# Global Level I and II Evidence of Stromal Vascular Fraction in treatment of Osteoarthritis

# CONTENT

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No serious adverse events were observed and significant improvement occurred for the clinical indicators of VAS, KOOS, WOMAC scores; in addition to cartilage improvement in joints by MRI, arthroscopy, WORMS and MOCART following treatment by autologous minimal manipulated SVF.

**Note:** In both the scientific and marketing literature there has been confusion on the correct use of terms. SVF may incorrectly be referred to as stem cells, mesenchymal stem cells, MSC, adipose derived stem cells (ADSC). In depth reading of articles is required to ascertain the correct source of the cells rather than the incorrect term which may have been used historically in the literature.

# Clinical Efficacy of Intra-articular Mesenchymal Stromal Cells for the Treatment of Knee Osteoarthritis

## A Double-Blinded Prospective Randomized Controlled Clinical Trial

Jaime R. Garza,* MD, Richard E. Campbell,† BS, Fotios P. Tjoumakaris,† MD, Kevin B. Freedman,† MD, Lawrence S. Miller,‡ MD, Daniel Santa Maria,§ MD, and Bradford S. Tucker,†|| MD

*Investigation performed at the Rothman Orthopaedic Institute, Philadelphia, Pennsylvania, USA; Cooper University Health Care, Camden, New Jersey, USA; and Texas Plastic Surgery, San Antonio, Texas, USA*

**Background:** Currently, there are limited nonoperative treatment options available for knee osteoarthritis (OA). Cell-based therapies have emerged as promising treatments for knee OA. Autologous stromal vascular fraction (SVF) has been identified as an efficient medium for intra-articular administration of progenitor cells and mesenchymal stem cells derived from adipose tissue.

**Hypothesis:** Patients receiving intra-articular SVF would show significantly greater improvement than patients receiving placebo injections, and this improvement would be dose dependent.

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

**Methods:** This was a multisite prospective double-blinded randomized placebo-controlled clinical trial. Adult patients with symptomatic knee OA were eligible. Thirty-nine patients were randomized to high-dose SVF, low-dose SVF, or placebo (1:1:1). SVF was obtained via liposuction, processed to create the cellular implant, and injected during the same clinical visit. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores and magnetic resonance images were obtained preoperatively and at 6 and 12 months after injection. The Wilcoxon rank sum nonparametric test was utilized to assess statistical significance, and the Hodges-Lehmann location shift was used to assess superiority.

**Results:** The median percentage change in WOMAC score at 6 months after injection for the high-dose, low-dose, and placebo groups was 83.9%, 51.5%, and 25.0%, respectively. The high- and low-dose groups had statistically significant changes in WOMAC scores when compared with the placebo group (high dose, $P = .04$; low dose, $P = .02$). The improvements were dose dependent. The median percentage change in WOMAC score from baseline to 1 year after injection for the high-dose, low-dose, and placebo groups was 89.5%, 68.2%, and 0%, respectively. The high- and low-dose groups displayed a greater percentage change at 12 months when compared with the placebo group (high dose, $P = .006$; low dose, $P = .009$). Magnetic resonance image review revealed no changes in cartilage thickness after treatment. No serious adverse events were reported.

**Conclusion:** Intra-articular SVF injections can significantly decrease knee OA symptoms and pain for at least 12 months. The efficacy and safety demonstrated in this placebo-controlled trial support its implementation as a treatment option for symptomatic knee OA.

**Registration:** NCT02726945 (ClinicalTrials.gov identifier)

**Keywords:** progenitor cells; stem cells; osteoarthritis; cartilage; knee; stromal vascular fraction; adipose

Knee osteoarthritis (OA) is highly prevalent among older adults in developed countries and is a significant cause of chronic pain and disability.[24] The definitive surgical treatment for knee OA is total knee arthroplasty, a major surgical procedure. Given the complications and intense rehabilitation process associated with total knee arthroplasty, physicians

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mpt to manage knee OA symptoms with multiple nonoperative modalities before surgery. These modalities include the use of anti-inflammatory medications, physical therapy, corticosteroid injections, and viscosupplementation. Without the ability to prevent cartilage loss, these treatment modalities only delay symptomatic progression to total knee arthroplasty.

Recently, cell-based therapies have emerged as possible disease-modifying treatments. Mesenchymal stem cells (MSCs) and adipose-derived stem cells (ASCs) have demonstrated chondrogenic potential.[5] However, the isolation of MSCs and ASCs may require multiple weeks and special laboratories for cell expansion.[17] A more efficient method for collection and administration of ASCs is the use of autologous stromal vascular fraction (SVF) cells. SVF consists of a heterogeneous concentration of nucleated stromal and vascular cells that are normally present in the stromal and vascular structures of adipose tissue, including stromal and vascular progenitor cells, as well as endothelial cells.[19] SVF does not contain adipocytes; it has a very low concentration of leukocytes and a very low presence of extracellular matrix. Adipose tissue is easily acquired through the use of a small liposuction harvest (requiring only local anesthetic), which can then be processed to isolate the SVF cells. Furthermore, SVF processing does not require cell expansion or culture.[4] SVF can be processed at the bedside.

Multiple studies have supported the use of intra-articular SVF injections for knee OA symptom management.[2,15,16,26] These studies demonstrated improvement in knee OA symptoms ranging from 1 month to 2 years after SVF injection, without an increased risk of adverse effects.[16,26] Unfortunately, the clinical interpretations of these studies are limited by small sample sizes and the lack of control group comparisons or evaluation of SVF in conjunction with other treatment modalities, such as platelet-rich plasma or arthroscopic debridement.

Previous randomized controlled trials have demonstrated increased efficacy of intra-articular injections of autologous bone marrow MSCs or ASCs as compared with hyaluronic acid and normal saline.[6,12,17] The primary aim of this study was to investigate the efficacy and safety of intra-articular autologous SVF injections at 6 months as compared with placebo injection. The secondary aims of this study were to determine if SVF injections continue to reduce knee pain at 1 year after treatment and to assess any effects of SVF injections on articular cartilage with magnetic resonance imaging (MRI) evaluations 6 months and 1 year after injection. We hypothesized that patients receiving intra-articular SVF will show significantly greater improvement in symptoms than patients receiving placebo injections and that this improvement will be dose dependent.

## METHODS

### Study Design and Participants

Before voluntary patient enrollment, this clinical trial was approved by the Institutional Review Board at each research site, as well as the US Food and Drug Administration (IDE 16347). This trial was listed on ClinicalTrials.gov (NCT02726945). The complete protocol will not be available for access. A synopsis is available on ClinicalTrials.gov. This study was sponsored and funded by the GID Group. This was a prospective double-blinded randomized placebo-controlled interventional safety and efficacy study conducted at multiple centers (3 sites). The dose-escalated study used a parallel-group design with 3 arms: high-dose treatment group ($3.0 \times 10^7$ SVF cells), low-dose treatment group ($1.5 \times 10^7$ SVF cells), and placebo control group (zero SVF cells).

English-speaking men and nonpregnant women between the ages of 40 and 75 years were screened. Patient eligibility was determined per the degree of clinical and radiographic disease. Eligibility criteria included (1) a Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain (A1) subscore $>6$ and $\leq 16$ on a 20-point scale in 1 knee and a WOMAC pain score $\leq 6$ for the contralateral knee; (2) grade 2 or 3 Kellgren-Lawrence OA on radiograph with no full-thickness lesion $>1$ cm in any dimension by MRI assessment; and (3) failure of a minimum of 2 nonoperative therapies (oral pain medications, physical therapy, corticosteroid injection, or viscosupplementation injection).

Exclusion criteria included the following: a body mass index $\geq 35$, American Society of Anesthesiologists score $\geq 3$, history of symptomatic OA (hips, spine, or ankle), rheumatologic disease, avascular necrosis, severe bone deformity, previous infection of the knee joint, pes anserine

‖Address correspondence to Bradford S. Tucker, MD, Rothman Orthopaedic Institute, 2500 English Creek Avenue, Building 1300, Egg Harbor Township, NJ 08234, USA (email: Bradford.Tucker@rothmanortho.com).

*School of Medicine, Tulane University, New Orleans, Louisiana, USA.

†Rothman Orthopaedic Institute, Philadelphia, Pennsylvania, USA.

‡Cooper University Hospital, Camden, New Jersey, USA.

§Sports Medicine Associates of San Antonio, San Antonio, Texas, USA.

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bursitis, pain attributed to diffuse edema, pain attributed to displaced meniscal tear or osteochondritis dissecans, neurogenic or vascular claudication, bleeding disorders, chemotherapy, radiation therapy to treatment leg or adipose harvest site, and tobacco use. Patients were also excluded if their target knee had an injection within 3 months before screening, surgery within 6 months before screening, or major injury within 12 months before enrollment. Those who could not discontinue use of the following drugs 7 days before injection have also excluded prescription pain medication, chronic oral steroids, anticoagulants, thrombolytics, or antiplatelet medication.

Patient screening and evaluations took place in private physician examination rooms. All procedures related to adipose collection, SVF processing, and SVF injection took place in private physician examination rooms. Patient-reported outcomes were collected during clinical visits.

## Randomization, Blinding, and Dose Escalation

Patients were randomized 1:1:1. Randomized opaque folders containing the treatment dose assignment were sent to each site. On the day of intervention, a folder was randomly selected for the patient and delivered to the site technician. Only the site technician had access to the randomization information. Investigators and participants were blinded to treatment group assignment. Once the appropriate SVF or placebo dose was created by the technician, the dose syringe and tip were completely wrapped in sterile white labels to mask the contents. After the 6-month follow-up evaluation, patients and physicians were unblinded. Patients were unblinded at this time because the primary efficacy endpoint was the 6-month follow-up.

Under the dose escalation protocol, the first 15 consecutive participants were randomized to either the low-dose or the placebo group and followed for 6 weeks with a safety and adverse events analysis. The remaining 24 patients were assigned to the high-dose, low-dose, or placebo group. The criterion for trial continuation was $\leq$2 adverse events of grade $<$4 on the Common Terminology Criteria for Adverse Events scale. No adverse events were observed, and the study proceeded. Adverse events were monitored continuously during the study period.

## Intervention

The complete adipose harvesting, processing, and injection procedure is described in the Appendix Methods (available in the online version of this article). Adipose was harvested from the abdomen with patients under local anesthetic. A mean 75 mL of adipose was aspirated directly into a sterile GID SVF-2 tissue-processing device (GID Group). The GID SVF-2 device is designed to produce a standardized and fully characterized dose of stromal vascular cells. The filled device was handed to a technician for tissue processing and cell characterization. Complete cell characterization and results are detailed in the Appendix Methods.

All tissue processing was done under sterile conditions within the single-use GID SVF-2 device. The appropriate dose (per treatment group) was created in a blin 5-mL syringe, and the total volume was brought to 3 ω 4 mL with lactated Ringer solution. The dose was then injected into the knee joint via a superior-lateral approach under sterile technique. Verification of joint space location of the needle was verified with ultrasound imaging or by aspiration of visible synovial fluid into the syringe. All participants were advised to maintain minimal weightbearing for 2 days. Full range of motion (nonweightbearing) was encouraged. Participants were advised to maintain only light activity and to avoid previously painful activities for the first 3 weeks after injection.

The cell dose was evaluated for viability, endotoxin level, and gram-negative contamination before release. A sample from each subject was sent to a central laboratory for evaluation of residual collagenase, cultured sterility, colony-forming unit analysis, phenotype analysis (flow cytometry), and cytokine/growth factor assessment, see Appendix Methods (available online).

## Outcome Measures

The prespecified primary efficacy outcome was the percentage change from baseline per the short-form WOMAC scale, a patient-reported OA symptom questionnaire. The WOMAC instrument consists of 3 subscores used to evaluate pain, stiffness, and functionality. Total scores range from 0 to 56 points. A decreasing score is indicative of decreased pain and stiffness and increased functionality. The total score was normalized to 100 points. The WOMAC was completed by the patient before intervention and at 6 weeks and 3, 6, and 12 months after injection.

MRI of the treatment knee was obtained before treatment and at 6 months and 1 year after treatment. MRI scans were taken according to the following parameters: sagittal plane only, 2.5-mm proton density fat saturation sequence, and 3.0 or 1.5 T with knee coil magnet (8-16 channels). The MRI studies were reviewed for anatomic changes and for cartilage changes in the anteroposterior dimension for medial and lateral tibiofemoral lesions via the sagittal view. Cartilage degeneration was rated with the modified Outerbridge classification. Two fellowship-trained radiologists reviewed all images independently and then reached consensus agreement. Reviewers were blinded to the treatment arm. The resolution of the MRI measurement was 1 mm.

## Statistical Analysis

The sample size determination was based on a difference to detect at least 17 points (representing a 33% change relative to baseline for a median baseline score of 50 points on the WOMAC scale [100 points, full scale]), a 1-sided superiority test, a standard deviation of 14 points, an $\alpha$ value of .05, and a power of 80%, resulting in 11 participants per group. To account for possible losses to follow-up, a loss rate of 20% was assumed, and an additional 2 participants per treatment group were added for a total

ple size of 13 per group and a total enrolled sample size of 39.

The hypothesis in the prespecified analysis was as follows: primary efficacy will be achieved if either dose group is shown to be superior to the placebo group at 6 months after treatment by using the percentage change from baseline as the primary variable and the following null and alternative hypotheses:

$H_0$: There is no difference between the dose group and the placebo group.

$$H_0: M_L = M_C \text{ and } M_H = M_C.$$

$H_a$: Either or both dose groups are superior to the placebo group.

$$H_a: M_L > M_C \text{ and/or } M_H > M_C.$$

C is control (placebo); L is low dose; H is high dose; and M is median.

Percentage change in total WOMAC score for each group was calculated and compared by between-group comparisons of each treatment group with the placebo per the Wilcoxon rank sum test (nonparametric). Hodges-Lehmann estimation (nonparametric) was used to construct 1-sided 95% CIs of the location shift (median of pairwise differences) to assess superiority. Concordance statistics (nonparametric) were used to calculate the area under the operating characteristic curve to evaluate the effect size. A Bonferroni-corrected $\alpha$ value of .025 (2.5%) was used for the primary efficacy evaluation to account for the multiple comparisons (2) of the null hypothesis.

