for the treatment of such cases in our institute [14]. Moreover, Desando et al. [9] reported that the healing proprieties of adipose-derived stem cells, including the promotion of cartilage and menisci repair and attenuation of inflammatory events in the synovial membrane, may facilitate the inhibition of OA progression.

In previous studies using bone marrow-derived MSCs for the treatment of cartilage defects, culture expansion of MSCs was performed to obtain a large number of cells [2, 11]. However, MSC culture expansion is costly, time-consuming, and carries some risk of contamination. In addition, MSC properties may be altered during culture by various elements of the local microenvironment that can affect MSC differentiation [7, 28]. In the present study, we could extract approximately 4.0×10^6 stem cells without culture (9.7 % of the 4.2×10^7 cells in the stromal vascular fraction). Consistent with our results, De Toni et al. [8] reported that adipose-derived stem cells represent 6.4 % of nucleated cells in the normal vascular fraction in adipose tissue, whereas MSCs represent only 0.0005 % of nucleated cells in the human bone marrow, which is a considerable difference. The advantages of our methods are that MSCs can be harvested in a minimally invasive manner and are easily isolated; in addition, an important advantage of this procedure is that, since time-consuming in vitro cell culture is not required, all procedures can be performed with a single admission.

Although the primary effects of stem cell treatment are generally believed to occur through tissue-specific differentiation [6, 23], new data suggest that the therapeutic potential of these cells may be related to their paracrine effect [6, 21]. Following second-look arthroscopy, only 10/16 patients in our cohort demonstrated cartilage formation. However, regardless of cartilage formation, almost all patients demonstrated improved clinical symptoms. In the some case, although radiological and second-look findings indicated worsening, the patient showed excellent clinical outcome and high satisfaction with her results. Thus, the main effect of this therapy appears to be the paracrine effect. Several studies have shown that MSCs can modulate the functions of adaptive immune system cells such as T cells and B cells [1]. Other studies have shown that these stem cells are also able to induce expression of anti-inflammatory mediators, such as IL-10 and IL-12p40, in macrophages [22]. Our stem cell therapy may act primarily through a long-lasting anti-inflammatory effect.

In this study, the effect of MSC insertion was maintained for 2 years. MSC therapy may be a new option for elderly patients who are not fully indicated for total knee arthroplasty. Menno et al. [34] reported that a single injection of adipose-derived stem cells into the knee joints of mice with early-stage collagenase-induced OA inhibits synovial thickening, formation of enthesophytes associated with ligaments, and cartilage destruction. Additionally, in contrast to early treatment, late injection of adipose-derived stem cells after OA induction showed no significant effect on synovial activation or joint pathology. Similar to the preceding findings, in our study, patients aged <70 years and K–L grade two patients achieved greater improvement in clinical outcomes than those aged >70 years or those with K–L grade 3. Therefore, we believe that MSCs may be particularly useful for delaying total knee arthroplasty in younger patients and cases of less severe OA.

The present study has some limitations. First and most importantly, our data lack quantitative evidence. MRI examination and biopsy should be performed. Second, this study is a level IV study, and therefore, no control group was included. Although we had earlier proved the effect of infrapatellar fat pad-derived adipose stem cells, we altered the source of the cells and used a novel method of arthroscopic-guided injection in the present study. Thus, we performed a new study without having a group for comparison, such as a pilot study. As the present study was designed as a pilot study, only patients aged >65 years who did not wish to undergo total knee arthroplasty were included. An additional study with a comparative design in patients with an early stage of OA will be performed. Third, our treatments were delivered during a single injection, although the possibility exists that optimal results can only be obtained by giving patients >1 injection within a certain time period. Fourth, only the effects of simultaneous treatment with both stem cells and PRP were focused on in the present study; additional work is needed to measure the effects of pure stem cell injections, distinguish the effects of stem cells from those of PRP, and determine the proper use of costimulators. Finally, the major limitation of the current study is the lack of matched control groups that would facilitate determining the efficacy of the stem cell therapy.

The clinical relevance of this study is that adipose-derived stem cell treatment may be a useful therapy for knee OA. Therefore, adipose-derived stem cell treatment appears to be a good option for OA treatment in elderly patients.

Conclusions

Adipose-derived stem cell therapy for elderly patients with knee OS was effective in cartilage healing, reducing pain, and improving function. Therefore, adipose-derived stem cell treatment appears to be a good option for OA treatment in elderly patients.

Conflict of interest None.

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