The data set was analyzed with an intent-to-treat principle and used the last observation carried forward method for missing data. To assess clinical meaningfulness of treatment, a threshold of 33% change from baseline in total WOMAC score was set as the minimal clinically important difference (MCID). This indicated that a 33% improvement in the WOMAC score was needed for patients to experience a clinically meaningful change in knee OA symptoms. The MCID for the percentage change in WOMAC score was based on an analysis of current treatments for OA of the knee. Ten peer-reviewed randomized and concurrent-controlled studies involving >2500 participants for treatment of OA of the knee with corticosteroids, hyaluronic acid, total knee replacement, and controls (saline)[¶] were analyzed (Table 1).

The MCID of 33% was selected as the largest of the 3 injection approaches. The primary endpoint was prespecified at 6 months with a safety follow-up at 1 year. Data at 6 weeks and 3 months were used descriptively but not prespecified for statistical comparison. Between-group differences in cartilage thickness and within-group differences in Outerbridge classifications were analyzed per the Mann-Whitney *U* and Wilcoxon signed rank tests, respectively.

[¶]References 1, 3, 7, 10, 13, 14, 18, 20, 23, 25.

TABLE 1
Previously Published WOMAC Percentage Change for MCID Calculation[a]

| Treatment | Percentage Change From Baseline at 6 mo | Participants, n |
|---|---|---|
| Total knee arthroplasty[7,10,13,14] | 54 | 1451 |
| Corticosteroids[3,18,20,23] | 26 | 362 |
| Hyaluronic acid[1,3,18,23] | 33 | 433 |
| Normal saline[1,20,25] | 22 | 250 |

[a]MCID, minimal clinically important difference; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

## RESULTS

Of the 329 patients screened, 67 consented for further MRI evaluation of their OA. After review, 1 patient unenrolled owing to relocation, and 27 were excluded for full-thickness lesions >1.5 cm in any dimension or displaced meniscal tears (Figure 1). A total of 39 patients (22 women and 17 men) were enrolled with 13 in each treatment group. Patients were enrolled between July 2016 and September 2017. The last patient completed 1-year follow-up in September 2018. Patient characteristics are summarized in Table 2.

### Six-Month WOMAC Evaluation

Of the 39 patients enrolled, 37 completed the 6-month WOMAC evaluation. Missing data were completed with the aforementioned last observation carried forward method. Two missing 6-month values were imputed, resulting in an imputation rate of 2.6% (2 of 78). Patient attrition and data carried forward are described in Figure 1. One patient in the placebo group was disqualified after receiving the knee injection because of a protocol error in the initial MRI evaluation, which was identified immediately after treatment. The distribution of studentized residuals of the primary variable (percentage change) was evaluated for normality with the Shapiro-Wilk test for normality, showing a strongly nonnormal distribution ($W = 0.907$, $P = .004$). The nonnormal distribution was partially caused by the floor effect of the WOMAC and partially by the nature of the WOMAC, which is a Likert-type ordinal scale. Parametric analysis was not tenable, and nonparametric methods (distributed free about medians) were used to evaluate the hypotheses.

The rate of 6-month WOMAC follow-up for the high-dose, low-dose, and placebo groups was 92.3% (12 of 13), 100% (13 of 13), and 92.3% (12 of 13), respectively. Six months after SVF injection, all groups displayed a reduction in total WOMAC score from baseline. The median percentage change in WOMAC score for the high-dose, low-dose, and placebo groups was 83.9%, 51.5%, and 25.0%, respectively (Table 3, Figures 2 and 3). The median percentage change in the WOMAC score for the high- and low-dose groups was greater than the MCID, and that for

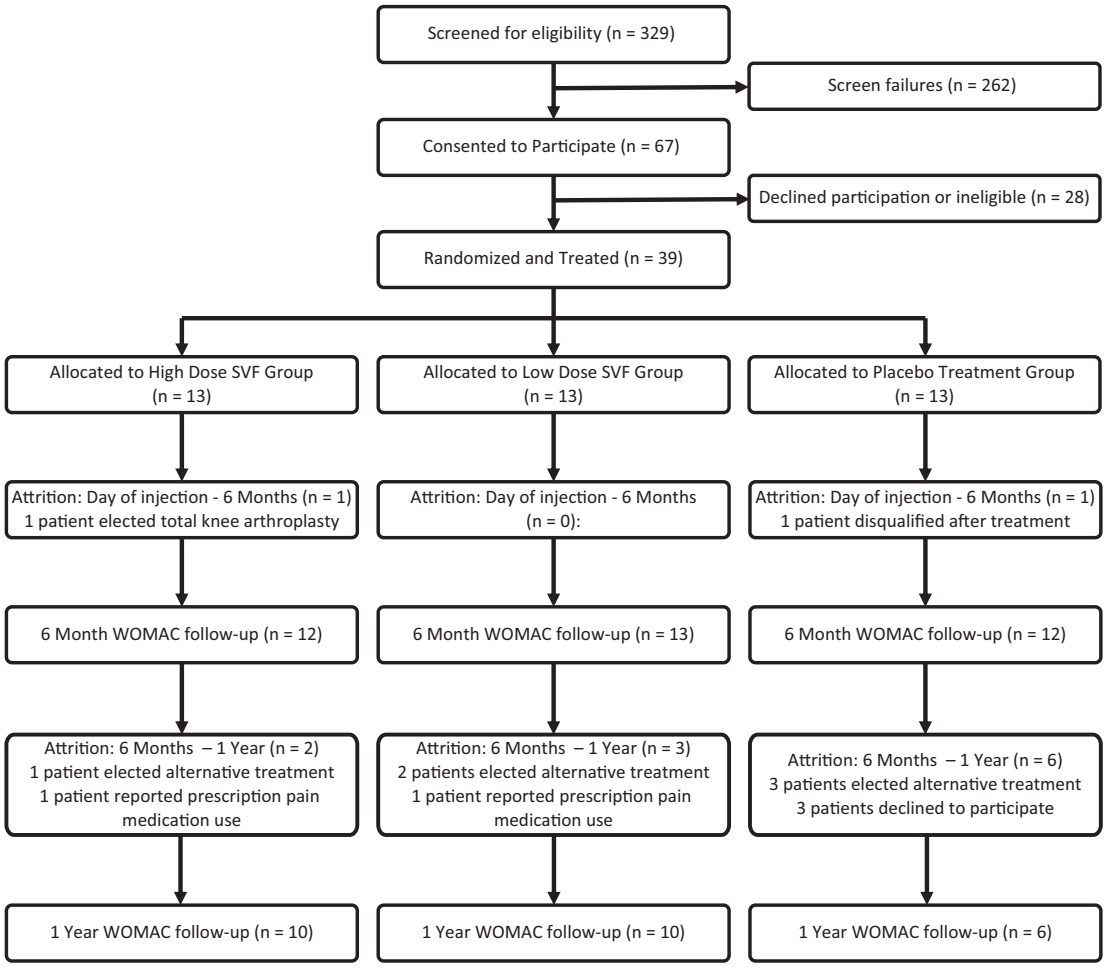


**Figure 1.** Study flow diagram depicts patient follow-up and attrition. SVF, stromal vascular fraction; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

TABLE 2
Patient Characteristics[a]

| | Treatment Group | | | |
|---|---|---|---|---|
| Parameter | Placebo | Low | High | Total |
| Age, y | 57.1 ± 9.1 (41-74) | 60.5 ± 7.9 (48-71) | 59.5 ± 11.7 (41-74) | 59.0 ± 9.9 (41-74) |
| BMI | 27.1 ± 2.7 (22.3-32.8) | 27.6 ± 4.1 (19.6-34.9) | 28.8 ± 4.3 (21.7-34.9) | 27.8 ± 3.9 (19.6-34.9) |
| Race/ethnicity | | | | |
| White | 69.2 (9) | 92.3 (12) | 84.6 (11) | 82.0 (32) |
| Black | 7.7 (1) | 0 (0) | 0 (0) | 2.6 (1) |
| Hispanic | 23.1 (3) | 7.7 (1) | 15.4 (2) | 15.4 (6) |
| Sex | | | | |
| Female | 53.8 (7) | 69.2 (9) | 46.2 (6) | 56.4 (22) |
| Male | 46.2 (6) | 30.8 (4) | 53.8 (7) | 43.6 (17) |
| Kellgren-Lawrence grade | | | | |
| 2 | 30.8 (4) | 30.8 (4) | 30.8 (4) | 30.8 (12) |
| 3 | 69.2 (9) | 69.2 (9) | 69.2 (9) | 69.2 (27) |
| Knee laterality | | | | |
| Right | 30.8 (4) | 76.9 (10) | 69.2 (9) | 59.0 (23) |
| Left | 69.2 (9) | 23.1 (3) | 30.8 (4) | 41.0 (16) |
| ASA score | | | | |
| I | 61.5 (8) | 69.2 (9) | 46.2 (6) | 59.0 (23) |
| II | 38.5 (5) | 30.8 (4) | 53.8 (7) | 41.0 (16) |

[a]Values are presented as mean ± SD (range) or % (n). ASA, American Society of Anesthesiologists; BMI, body mass index.

TABLE 3
WOMAC Total Scores for Groups: 100-Point Full Scale[a]

| Group: Time Point | Mean | Median (IQR) | Median Percentage Change | Minimum | Maximum |
|---|---|---|---|---|---|
| High dose | | | | | |
| Baseline | 47.1 | 49.8 (35.6-55.2) | 0 | 19.6 | 69.4 |
| 6 wk | 25.7 | 27.0 (14.2-36.0) | 37 | 0.0 | 55.2 |
| 3 mo | 26.5 | 27.0 (10.7-34.7) | 56 | 3.6 | 60.5 |
| 6 mo | 20.0 | 8.9 (3.6-32.0) | 84 | 0.0 | 53.4 |
| 1 y | 13.2 | 3.6 (0.0-26.7) | 89 | 0.0 | 53.4 |
| Low dose | | | | | |
| Baseline | 56.2 | 51.6 (46.3-62.3) | 0 | 39.2 | 99.7 |
| 6 wk | 24.8 | 20.0 (10.7-37.4) | 50 | 0.0 | 64.1 |
| 3 mo | 19.7 | 14.0 (5.3-35.6) | 75 | 0.0 | 64.1 |
| 6 mo | 23.7 | 26.7 (8.9–32.0) | 52 | 0.0 | 60.5 |
| 1 y | 21.8 | 12.5 (7.1-35.6) | 68 | 0.0 | 60.5 |
| Placebo | | | | | |
| Baseline | 49.3 | 49.8 (37.4-57.0) | 0 | 28.5 | 80.1 |
| 6 wk | 26.0 | 23.0(14.2-37.4) | 46 | 6.2 | 55.2 |
| 3 mo | 22.9 | 20.0 (16.0-32.0) | 62 | 0.0 | 55.2 |
| 6 mo | 37.2 | 30.2 (21.4-55.2) | 25 | 16.0 | 81.9 |
| 1 y | 41.9 | 41.0 (19.5-55.2) | 0 | 5.3 | 81.9 |
| Treatment[b] | | | | | |
| Baseline | 51.7 | 51.0 (41.4-58.7) | 0 | 19.6 | 99.7 |
| 6 wk | 25.2 | 24.0 (14.2-37.0) | 45 | 0.0 | 64.1 |
| 3 mo | 23.1 | 20.0 (7.1-35.4) | 61 | 0.0 | 64.1 |
| 6 mo | 21.8 | 22.0 (4.0-32.0) | 62 | 0.0 | 60.5 |
| 1 y | 17.5 | 8.0 (0.9-29.6) | 85 | 0.0 | 60.5 |

[a]IQR, interquartile range; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.
[b]Treatment group comprises both the high- and low-dose groups.

the placebo group was below the MCID. Sixty-two percent of patients in the treatment groups (high and low doses) had a response greater than the MCID, in contrast to only 38% participants in the placebo group. Three patients in the high-dose treatment group and 3 in the low-dose group experienced a 100% reduction in WOMAC score, while no patients in the placebo group did.

In the comparative analysis, the high- and low-dose groups displayed statistical significance as compared with the placebo group (high dose, $P = .043$; low dose, $P = .023$; below Bonferroni-corrected value for multiple comparisons). The lower-bound 95% 1-sided confidence intervals (CIs) of the location shift showed that the high dose and the low dose were superior to placebo (location shift >0): high dose, 0.339 (95% CI, 0.012-0.662); low dose, 0.314 (95% CI, 0.042-0.606). The effect sizes for the high and low doses were 0.701 and 0.734, respectively, indicating large effect sizes for both doses relative to placebo. Both dose groups showed statistical significance relative to placebo (with superiority based on CIs relative to placebo), had similar large effect sizes, and were combined in a treatment group (see Table 3).

## One-Year WOMAC Evaluation

Of the initial 39 patients, 37 were available for follow-up 1 year after SVF injection; however, only 26 were able to complete the WOMAC. The 1-year WOMAC follow-up rate in the high-dose, low-dose, and placebo groups was 76.9% (10 of 13), 76.9% (10 of 13), and 46.2% (6 of 13), respectively. Missing data were completed with the last observation carried forward method. Reasons for patients' inability to complete the WOMAC at 1 year are displayed in Figure 1.

All groups continued to demonstrate lower total WOMAC scores 1 year after injection as compared with baseline scores. The percentage change from baseline for the high-dose, low-dose, and placebo groups was 89.5%, 68.2%, and 0%, respectively (Table 3). The high- and low-dose groups continued to display significantly greater percentage improvement in WOMAC scores as compared with the placebo group (high dose, $P = .006$; low dose, $P = .009$).

The lower-bound 95% 1-sided CIs of the location shift showed that the high dose and the low dose were superior to placebo (location shift >0): high dose, 0.524 (95% CI, 0.252-0.917); low dose, 0.435 (95% CI, 0.122-0.810). The effect sizes for the high and low doses were 0.793 and 0.775, respectively, indicating large effect sizes for both doses relative to placebo.

The analysis at 1 year showed continued improvement from 6 months to 1 year for both high- and low-dose groups and a return toward baseline for the placebo group (Figure 2). Both treatment groups maintained statistical significance and superiority relative to the placebo group and large effect sizes at 1 year.

## MRI Review

Of the initial 39 patients, 37 completed MRI evaluation 6 months after SVF injection. There were no signs of new cyst

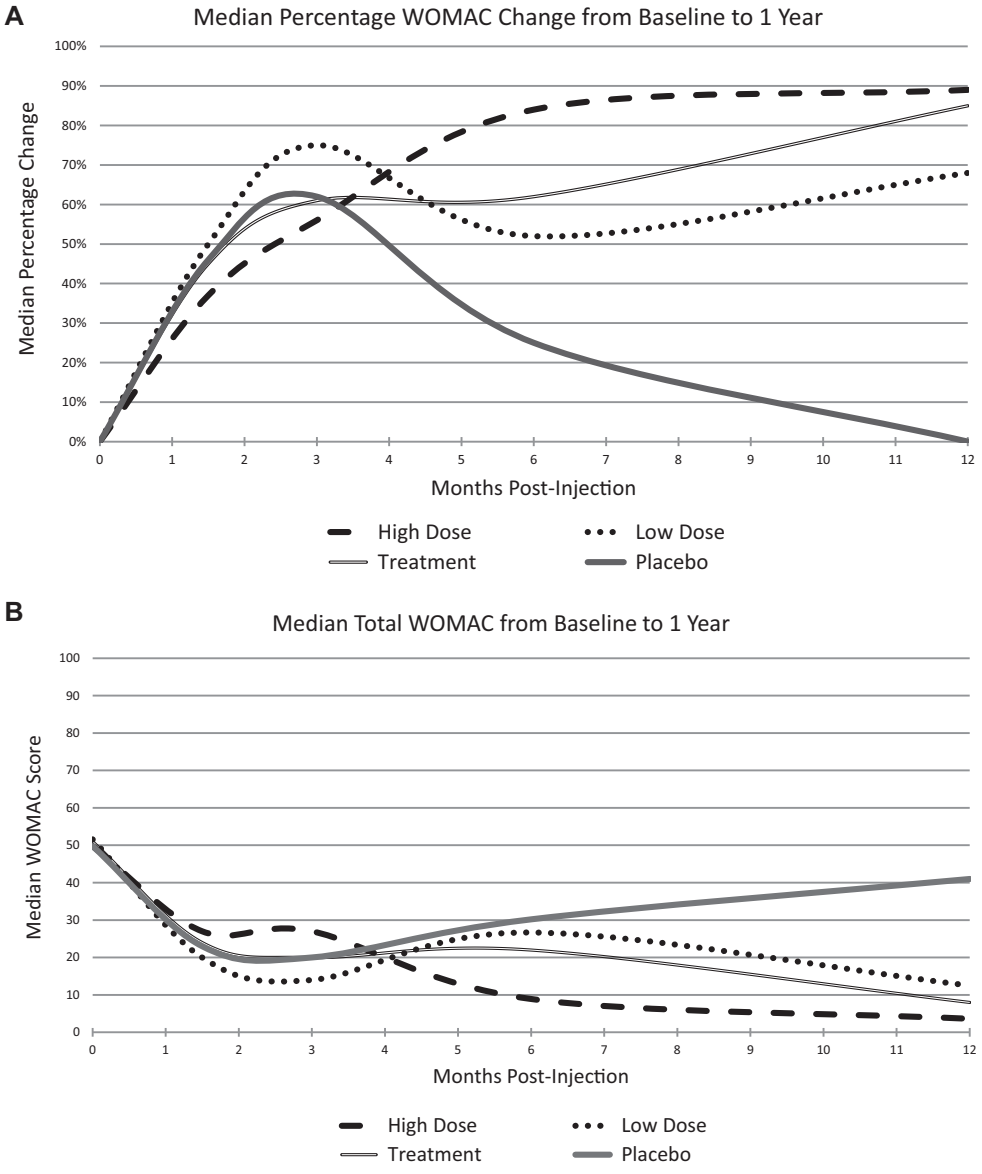


**Figure 2.** (A) Median overall percentage improvement in WOMAC scores over time. (B) Median total WOMAC scores over time. The high- and low-dose groups demonstrated an improvement in WOMAC scores 6 months and again at 1 year after injection. Treatment group represents the low- and high-dose groups combined. WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

formation, heterotopic ossification, or neoplasms (benign or malignant) of the bone, cartilage, synovium, or vasculature. Sixty lesions in the 39 patients were evaluated for changes in cartilage thickness and changes in Outerbridge classification. Outerbridge classification ranged from 1 to 4, with patients having 1 to 4 lesions of various grades. At 6-month follow-up, the mean change in cartilage thickness for all participants was 0 mm (Table 4). The mean changes in cartilage thickness for the treatment group and the placebo group were –0.2 mm and 0.5 mm, respectively, with no statistical difference between groups ($U = 316$, $P = .89$). The median change in Outerbridge classification at 6 months was 0 for the treatment group and the placebo group, with no statistically significant difference between baseline and 6 months per within-group evaluation ($V = 30$, $P = .46$; $V = 0$, $P \geq .99$ [respectively]).

Of the initial 39 patients, 23 completed MRI evaluation at 1 year. Patient attrition is detailed in Table 5. There were no visibly quantifiable changes in knee cartilage thickness (Table 6). One MRI scan (high-dose group) was notable for showing new subchondral cystic changes, and another (placebo group) was notable for showing a new paramenisical cyst. All other MRI scans revealed no changes from baseline or any evidence of disease progression.

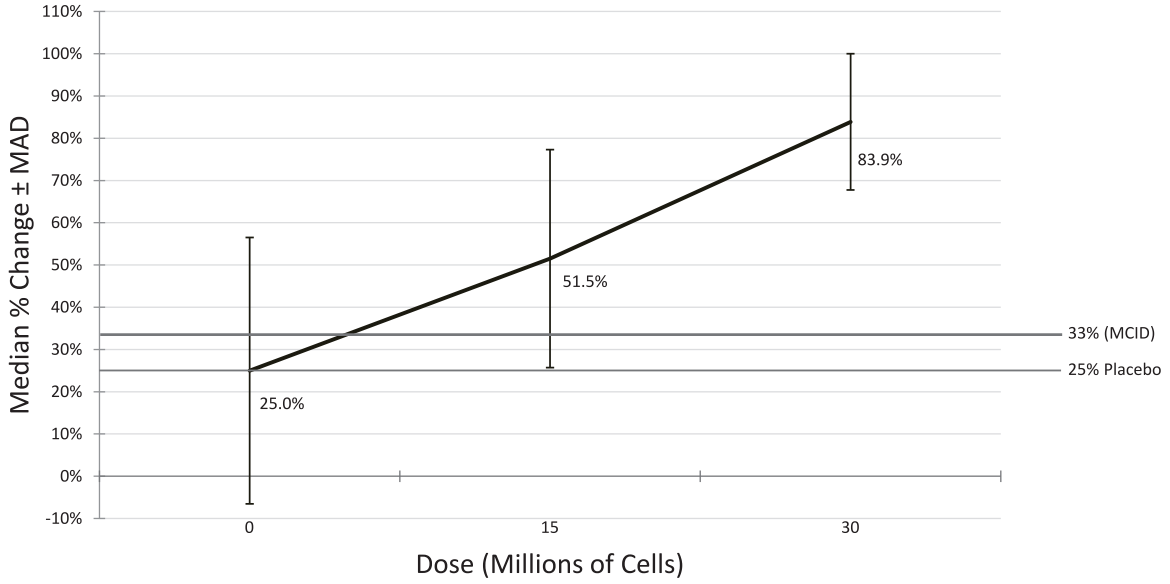


**Figure 3.** Dose-response curve at 6 months. Error bars represent median absolute deviation (MAD), a measure of variation around the median, representing the median of the values on each side of the group median. MCID, minimal clinically important difference.

TABLE 4
Changes in Cartilage from Baseline to 6 Months using MRI[a]

| | | Cartilage Loss | | Outerbridge Classification | |
|---|---|---|---|---|---|
| Group | Lesions, n | Baseline Mean, mm | Mean Change at 6 mo, mm | Baseline Median (Range) | Median Change at 6 mo |
| All | 60 | 12.6 | 0 | 3 (1-4) | 0 |
| Treatment group | 46 | 11.5 | −0.2 | 3 (1-4) | 0 |
| Placebo group | 14 | 16.3 | 0.5 | 4 (1-4) | 0 |
| Responders, >MCID | 38 | 13.2 | 0.2 | 3 (1-4) | 0 |
| Nonresponders, <MCID | 22 | 11.6 | −0.4 | 3 (1-4) | 0 |

[a]MCID, minimal clinically important difference.

## Adverse Events

During the initial 6 months, no serious adverse events were reported, and 3 adverse events were reported with none greater than grade 1 on the common terminology criteria for adverse events rating scale.[21] One patient from the high-dose group reported knee swelling, and aspirated fluid was sent for culture, with no growth. Two SVF sample cultures at the central laboratory for the study indicated possible bacteria growth, having only 1 colony in the culture plate. Those patients were evaluated, with no infection identified. None of these events were associated with infections. No adverse events of any type were reported during the 6-month to 1-year follow-up period.

## DISCUSSION

Nonoperative management is the primary treatment for knee OA symptoms. While current nonoperative modalities can offer symptomatic relief, these treatment modalities often fail, ultimately leading to knee arthroplasty. There is a need for more effective nonoperative knee OA treatment modalities, especially ones that may arrest or even reverse disease progression. The results from our study demonstrate a clinically meaningful improvement in knee OA symptoms and pain 6 months and 1 year after intra-articular injection of a high dose ($3.0 \times 10^7$ cells) or low dose ($1.5 \times 10^7$ cells) of SVF cells. The percentage improvement in WOMAC scores for both SVF dose treatment groups was >33%, the predetermined MCID for this study, at 6 months and 1 year. The MCID of 33% represents the magnitude of improvement needed for patients to experience a clinically meaningful improvement in their symptoms; therefore, the superiority of their improvement was clinically meaningful. The improvements in WOMAC scores in the treatment groups were significantly greater than the improvement experienced by patients in the placebo treatment group at 6 months and 1 year. Furthermore, WOMAC scores continued to improve for the high- and low-dose SVF groups from 6 months to 1 year after treatment. In contrast, the WOMAC scores for the placebo group declined after 3

TABLE 5
Six-Month and 1-Year Magnetic Resonance Imaging Attrition

| | | | Attrition Cause | | |
|---|---|---|---|---|---|
| Treatment Group | Initially Enrolled | Completed Assessment | Patient Exited Study | Alternative Treatment[a] | Declined to Participate |
| 6 mo | | | | | |
| High dose | 13 | 12 | 1 | 0 | 0 |
| Low dose | 13 | 13 | 0 | 0 | 0 |
| Placebo | 13 | 12 | 0 | 0 | 1 |
| 1 y | | | | | |
| High dose | 13 | 9 | 1 | 1 | 2 |
| Low dose | 13 | 10 | 0 | 2 | 1 |
| Placebo | 13 | 4 | 1 | 3 | 5 |

[a]Total knee replacement or intra-articular injection (corticosteroids, hyaluronic acid, or platelet-rich plasma).

TABLE 6
Changes in Cartilage from Baseline to 6 Months using MRI[a]

| Group | Lesions, n | Mean at Baseline, mm | Mean Change, mm |
|---|---|---|---|
| All | 38 | 10.4 | 0.0 |
| Treatment group | 33 | 9.9 | −0.1 |
| Placebo group | 5 | 14.2 | 0.8 |
| Responders, >MCID | 27 | 10.6 | 0.1 |
| Nonresponders, <MCID | 11 | 10.2 | −0.2 |

[a]MCID, minimal clinically important difference.

months and continued to decline toward baseline during the 6-month to 1-year period. This demonstrated the potential for SVF to provide symptomatic relief for a greater time frame than other OA treatments.

Our results are similar to previous studies assessing the efficacy of SVF injections. Fodor and Paulseth[8] and Garza et al[11] identified similar results in pilot studies assessing the safety and feasibility of intra-articular SVF injections in 6 patients (8 knees) and 6 patients (10 knees) with knee OA via the same method of SVF preparation, respectively. Similarly, Yokota et al[26] identified a significant 32% improvement in WOMAC scores and 40% improvement in pain visual analog scale scores 6 months after SVF injection in 13 patients.

In this investigation, the treatment (both doses) and placebo groups obtained the majority of improvement during the first 3 months; however, knee function in the treatment group continued to improve between 3 and 6 months and thereafter to 1 year. In contrast, knee function in the placebo group began to decline after the 3-month point, with continued decline toward baseline at 6 months and 1 year. At 1 year after injection, the treatment group showed a median improvement of 85%, the placebo group showed a median improvement of 0%. Similarly, previous studies have also identified a sustained improvement in knee pain and function 1 year after SVF injections.[8,12] In contrast, the efficacy of corticosteroid or hyaluronic acid injections 1 year after treatment has not been established.

Although patients receiving SVF injections had significantly better knee function, MRI review revealed no changes in modified Outerbridge classifications over time and no differences in the changes in chondral thickness between groups. However, it should be noted that the mean change in cartilage thickness (anteroposterior dimension) for all groups was less than the resolution of the MRI measurement. Our results contrast with those from a study performed by Hong et al,[12] which evaluated the efficacy of SVF injections for knee OA as compared with hyaluronic acid injections. MRI performed at 1-year follow-up demonstrated significantly better defect filling and cartilage repair in knees that received SVF as compared with those that received hyaluronic acid. However, these patients underwent arthroscopic debridement before treatment injection, and MRI scans were evaluated with WORMS (whole-organ magnetic resonance imaging score) and the MOCART score (magnetic resonance observation of cartilage repair tissue) for MRI analysis. This may account for the differences in results. Bansal et al[2] also performed MRI analysis 1 year after SVF injections. They observed an increase in cartilage thickness of at least 0.2 mm in 6 patients, no change in 2 patients, and a decrease in cartilage thickness of 0.2 mm in 2 patients. However, the mean change in cartilage thickness was not reported, and platelet-rich plasma injections were administered concomitantly with the SVF injections; therefore, direct comparisons are not possible.

The large effect sizes observed in this clinical trial are noteworthy, with a large area under the curve (>0.70) in both dose groups. This indicates that the statistical superiority of SVF as compared with placebo was large and likely had a clinically meaningful effect on patients' symptoms. The effect size observed in this study can also be compared with previous studies investigating the efficacy of bone marrow–derived MSCs. Emadedin et al[6] reported the effect size of bone marrow–derived MSCs as compared with saline placebo injections as medium to large at 6 months after injection, with a Hedges *g* of 0.7 for function measured on the WOMAC. Of note, the effect size of SVF injections identified in this study and that of bone marrow–derived MSC injections observed by Emadedin et al

similar; however, the MSCs used by Emadedin et al were isolated and cultured in a separate laboratory, while SVF was obtained and injected at the same clinical visit.

The dose-response curve provides meaningful guidance with regard to a dose-response relationship. Both dose groups were shown to be safe with respect to adverse events and to have similar statistical comparisons relative to placebo. The dose-response curve and the superiority and effect size assessments show that the high dose provided additional therapeutic relief of OA pain over the low dose.

While multiple studies have reported outcomes after SVF injections, differences in methodology make our trial unique. To our knowledge, this is the first randomized blinded multisite trial to assess the efficacy of SVF injection as compared with intra-articular placebo injections. Given the known positive response to placebo injections within populations with knee OA, the inclusion of a placebo arm in this trial helped strengthen the conclusions.[22] Our study similarly showed symptomatic improvement with placebo injections, although significantly less than our treatment groups, beyond 3 months. Although only 39 patients were included in this trial, it represents one of the largest to assess the utility of SVF injections. Moreover, the SVF suspension was not combined with any other treatment modalities, allowing for specific evaluation of SVF therapy. We collected, processed, and injected SVF cells during 1 patient visit, simulating the ideal treatment scenario. The multisite design increased the generalizability of our results. Finally, the comparison of percentage reduction in WOMAC scores with the calculated MCID allowed for clear clinical interpretation of our results.

While informative, this trial does have limitations. The high percentage of Caucasians in this study may limit its generalizability. Furthermore, patients with a body mass index $\geq$35 and other comorbidities were excluded, thus limiting generalizability. The primary purpose of the MRI scans was to assess safety and not for statistical analysis of efficacy (pain/function) among groups. Patients were also unblinded after 6 months, potentially biasing the 1-year results. Finally, there was considerable attrition in the control group at 1 year, which may have biased the results; however, its 6-month WOMAC scores were imputed for the 1-year results. Four of the 6 patients lost to follow-up sought additional therapy for their knee pain in the 6-month to 1-year period and thus were lost to follow-up. The use of the 6-month imputed scores for the 12-month missing values is considered conservative given the additionally sought therapies. Further research is needed to assess the efficacy of SVF treatment in patients with other comorbidities. Long-term outcomes and delay or elimination of progression to total knee arthroplasty after SVF treatment should also be investigated. Finally, the cost and risks of any treatment should be weighed against the benefit. While this trial demonstrated that SVF injections are safe and efficacious, the cost cannot be accurately estimated at this time. If SVF injections become commercially available for the treatment of knee OA, a cost analysis should be performed for comparison with other available treatment options.

## CONCLUSION

In conclusion, intra-articular SVF injections can significantly decrease knee OA symptoms and pain at 6 months and 1 year. Both low- and high-dose treatments had a large effect size, with the greatest change in the high-dose group. The efficacy and safety of SVF support its use as a treatment option for symptomatic OA of the knee. Longer-term results are needed to determine if there is any effect of SVF on disease progression.

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PLOS ONE

RESEARCH ARTICLE

# Clinical efficacy and safety of mesenchymal stem cell transplantation for osteoarthritis treatment: A meta-analysis

**Ma Yubo[1☯‡], Li Yanyan[2☯‡], Li Li[3], Sun Tao[4], Lin Bo[1], Chen Lin[5]***

**1** Department of Orthopaedics, Hongqi Hospital, Mudanjiang Medical University, Mudanjiang City, Heilongjiang Province, China, **2** Department of Neurology, The Second People Hospital of Mudanjiang, Mudanjiang City, Heilongjiang Province, China, **3** Department of Basic Medicine, Mudanjiang Medical University, Mudanjiang City, Heilongjiang Province, China, **4** Department of Radiology, Hongqi Hospital, Mudanjiang Medical University, Mudanjiang City, Heilongjiang Province, China, **5** Department of Orthopaedics, The 2nd Affiliated Hospital of Harbin Medical University, Harbin City, Heilongjiang Province, China

☯ These authors contributed equally to this work.
‡ These authors are co-first authors on this work.
* dr_chenlin@yeah.net





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## Abstract

### Purpose

The aim of this study was to evaluate the therapeutic efficacy and safety of mesenchymal stem cells (MSCs) for the treatment of patients with knee osteoarthritis (OA).

### Materials

We performed a meta-analysis of relevant published clinical studies. An electronic search was conducted for randomized controlled trials (RCTs) of MSC-based therapy in knee OA. The visual analogue scale (VAS), International Knee Documentation Committee (IKDC) form, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Lequesne algofunctional indices (Lequesne), Lysholm knee scale (Lysholm), Tegner activity scale (Tegner) and adverse events (AEs) were evaluated.

### Results

Eleven eligible trials with 582 knee OA patients were included in the present meta-analysis. We demonstrated that MSC treatment could significantly decrease VAS and increase IKDC scoresafter a 24-month follow-up compared with controls ($P<0.05$). MSC therapy also showed significant decreases in WOMAC and Lequesne scores after the 12-month follow-up ($P<0.01$). Analysis of Lysholm (24-month) and Tegner (12- and 24-month) scores also demonstrated favorable results for MSC treatment ($P<0.05$).

### Conclusion

Overall, MSC transplantation treatment was shown to be safe and has great potential as an efficacious clinical therapy for patients with knee OA.

## 1. Introduction

The knee is a marvel of engineering that enables sophisticated movements and also acts as a conduit for transferring body weight in a way that is essential for normal human mobility [1,2]. Knee osteoarthritis(OA) is a chronic disease which affects all races, genders and ages but is known to be most in obese and in elderly people [3]. Knee OA includes self-reported knee OA, radiographic definitions of knee OA, and symptomatic knee OA (self-reported joint pain, stiffness, tenderness, and radiographic evidence) [4]. The menisci are known to maintain the normal function of the knee, distribute loads, lubricate the joint, and facilitate joint stability [5–7]. In general, partial or total meniscectomy causes OA of the knee [8,9]. Worldwide, arthritis is considered to be the fourth leading cause of disability [10,11]. In developing and developed countries, OA may cause a significant decline in the quality of life for individuals above the age of 65 due to joint pain and disability [2,12–15].

The basic pathophysiological characteristic of OA is a loss of articular cartilage, although the synovial membrane, bone or other components of the joint may also be affected [2,16–18]. Chondrocytes are the main component of the cartilage. These cells are relatively inert, and rarely regenerate [13–15]. The outer third of the meniscus (also known as the red-red zone) has better self-healing capabilities compared with other regions due to a good blood supply. Conventional therapies for OA include physiotherapy, anti-inflammatory drugs, pain-relieving drugs, hyaluronic acid, platelet-rich plasma or corticosteroid-based intra-articular injections, and knee arthroscopic surgery [19–21]. Unfortunately, these treatments have demonstrated modest clinical benefits compared with controls, and articular replacement by prosthesis is recommended as a last therapeutic option [2,3,5].

Medical researchers believe that tissue engineering, an innovative and effective therapy method, is the next logical step in the progression of surgical intervention [5,22,23]. There are three main types of cells used in the clinical trials for knee OA or degenerative conditions, including mesenchymal stem cells (MSCs), articular chondrocytes, and meniscal fibrochondrocytes (MFCs). Among the various cell therapies, MSC therapies are promising for the treatment of OA and have shown encouraging results. Clinicaltrials.gov lists 125 registered trials of knee OA with the key words of "MSCs" and "knee osteoarthritis"until October 2016, including umbilical cord-derived mesenchymal stem cells (UCMSCs), bone marrow-derived mesenchymal stem cells (BMSCs), adipose-derived stem cells (ADSCs), synovium-derived mesenchymal stem cells (SMSCs), and meniscus-derived mesenchymal stem cells (MeMSCs). In 2011, Cupistem (Anterogen) was approved by the Korean Food and Drug Administration (FDA) for the treatment of OA, and UCMSCs were the main ingredient of this drug.

In this study, we performed a systematic review and meta-analysis of randomized controlled trials (RCTs) to assess the efficacy and safety of MSC-based stem cell therapy in knee OA treatment and to provide additional treatment options for patients with knee OA. The goal of the present study was to evaluate the clinical response to MSC-based stem cell therapy by using the Lysholm knee scale (Lysholm), Tegner activity scale (Tegner), visual analogue scale (VAS), International Knee Documentation Committee (IKDC) form, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Lequesne algofunctional indices (Lequesne), and adverse events (AEs).

## 2. Materials and methods

### 2.1. Search strategy, study design, and eligibility criteria

Science Direct, Springer-Link, PubMed, the Wangfang Database, the China Science and Technology Journal Database, and China Journal Net were searched for the relevant studies published from 1980 to October, 2016. The search strategy included the keywords ("mesenchymal

stem cells" OR "MSCs") AND ("knee osteoarthritis" OR "knee articular cartilage regeneration" OR "knee cartilage defect") AND clinical trial, without language restriction. We also searched the Clinicaltrials.gov for information on ongoing trials, using the keywords ("MSCs") AND ("knee osteoarthritis"). Publication citations were displayed at the bottom of the "Full Text View" tab of a study record, under the "More Information" heading. Furthermore, previously published clinical trials, relevant review articles, and postgraduate papers were examined to identify further relevant studies. Studies were eligible for inclusion if: (1) they were published RCTs in humans of MSC transplantation therapy for patients with knee OA, (2) the patient's detailed information was reported both prior to and after therapy, and (3) the study enrolled 10 or more patients. Phase IMSC-based stem cell therapy trials and review studies were excluded. In addition, case reports, studies on animal models and cell lines, and studies with no appropriate control arm were excluded.

## 2.2. Data selection criteria and quality assessment

Study selection and data extraction were independently conducted by two reviewers (Li Yan-yan and Li Li) using a standardized approach. Any differences were adjudicated by a third reviewer (Ma Yubo) after referring back to the original publication. The extracted study data features included the first author name, year and country of publication, clinical trial phase, sample size per arm, mean patient age, previous treatments, follow-up time, and dose and route of MSCs administration. The overall quality of each included paper was evaluated by the Jadad scale [24]. Several major criteria were employed in a grading scheme: (1) randomization, (2) allocation concealment, (3) blinding, (4) lost to follow up, (5) intention to treat (ITT), and (6) baseline.

## 2.3. Definition of outcome measures

VAS improvement was defined as the mean change in VAS from baseline. IKDC and WOMAC improvement were defined as the mean changes in IKDC and WOMAC from baseline, respectively. Lequesne reduction was defined as the mean change in Lequesne from baseline. The primary outcome measures were absolute change in VAS, IKDC, WOMAC, and Lequesne. Lysholm and Tegner improvement were defined as the mean changes in Lysholm and Tegner from baseline, respectively. Secondary outcome measures were absolute change in Tegner and Lysholm clinical scores.

## 2.4 Statistical analysis

In this meta-analysis, we compared the MSC treatment groups from the identified trials with their respective control groups using Review Manager Version 5.0 (Nordic Cochran Centre, Copenhagen, Denmark). Heterogeneity among the trials was assessed with the $\chi^2$-based Q-test and the $I^2$ statistic, such that $I^2>50\%$ was considered to indicate a high level of heterogeneity. Fixed- and random-effects models were used to estimate MSC treatment effects. Afixed-effects model was used when statistical heterogeneity was not confirmed; otherwise, a random-effects model was employed. The MSC treatment effects were reflected by the mean differences (MDs) with 95% confidence intervals (CIs). $P\leq0.05$ was considered to be statistically significant in all analyses, and all reported $P$-values resulted from two-sided version tests of the respective tests. To assess the possibility of publication bias, Egger's test and Begg's test were used (Stata version12.0, Stata Corporation, USA). We also used a funnel plot to evaluate publication bias.

## 3. Results

### 3.1. Trial selection

The data search yielded 84 references, 41 of which were excluded for various reasons (21 review articles, 13 in vitro experiments or animal models, 5 case reports, and 2 meta-analyses). A further 32 studies were excluded because they did not provide clinical data with enough detail or they were not RCTs. Finally, 11 trials met the specified inclusion criteria [25–35]. Fig 1 provides a flow-chart illustrating the search results and the exclusion mechanisms for certain studies. The quality assessment of the 11 trials is summarized in Table 1. Six of the included studies scored an A on the Jadad scale [26,27,28,30,31,32], and fivescored a B [25,29,33,34,35]. The funnel plots for the six analyses regarding VAS, IKDC, WOMAC, Lequesne, Lysholm, and Tegner were largely symmetrical (S1 Fig). Egger's test and Begg's test showed that there was no evidence of publication bias ($P>0.05$). Thus, publication bias did not seem to be present in our study.

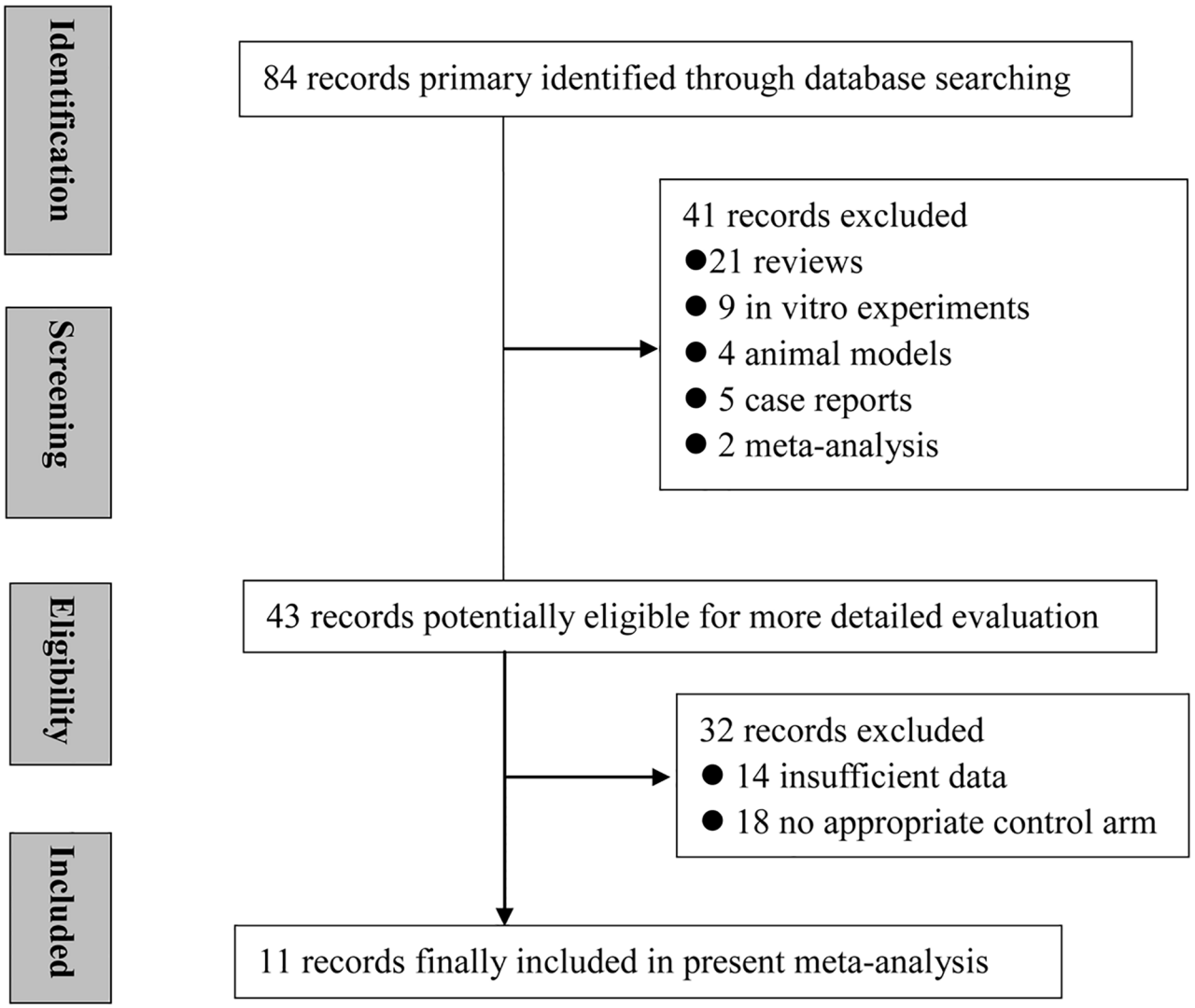


**Fig 1. Flow diagram showing the study identification, screening, and inclusion process.**

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**e 1. Jadad scale for the eligible trials.**

| Studies | Randomization | Allocation concealment | Blinding | Lost to follow up | ITT analysis | Baseline | Quality grading |
|---|---|---|---|---|---|---|---|
| **Nejadnik H 2010** [25] | B | A | B | A | A | A | B |
| **Koh YG 2012** [26] | A | A | A | A | A | A | A |
| **Saw KY 2013**[27] | A | A | A | A | A | A | A |
| **Wong KL 2013** [28] | A | A | A | A | A | A | A |
| **Tan YH 2013** [29] | A | A | B | A | A | A | B |
| **Koh YG 2014** [30] | A | A | A | A | A | A | A |
| **Vangsness CT Jr 2014** [31] | A | A | A | A | A | A | A |
| **Akgun I 2015** [32] | A | A | A | A | A | A | A |
| **Liang HS 2015** [33] | A | A | B | A | A | A | B |
| **Lv XX 2015** [34] | A | B | B | A | A | A | B |
| **Vega A 2015** [35] | A | A | B | A | A | A | B |

Abbreviations: A, adequate, with correct procedure; B, lacks a description of the methods; C, inadequate procedures, methods, or information; ITT, intention to treat. Each criterion was graded as follows: A, studies with a low risk of bias that were scored as grade A for all items; B, studies with a moderate risk of bias with one or more grades of B; and C, studies with a high risk of bias with one or more grades of C.

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## 3.2. Baseline patient characteristics

The baseline characteristics of the patients in the 11 selected publications are listed in Table 2. The trials involved a total of 582 patients with knee OA. All 11of the papers were fully

**Table 2. Clinical information from the eligible trials in the meta-analysis.**

| Author and Year | Clinical trial phase | No. of patients (male) and control | Age (years, mean) and control | Follow up (months) | Control arm | Stem cell arm (Injection) | Regimens dose |
|---|---|---|---|---|---|---|---|
| **Nejadnik H 2010 (Singapore)** [25] | III | 36(20); 36(18) | 44.0; 42.5 | 25 | ACI | BMSCs (i.a) | $1\sim1.5\times10^7$ |
| **Koh YG 2012 (Korea)** [26] | II | 25(8); 25(8) | 54.2; 54.4 | 17.2 | AO+PRP | AO+ADSCs (i.a) | $1.89\times10^6$ |
| **Saw KY 2013 (Malaysia)]** [27 | II | 25(10); 24(7) | 38; 42 | 18 | AO +HA | AO+HA+PBSCs (i.a) | UK |
| **Wong KL 2013 (Singapore)** [28] | II | 28(15); 28(14) | 53; 49 | 24 | AO +HA | AO+BMSCs (i.a) | $1.46\times10^7$ |
| **Tan YH 2013 (China)** [29] | II | 36(10); 36(9) | 53.4; 53.8 | 12 | AO | AO+BMSCs (i.a) | $2\sim3\times10^7$ |
| **Koh YG 2014 (Korea)** [30] | II | 21(5); 23(6) | 54.2; 52.3 | 25.7 | AO+PRP | AO+ADSCs (i.a) | $4.11\times10^6$ |
| **Vangsness CT Jr 2014 (USA)** [31] | II | 18; 19 | 46.0 | 24 | placebo | BMSCs (i.a) | $5\times10^7$ |
| | | 18; 19 | | | | | $1.5\times10^8$ |
| **Akgun I 2015 (Turkey)** [32] | II | 7(4); 7(4) | 32.3; 32.7 | 24 | ACI | SMSCs (i.a) | $8\times10^6$ |
| **Liang HS 2015 (China)** [33] | II | 30(19); 30(18) | 36.2; 35.8 | 16.4 | AO | AO+BMSCs (i.a) | $1\times10^6$ |
| **Lv XX 2015 (China)** [34] | III | 40(14); 40(13) | 55.9; 55.1 | 12 | HA | BMSCs (i.a) | $1.15\times10^8$ |
| **Vega A 2015 (Spain)** [35] | II | 15(6); 15(5) | 56.6; 57.3 | 12 | HA | BMSCs (i.a) | $4\times10^7$ |

Abbreviations: ACI, autologous chondrocyte implantation; ADSCs, adipose-derived stem cells; AO, arthroscopic operation; BMSCs, bone marrow-derived mesenchymal stem cells; HA, hyaluronic acid; i.a., intra-articular injection; PBSCs, peripheral blood stem cells; PRP, platele-rich plasma; SMSCs, synovium-derived mesenchymal stem cells; UK, Unknown.

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published during the period from 2010 to 2015 and described nine Phase II trials. The mean ages of patients enrolled were between 32 and 57 years. Sample size ranged from a minimum of 14 to a maximum of 80 patients. The percentage of male patients ranged from 25% to 62%. In all of the trials, MSC transplantation therapy was evaluated in knee OA patients with BMSCs in 7 studies [25,28,29,31,33,34,35], ADSCs in 2 studies [26,30], peripheral blood stem cells (PBSCs) in 1 study [27], and SMSCs in 1 study [32]. The patients received cell infusions from$1\times10^6$ to $1.5\times10^8$ cells. The injected route for MSC therapy was intra-articular injection (i.a.).

### 3.3. Visual analogue scale

Information on the 6-month VAS improvement was available from two trials [31,32]. These two trials contained a total of 88 patients, of whom 43 patients received MSC treatment, and 45 control patients did not receive MSC transplantation. The MD of changes in VAS of patients receiving MSC treatment was a non-significant decrease of -10.55 (95%CI -21.86–0.77, $P = 0.07$, $I^2 = 94\%$) compared with that of the controls. In three trials that reported 12-month VAS, the MD of changes in VAS was -10.22 (95%CI -22.48–2.04, $P = 0.10$, $I^2 = 95\%$). Information on the 24-month VAS improvement was available from five trials [26,30,31,32,33]. These five trials contained a total of 242 patients, of whom 119 patients received MSC treatment. The MD of changes in VAS of patients receiving MSC treatment was a significant decrease of -5.78 (95%CI -8.05- -3.52, $P<0.00001$) compared with that of the controls. Additionally, the corresponding $I^2$ was 97% (Fig 2).

### 3.4. International Knee Documentation Committee

Information on the 6-, 12-, and 24-month IKDC improvement was available from three trials [25,27,28], totaling 177 patients (89 of whom received MSC treatment; Fig 3). MSC therapy led to a 6-month IKDC increase of 1.41 (95%CI -2.76–5.58, $P>0.05$, $I^2 = 44\%$) in patients with knee OA. The MD of changes in 12-month IKDC was 2.21 (95% CI -2.78–7.21, $P>0.05$, $I^2 = 59\%$). The MD of changes in 24-month IKDC was statistically significant at 4.89 (95% CI 0.36–9.42 $P = 0.03$). Additionally, the corresponding $I^2$ was 57%.

### 3.5. Western Ontario and McMaster Universities Osteoarthritis

Information on the 12-month WOMAC improvement was available from two studies [34,35], which included a total of 110 patients (55 of whom received MSC treatment; Fig 4). The MD of WOMAC changes was statistically significant at -11.05 (95% CI -15.97- -6.14, $P<0.0001$). Additionally, the corresponding $I^2$ was 0%, indicating that the degree of variability between the trials was consistent with what would be expected by chance alone.

### 3.6. Lequesne algofunctional indices

Information on the 12-month Lequesne improvement was available from two studies [29,35], which included a total of 102 patients (51 of whom received MSC treatment; Fig 5). The MD of Lequesne changes was statistically significant at -5.32 (95% CI -5.91- -4.74, $P<0.00001$). Additionally, the corresponding $I^2$ was 0%.

### 3.7. Lysholm knee scale

The MD of changes in 6-month Lysholm was 2.21 (95%CI -3.52–7.95, $P>0.05$, $I^2 = 36\%$). In three trials that reported 12-month outcomes, the MD of changes in Lysholm was 2.02 (95%CI -6.25–10.30, $P>0.05$, $I^2 = 63\%$) [25,28,31]. Information on the 24-month Lysholm was

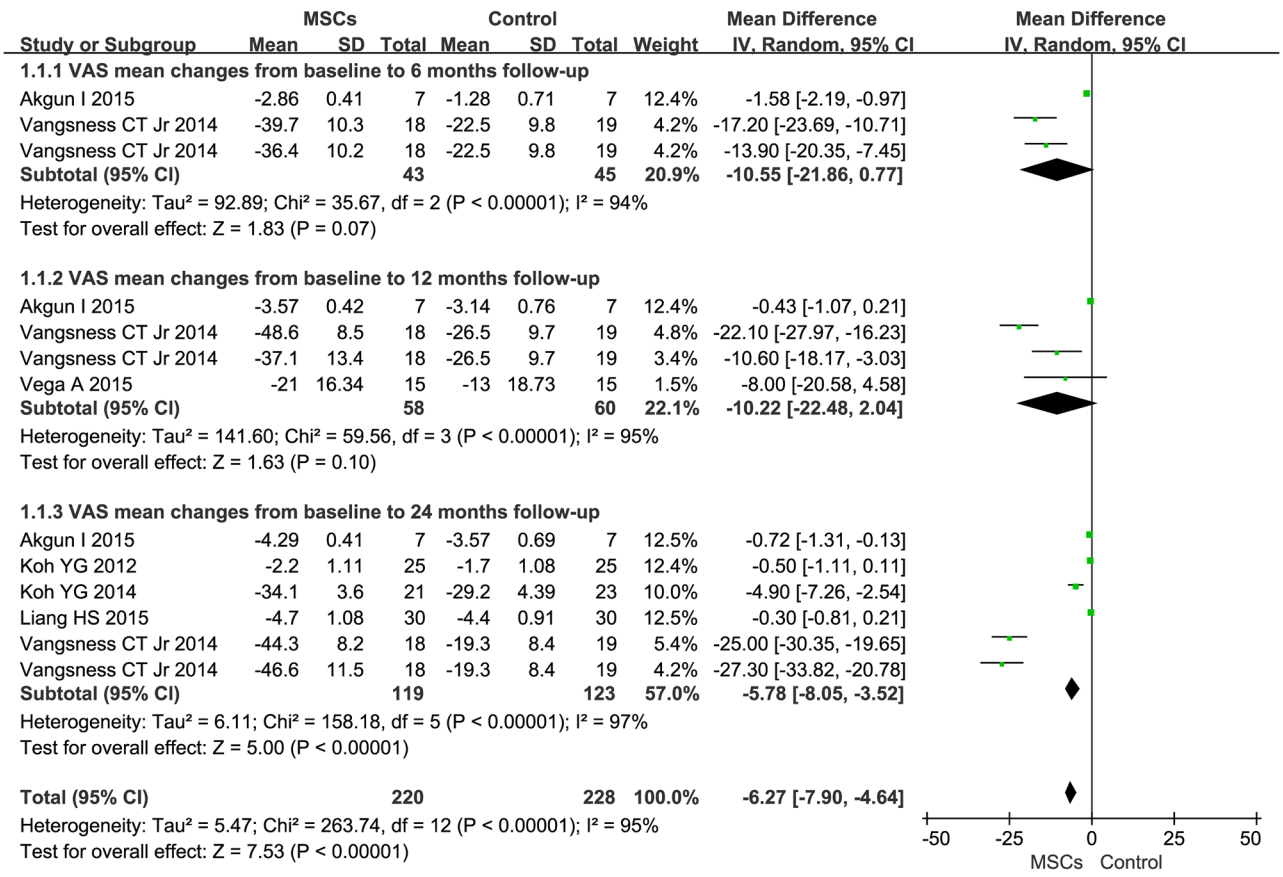


**Fig 2. Forest plots of mean difference (MD) with 95% confidence interval (CI) in VAS between patients undergoing MSC therapy and controls at: (1) 6 months, (2) 12 months, and (3) 24 months.** Random-effects models (Mantel-Haenszel method) were used. Each trial is represented by a square, and the size of the square is proportional to the information in that trial. The ends of the horizontal bars denote 95% confidence intervals (CIs). Black diamonds give the overall results of all trials.

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available for six trials [25,26,28,30,31,33]. These 6 trials contained a total of 356 patients (176 of whom received MSC treatment and 180 controls who did not receive this treatment). The MD of changes in Lysholm was 7.96 (95%CI 4.24–11.68, $P<0.0001$, $I^2 = 44\%$). (Fig 6)

## 3.8. Tegner activity scale

The MD of changes in 6-month Tegner was 0.40 (95%CI -0.18–0.98, $P>0.05$, $I^2 = 68\%$). A pooled analysis of the data at 12 months showed that Tegner score increased significantly (MD 0.44, 95%CI 0.05–0.83, $P = 0.03$, $I^2 = 22\%$). A pooled analysis was performed on four trials at 24 months. The MD of Tegner changes was statistically significant at 0.46 (95% CI 0.21–0.72, $P = 0.0004$). Additionally, the corresponding $I^2$ was 0%, indicating that the degree of variability between the trials was consistent with what would be expected by chance alone.

## 3.9 Toxicity and adverse reactions

The clinical trials included in this meta-analysis reported several AEs, including pain at injection site, persistent bleeding, knee swelling, warmth in the knee, fracture, difficulty moving the knee, infection in the knee, nervous system disorders, acute myocardial infarction, ileus, and small-intestine obstruction [25–35]. However, there was no statistical difference between the

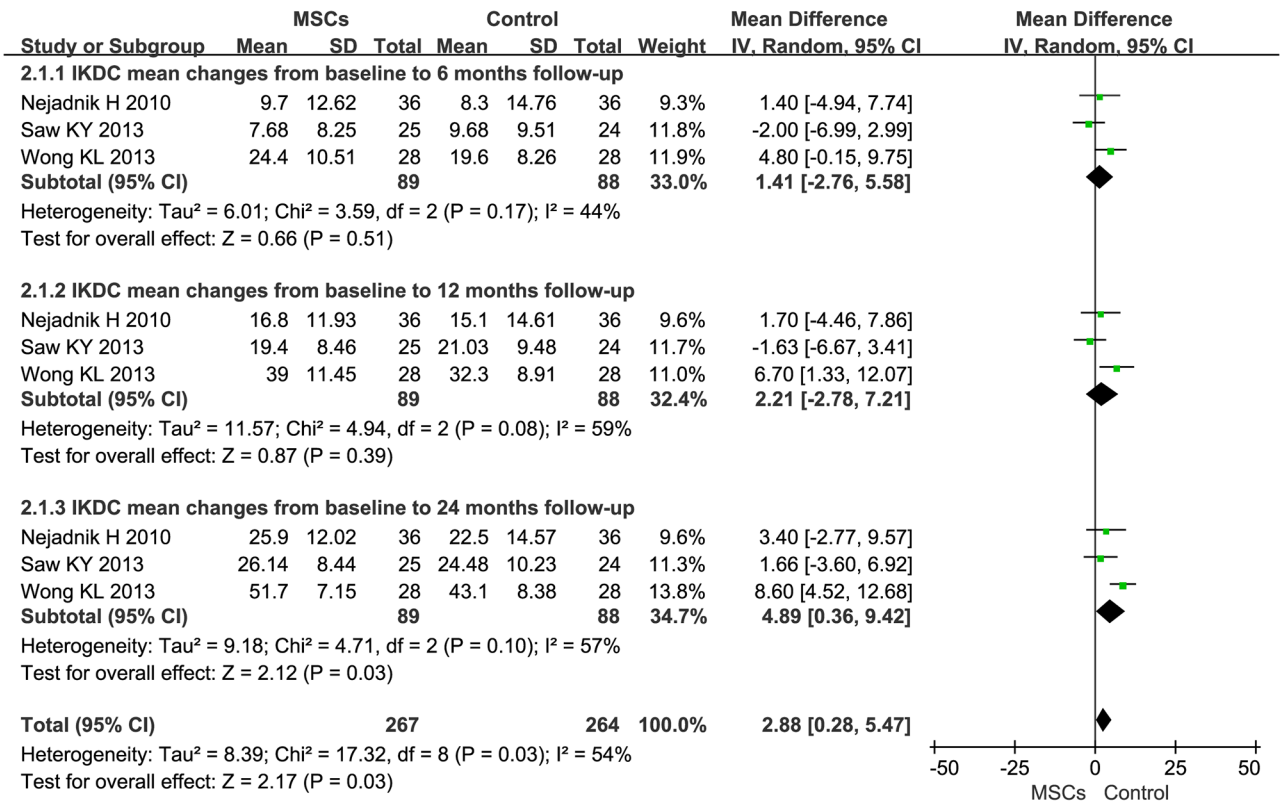


**Fig 3. Forest plots of MD with 95% CI in IKDC between patients undergoing MSC therapy and controls at: (1) 6 months, (2) 12 months, and (3) 24 months.** Random-effects models were used.

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MSC treatment groups and controls [27,31]. Moreover, no serious AEs related to MSC implantation were developed in the 11 selected publications. Another review also reported that the application of cultured stem cells in joints appeared to be safe [36].

## 4. Discussion

Knee OA is a progressive and degenerative condition, which will remain a serious clinical problem in orthopedics unless significant advancements are made in regeneration

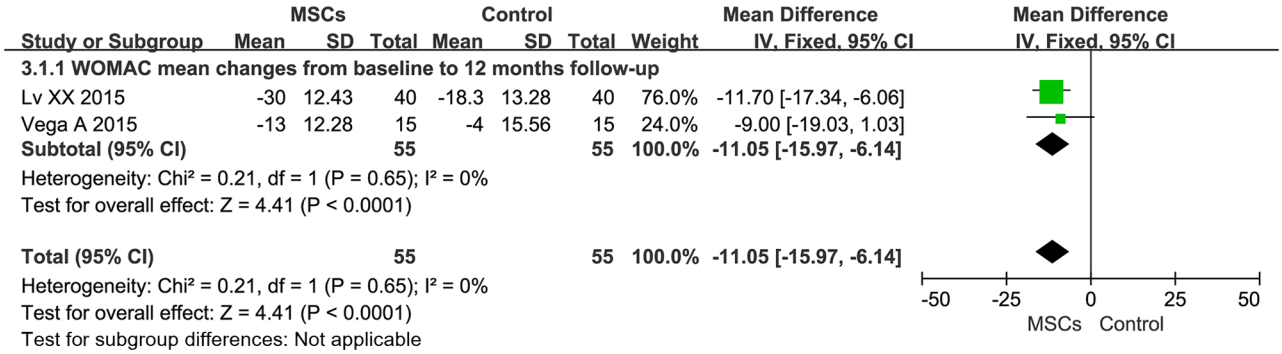


**Fig 4. Forest plots of MD with 95% CI in WOMAC between patients undergoing MSC therapy and controls at 12 months.** Fixed-effects models were used.

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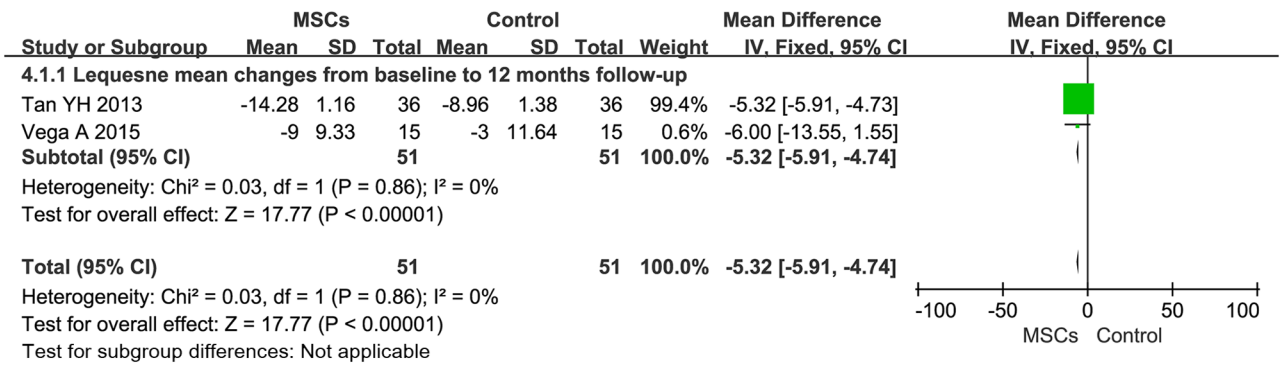


**Fig 5. Forest plots of MD with 95% CI in Lequesne between patients undergoing MSC therapy and controls at 12 months.** Fixed-effects models were used.

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technologies [2,37,38]. In fact, all of the currently accepted treatments are aimed at symptom control, rather than disease prevention [4,5]. MSCs are positive for the stromal cell markers CD13, CD29, CD73, CD90, and CD105 and negative for the hematopoietic markers CD31, CD34, CD45, and HLA-DR [2,39]. MSCs can inhibit the proliferation of

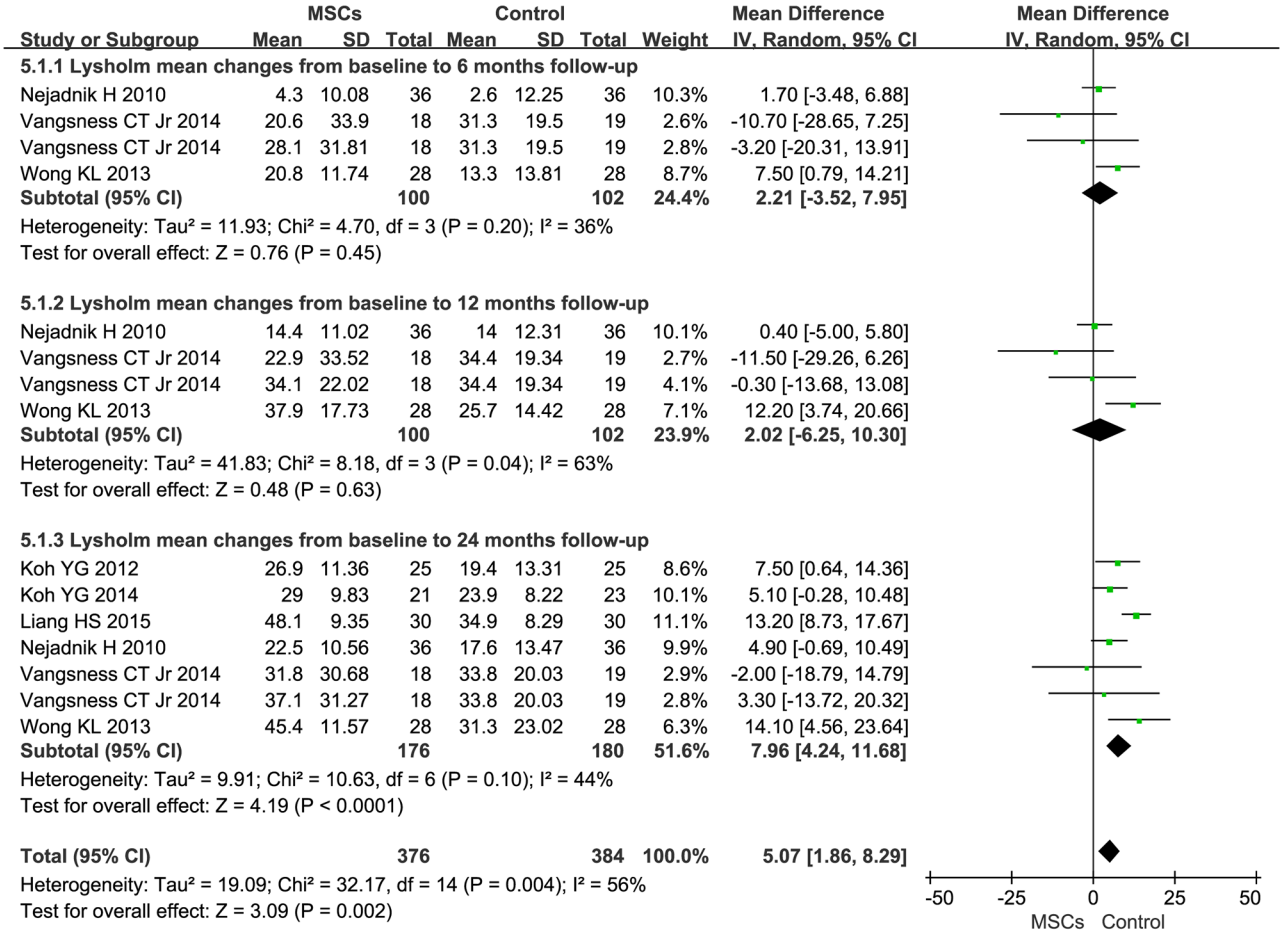


**Fig 6. Forest plots of MD with 95% CI in Lysholm between patients undergoing MSC therapy and controls at: (1) 6 months, (2) 12 months, and (3) 24 months.** Random-effects models were used.

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allogeneic T cells and express low levels of major histocompatibility complex (MHCI), MHCII, and vascular cell adhesion molecule-1 (VCAM-1), so it has low immunogenicity. The self-renewing ability of MSCs and differentiation potential to become adipocytes, osteocytes, and chondrocytes has been well documented [40]. Furthermore, the homing, survival, and ability to produce extracellular matrices of MSCs in vivo have been confirmed. Previous clinical studies have shown that MSCs provide an excellent therapeutic alternative for the treatment of knee OA [41,42]. Importantly, the recent limited case series evidence has shown the cartilage volume regeneration and the disease modification after MSC injections [4,5]. MSC-based stem cell therapy could represent one of the most promising solutions for knee OA. So far, data collected from clinical trials support the following assumptions: MSCs administered into the knee adhered to and persisted on the surface of a damaged meniscus, differentiated into chondrocytes, and expressed appropriate extracellular matrix proteins (i.e. collagen I and II), resulting in a regeneration of meniscal tissue, which, with an improved meniscus, could ultimately lead to long-term chondroprotection [31]. In the present study, we performed a systemic analysis of multinational, published RCTs to assess the efficacy and safety of MSC treatment for knee OA patients using VAS, IKDC, WOMAC, Lequesne, Lysholm, and Tegner scores.

Our analysis yielded several findings. First, we demonstrated that MSC treatment could significantly decrease VAS after a 24-month follow-up (Fig 2). The estimated pooled MD showed a significant increase in IKDC after the 24-month follow-up of MSC therapy (Fig 3). WOMAC and Lequesne also showed significant decrease after the 12-month follow-up of MSC therapy (Figs 4 and 5, respectively). However, the primary endpoints did not show significant changes at other time points. The positive trend was proven to exist. Our logistic regression results showed that MSC therapy could significantly change the long-term primary endpoints of knee OA patients. The effects of MSC therapy on short-term (6-month) primary endpoints still needs to be evaluated in a larger number of patients. A recently published study by Emadedin *et al.* on autologous BMSC transplantation in knee OA patients reported that VAS and WOMAC showed a significant decrease after the 6- and 12-month follow-up [43]. Thus, a larger sample size and more elegant clinical trials are needed. Patient knee pain, stiffness, and function was assessed with the use of VAS, IKDC, WOMAC, and Lequesne. The results of our analysis indicated that MSC treatment could significantly reduce pain, improve symptoms, and improve the function of a patient's knee OA.

Second, the secondary outcomes of Lysholm and Tegner scores showed favorable results after MSC treatment. The estimated pooled MD showed a significant increase in Lysholm after the 24-month follow-up but not after the 6-, and 12-month follow-up (Fig 6). Our pooled analysis of the collected data showed a significant increase in Tegner after the 12- and 24-month follow-up but not after the 6-month follow-up (Fig 7). This result might be due to the small number of patients in the analysis. Thus, based on logistic regression, we concluded: MSC therapy might improve signs and symptoms of knee OA patients. Additionally, MSC therapy was shown to be safe. These scales were all subjective evaluations of knee function for patients with OA. There are, however, some reports with objective assessments of cartilage volume and quality in the eligible trials. Vangsness *et al.* reported that the cartilage volume in MSC treatment groups showed a significant decrease, observed in MRI, after the 12-month follow-up [31]. But in another trial, all MSC treatment patients showed signs of cartilage regenerationin MRI after the 12-month follow-up [27]. Vega *et al.* also reported that the cartilage quality in MSC-treated patients showed a significant improvement [35], which suggests that MSC therapy is a potential therapy for knee OA to some extent.

There are some points that may explain these results. First, transplanted MSCs could differentiate into chondrocytes directly and promote cartilage regeneration. Horie and Mizuno

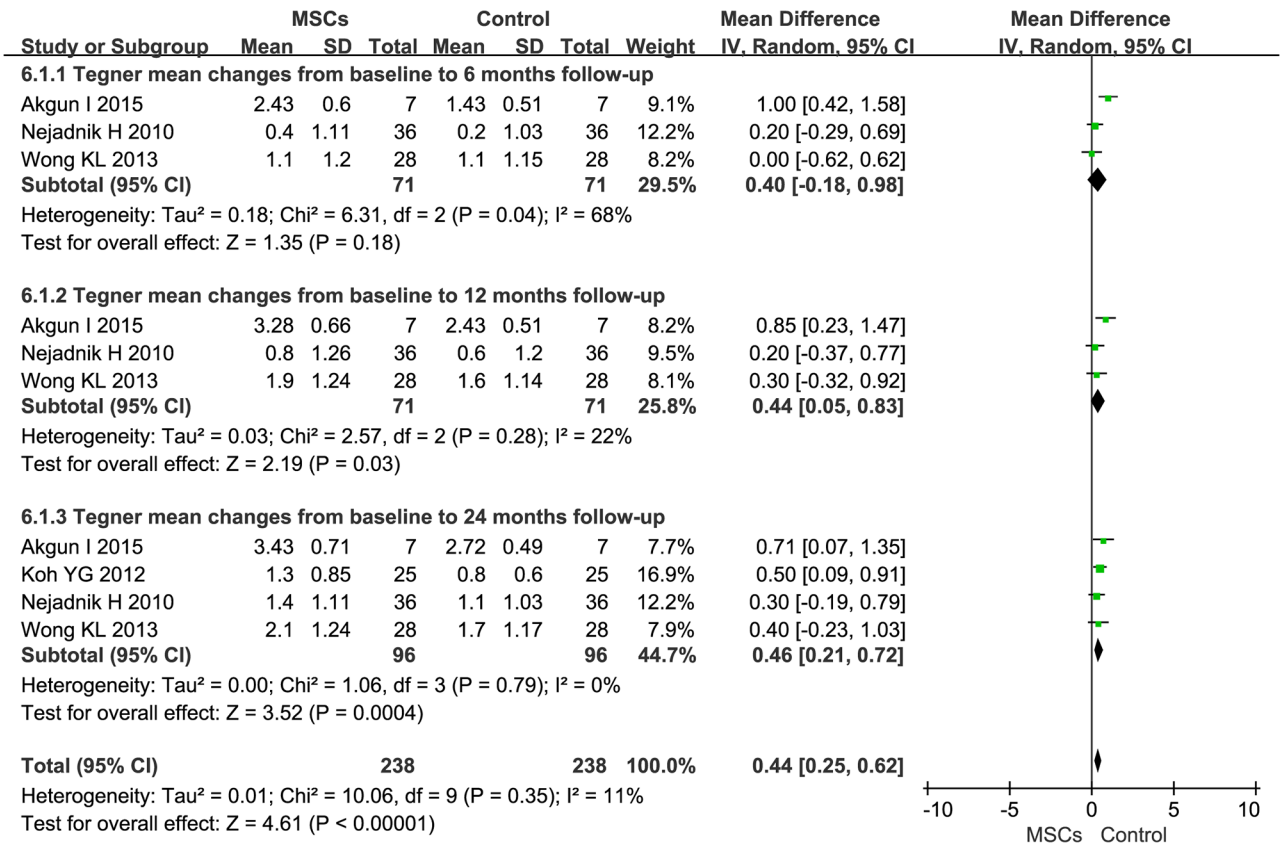


**Fig 7. Forest plots of MD with 95% CI in Tegner between patients undergoing MSC therapy and controls at: (1) 6 months, (2) 12 months, and (3) 24 months.** Random-effects models were used.

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reported that SMSCs injected into rat knees adhered to the lesion, differentiated into chondrocytes directly, and promoted cartilage regeneration without traveling to distant organs [44,45]. Another study showed that precultured BMSCs resulted in the regeneration of meniscal tears in a rabbit model [46]. Second, transplanted MSCs have trophic and paracrine effects on the existing cartilage. MSCs could secrete an array of growth factors and cytokines, including vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) for neovascularization andtransforming growth factor β (TGF-β), platelet-derived growth factor (PDGF), and epithelial growth factor (EGF) to augment natural regenerative pathways [47,48]. PDGF is the most potent factor analyzed, and may be useful to promote tissue integration during cartilage repair or tissue engineering. In contrast, several studies have shown that low physiologic doses of dexamethasone could ensure that MSCs differentiate toward chondrocytes or osteogenic cells [3]. MSCs could be procured from umbilical cord, placenta, bone marrow, and fat and can easily proliferate without the use of other supportive cells. Thus, we believe that MSCs are the most suitable for knee OA treatment, considering the multiple sources and therapeutic effect.

In short, our meta-analysis demonstrated that MSC-based stem cell therapy for patients with knee OA was associated with significantly decreased VAS, WOMAC and Lequesne scores; increased IKDC, Lysholm, and Tegner scores; and low rates of AEs.

## 5. Limitations

The therapeutic effects should be interpreted with caution. The reliability of this study might be influenced by several factors. (1) *Evaluation standards* The six scales used in the selected studies are all subjective evaluations. Although patients were asked to answer all questionnaires truthfully and to the best of their ability, our study may have a moderate risk of bias. (2) *Multicenter* Eight of the selected publications in this meta-analysis were conducted in Asia, and the other three were performed in the USA, Spain, and Turkey, respectively. There is no multinational large-sample multicenter clinical research regarding MSC therapy for knee OA. Thus, the results of this analysis could not be extended to all knee OA patients across the world. (3) *Blinding and Randomization* Half of the selected studies did not use the blind method. Not all selected publications demonstrated randomization, and the sample sizes of all selected trials were not large enough. These might lead to patient, distribution, or observer biases. (4) *Heterogeneity* The high heterogeneity limits the interpretation of our results. In addition, negative trial outcomes often remain unpublished, and some good efficacy articles were excluded because they lacked appropriate control arms. Thus, the results of our meta-analysis might be misleading. We expect that our study will be useful for the design of higher quality RCTs.

## 6. Future perspectives

In the near future, MSC-based stem cell therapy could be widely used as it potentially offers substantial benefits for knee OA patients and may reduce the cost of therapy. However, there are still some unanswered questions regarding the treatment mechanism, methodology for transplanting cells, and efficacy that need to be resolved before their widespread use. First, the use of allogeneic MSCs product would have several advantages compare with autologous MSCs. Induction of humoral and/or cellular alloimmunity by allogeneic MSCs would limit their therapeutic efficacy and might provoke adverse effects [49,50]. We urgently need large RCTs utilizing standardized and established outcome scores to evaluate the clinical benefits of MSCs in cartilage repair. MRI as an objective assessment is considered to be the best way to evaluate cartilage repair. Furthermore, we still need to explore the best cell dose and culture conditions and choose the best cell infusion method for MSC therapy. In addition, the combination of MSCs with scaffolds, PRP, growth factors, and even gene therapy is also being investigated to achieve the best therapeutic effect. Moreover, the regulation of MSC treatment for knee OA is a major challenge. This requires scientists and clinicians to develop a minimum set of safety and efficacy parameters. Finally, with the continuous progress that is being made in biomedical technology, the future of MSC therapy for patients with knee OA will move toward individualized treatment.

## 7. Conclusion

Eleven selected publications regarding knee OA with 582 patients were included in the present meta-analysis. This analysis of MSC therapy in knee OA patients yielded encouraging results, with superiority in VAS, WOMAC and Lequesne scores; improvements in IKDC, Lysholm, and Tegner scores; and low rates of AEs. Hence, these results suggest that MSC therapy has great potential as an efficacious treatment for patients with knee OA. However, the safety and efficacy must be evaluated with a more rigorous, larger sample size validation before MSC therapy can be used in clinical practice.

## Supporting information

**S1 Fig. A funnel plot of VAS, WOMAC, Lequesne, IKDC, Lysholm, and Tegner scores (tif) generated by Review Manager Version 5.0.**
(TIF)

**S2 Fig. Language edit certification (tif).**
(PDF)

**S1 File. PRISMA 2009 checklist.**
(DOC)

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## Author Contributions

**Conceptualization:** MY.

**Data curation:** LY LL.

**Formal analysis:** LB.

**Funding acquisition:** CL.

**Investigation:** LY LL.

**Methodology:** LY LL.

**Project administration:** CL.

**Resources:** ST.

**Software:** LY LL.

**Supervision:** CL.

**Validation:** ST.

**Visualization:** LY.

**Writing – original draft:** MY.

**Writing – review & editing:** CL.

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# ..ntra-articular Injection of Autologous Adipose-Derived Stem Cells or Stromal Vascular Fractions: Are They Effective for Patients With Knee Osteoarthritis?

## A Systematic Review With Meta-analysis of Randomized Controlled Trials

Kang-Il Kim,*† MD, PhD, Myung-Seo Kim,* MD, and Jun-Ho Kim,*‡ MD, PhD
*Investigation performed at Kyung Hee University Hospital at Gangdong, Seoul, Republic of Korea*

**Background:** Intra-articular injection of adipose-derived stem cells, which are divided into adipose-derived mesenchymal stem cells (ASCs) and adipose-derived stromal vascular fractions (ADSVFs), has been reported to be a viable treatment modality for knee osteoarthritis (OA); however, its efficacy remains limited.

**Purpose:** This study aimed to provide comprehensive information about the efficacy and safety of intra-articular injections of autologous ASCs and ADSVFs without adjuvant treatment in patients with knee OA.

**Study Design:** Meta-analysis; Level of evidence, 1.

**Methods:** A systematic search of the MEDLINE, Embase, Web of Science, and Cochrane Library databases was performed to identify randomized controlled trials (RCTs) that evaluated the efficacy and safety of intra-articular injections of autologous ASCs or ADSVFs without adjuvant treatments compared with placebo or hyaluronic acid in patients with knee OA. Clinically, the 100-mm visual analog scale for pain relief and the Western Ontario and McMaster Universities Osteoarthritis Index for functional improvement were implemented. Radiologically, cartilage status was assessed using magnetic resonance imaging (MRI). Procedure-related knee pain, swelling, and adverse events (AEs) were evaluated for safety. Additionally, we performed subgroup analyses comparing ASCs versus ADSVFs. Methodological quality was assessed using the modified Coleman Methodology Score (mCMS).

**Results:** A total of 5 RCTs were included in this study. Based on the meta-analysis, ASCs or ADSVFs showed significantly better pain relief at 6 months ($Z = 7.62$; $P < .0001$) and 12 months ($Z = 7.21$; $P < .0001$) and functional improvement at 6 months ($Z = 4.13$; $P < .0001$) and 12 months ($Z = 3.79$; $P = .0002$), without a difference in procedure-related knee pain or swelling compared with controls. Although a meta-analysis with regard to cartilage improvements was not performed owing to heterogeneous MRI assessment, 3 studies reported significantly improved cartilage status after the injection. No serious AEs associated with ASCs or ADSVFs were reported. Subgroup analyses showed similar efficacy between ASC and ADSVF treatments. The median mCMS was 70 (range, 55-75).

**Conclusion:** For patients with knee OA, intra-articular injection of autologous ASCs or ADSVFs without adjuvant treatment showed remarkable clinical efficacy and safety at short-term follow-up. Some degree of efficacy has been shown for cartilage regeneration in knee OA, although the evidence remains limited. Further RCTs that directly compare ASCs and ADSVFs are needed.

**Keywords:** adipose-derived stem cells; adipose tissue; knee osteoarthritis; mesenchymal stem cell; stromal vascular fraction

Knee osteoarthritis (OA) is a highly prevalent degenerative joint disorder that affects patients' quality of life.[12,25,40] OA is a progressive disease characterized by an imbalance between degeneration and regeneration that limits the knee cartilage potential for self-regeneration owing to the avascular nature of cartilage.[19,41,42] The

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eventual treatment for severe OA with intractable symptoms is surgery, such as knee arthroplasty; however, several concerns exist regarding surgery, such as comorbidities, surgical complications, and revision issues.[3,9,21,22,37,43,44] Various nonoperative treatments, including anti-inflammatory medications, physical therapy, and intra-articular (IA) injections of corticosteroids or hyaluronic acid (HA), have been used to manage knee OA symptoms and to delay surgery.[4,35] However, these modalities are palliative and not disease-modifying treatments to address the irreversible damage to cartilage and the associated structural abnormalities.[12,50]

Recently, cell-based therapies have gained attention as a disease-modifying treatment, and mesenchymal stem cells (MSCs) are particularly interesting, given their potential properties of regeneration, multilineage differentiation, and immunomodulatory capacity.[13,32,39] Although MSCs are commonly extracted from the bone marrow, adipose tissue, synovium, and umbilical cord, adipose tissue has become an attractive option owing to its easy accessibility and abundance.[19,41,50] Although the superiority of MSC chondrogenic potential is still debated,[17] several studies have reported that the application of MSCs from adipose tissue to patients with knee OA showed better clinical improvements than injections from other sources.[19,48]

Adipose-derived mesenchymal stem cells (ASCs) and adipose-derived stromal vascular fractions (ADSVFs) are common sources of MSCs from the adipose tissue, and their procurement depends on culture with cell expansion and heterogeneity in cells.[13,46,50] Many systematic reviews and meta-analyses have assessed the efficacy of MSCs, including those from adipose tissue, but most studies erroneously and confusingly used the terms *ASC* and *ADSVF* and otherwise were heterogeneous in terms of autologous or allogenic MSCs, adjuvant treatments, delivery methods, and level of evidence (LOE) of included studies.[8,13,17,19,35,41,48] In this regard, the results of systematic reviews and meta-analyses are still inconsistent with regard to the efficacy of MSCs from adipose tissue.[8,13,17,19,35,41,48]

Therefore, we performed a systematic review and meta-analysis of randomized controlled trials (RCTs) to provide comprehensive information about the efficacy and safety of autologous ASC or ADSVF IA injection without adjuvant treatments in patients with knee OA. We also indirectly compared the efficacy of ASC and ADSVF use through subgroup analyses. The primary purpose of the current study was to use meta-analysis to assess the efficacy (including pain relief, functional improvement, and cartilage change using magnetic resonance imaging [MRI] assessment) and safety of ASC and ADSVF treatment.

## METHODS

### Literature Search

This systematic review and meta-analysis followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines,[36] and the protocol for review was registered with the International Prospective Register of Systematic Reviews (PROSPERO, registration no. CRD42021226770). Two independent reviewers (J.-H.K. and M.-S.K.) systematically searched for articles using the PubMed (MEDLINE), Embase, Cochrane Library, and Web of Science databases from study inception to December 17, 2020, using an a priori search strategy. The following keywords were used in the search: "knee joint," "osteoarthritis," "adipose derived mesenchymal stem cell," "adipose derived culture expanded mesenchymal stem cell," "adipose derived stem cell," "stromal vascular fraction," and "adipose tissue stromal vascular fraction" aided by the use of Boolean operators "AND" or "OR." The bibliographies of the initially retrieved studies were manually cross-checked to find additional, relevant articles that could have been missed by electronic searches. No language restrictions were imposed.

### Study Selection

Two reviewers (J.-H.K. and M.-S.K.) independently screened the titles and abstracts of the retrieved articles; full manuscripts were reviewed if the abstract provided insufficient data for study inclusion. Any disagreement was resolved by consensus or consultation with another author (K.-I.K). Studies were included in the current study if they met the PICOS (patients, intervention, comparison, outcome, and study design) criteria[27] (Table 1). The exclusion criteria consisted of (1) conference abstracts; (2) clinical trial abstracts; (3) insufficient statistics or inability to reproduce statistics; (4) animal study or in vitro study; (5) allogenic cell therapy; (6) concomitant treatments, such as platelet-rich plasma (PRP), high-tibial osteotomy (HTO), or cartilage repair procedures, and biologic adjuvants, such as fibrin; (7) comparison group of other cell-based therapy or PRP; and (8) LOE 2, 3, 4, or 5. No minimum follow-up period was required for inclusion, because few RCTs existed and all had short-term follow-up.

### Assessment of Literature and Methodological Quality

The literature quality was assessed using the LOE determined by 2 reviewers (J.-H.K. and M.-S.K.) based on

‡Address correspondence to Jun-Ho Kim, MD, PhD, Department of Orthopaedic Surgery, Kyung Hee University Hospital at Gangdong, 892 Dongnam-ro, Gangdong-gu, Seoul 134-727, Republic of Korea (email: junojuno49@gmail.com).
*Department of Orthopaedic Surgery, Center for Joint Diseases, Kyung Hee University Hospital at Gangdong, Seoul, Republic of Korea.
†Department of Orthopaedic Surgery, School of Medicine, Kyung Hee University, Seoul, Republic of Korea.
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TABLE 1
Inclusion and Exclusion Criteria Based on PICOS[a]

| PICOS | Inclusion Criteria | Exclusion Criteria |
|---|---|---|
| Population | Patients with knee osteoarthritis | Animal study or in vitro study |
| Intervention | Intra-articular injection of autologous stromal vascular fraction or culture-expanded mesenchymal stem cells from adipose tissue | Adjuvant treatments such as platelet-rich plasma, cartilage repair procedures, or high-tibial osteotomy<br>Allogenic cell therapy<br>Biologic adjuvants such as fibrin |
| Comparison | Placebo or control group | Other cell-based therapy or platelet-rich plasma |
| Outcome | Patient-reported outcome measure (function and pain); magnetic resonance imaging; adverse effect | |
| Study design (level of evidence) | 1 | 2, 3, 4, or 5 |

[a]PICOS, population, intervention, comparison, outcome, study design.

previously published criteria.[31] The methodological quality was assessed by 2 reviewers (J.-H.K. and M.-S.K.) based on the modified Coleman Methodology Score (mCMS).[6,7] This score evaluates the included studies for items such as inclusion criteria, sample size calculation, randomization, follow-up, patient analysis, blinding, similarity in treatment, treatment description, group comparability, outcome assessment, description of rehabilitation protocol, clinical effect measurement, and the number of patients treated. The mCMS ranges from 0 to 100 for grading the quality of studies. The grading was considered as follows: a score of $>85$ was excellent; 70-84, good; 55-69, fair; and $\leq 54$, poor.[7] Any disagreement was resolved by consensus or consultation with the other author (K.-I.K).

## Assessment of Risk of Bias

The Cochrane Handbook for Systematic Reviews of Interventions was used to evaluate the risk of bias in the included RCTs.[15] This risk assessment was based on the following types of bias: selection, performance, detection, and attrition. Two reviewers (J.-H.K. and M.-S.K.) independently assessed the studies, and any discrepancies in scores between the 2 reviewers were resolved by discussion or consultation with the other author (K.-I.K).

## Data Extraction

The same reviewers independently collected available data from the included studies, and any disagreement was resolved by discussion or consultation with the third author. The basic characteristics of the study (author, year of publication, country of investigation, sample size, and LOE), details of patient characteristics (mean age, sex, mean body mass index, lower limb alignment, follow-up duration, and OA grading of involved patients), and details of cell therapy from adipose tissue (entity of cells, control group, delivery methods, culture with cell expansion, cell count, and adipose donor site) were collected. In addition, inclusion and exclusion criteria of included studies were collected. For outcome measurements, pain (100-mm visual analog scale [VAS] score), function (total Western Ontario and McMaster Universities Osteoarthritis Index [WOMAC] score), MRI assessment (cartilage improvement or structural change), and safety (procedure-related pain or swelling, adverse events [AEs], and serious AEs [SAEs]) were considered and extracted to a predefined data form. For missing data, we tried to contact the author of the article first; if this failed, we calculated the missing values from other available data using formulas in the Cochrane Handbook for Systematic Reviews of Interventions.[15] The cell type was determined according to a consensus statement regarding nomenclature by the International Society of Cellular Therapy.[2] Cellular therapy from adipose tissue was classified as that using ASCs and ADSVFs.

## Statistical Analysis

The primary outcomes of this systematic review were the efficacy of MSC-based therapy from adipose tissue, namely ASCs or ADSVFs, with respect to pain relief, functional improvement, cartilage, or structural change on MRI assessment, and the safety of this therapy. If possible, a meta-analysis was performed to show the standardized mean difference (SMD) with 95% CI for continuous variables and the risk ratio with 95% CI in dichotomous variables. If a meta-analysis was not possible because of a lack of variables, a qualitative description of the outcome was performed. A subgroup analysis was performed to indirectly compare ASCs and ADSVFs with SMD and standardized variance, which were calculated from the weighted estimate, standard error, and sample size of each cohort using a logit model.[20,47] Publication bias was not assessed because it was not considered necessary if there were $<10$ studies in a comparison.[15] Heterogeneity was assessed by estimating the proportion of between-study inconsistencies because of actual differences between studies using the $I^2$ statistic.[34] A fixed-effects meta-analysis model was performed to pool outcomes across the included studies. Forest plots were used to show outcomes, pooled estimate of effect, and an overall summary effect of each study and were constructed using RevMan (Version 5.4; The Cochrane Collaboration) and

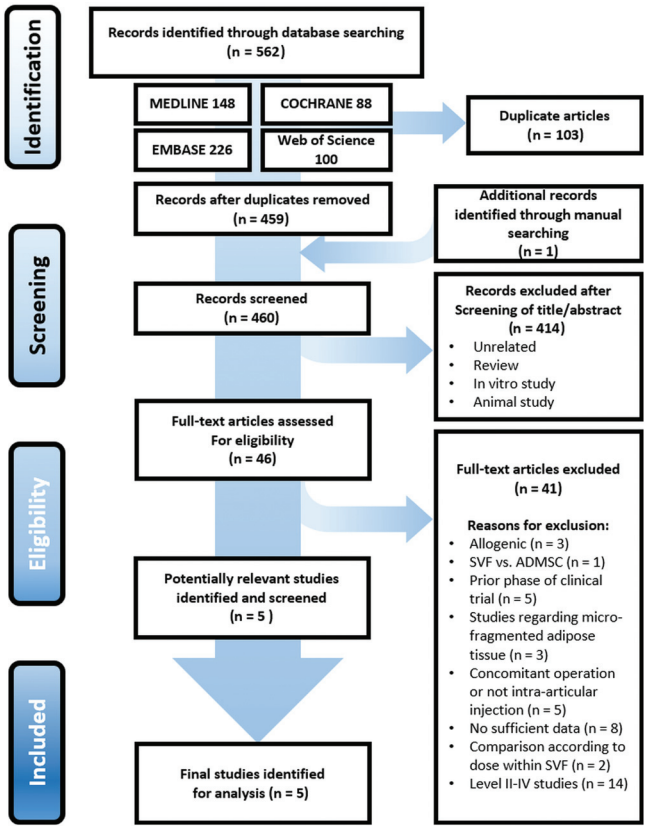


**Figure 1.** PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram for identification and selection of studies. ADMSC, adipose-derived mesenchymal stem cell; SVF, stromal vascular fraction.

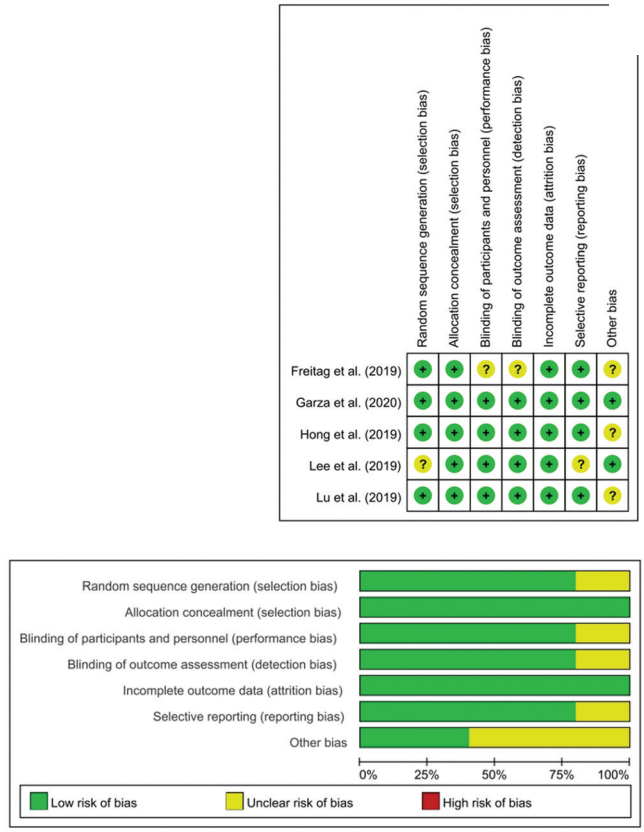


**Figure 2.** Risk of bias assessment of the included studies, involving a risk of bias graph and summary.

Open Meta-Analyst (http://www.cebm.brown.edu/open-meta). Statistical significance was set at $P < .05$.

## RESULTS

### Identification of Studies

The initial electronic search yielded 562 studies, and 1 study was identified from an additional manual search. After removal of 103 duplicates, 460 studies remained. We excluded 414 studies after reading the title or abstract, and 41 studies were excluded after a full-text review. Finally, 5 RCTs were included in this systematic review (Figure 1).

### Study Characteristics

Of the 5 RCTs, 3 studies compared ASC treatment versus no injection[10] and placebo,[26,30] and 2 studies compared ADSVF treatment versus placebo[11] and HA.[16] A total of 177 knees with OA were included, with a mean patient age of 56.8 ± 9.0 years. Follow-up was conducted for up to 6 months in 1 RCT[26] and 12 months in 4 RCTs.[10,11,16,30] All knees ranged from I to III on the Kellgren-Lawrence grading scale, with the exception of 1 knee that was grade IV. Details of the study characteristics, patient characteristics, and therapy protocol are presented in Table 2. Inclusion and exclusion criteria of included studies are presented in Appendix Table A1 (available in the online version of this article).

### Assessment of Literature and Methodological Quality and Risk of Bias

All of the 5 studies[10,11,16,26,30] included had LOE 1. Regarding mCMS for quality assessment, no study was of excellent quality, whereas 3 studies[11,26,30] were of good quality and 2 studies[10,16] were of fair quality (Table 2). The median mCMS was 70 (range, 55-75). The included studies showed a low risk of bias, and there was no high risk of bias in the properties evaluated (Figure 2).

### Pain Improvement (100-mm VAS)

In total, 4 studies reported 100-mm VAS scores at 6 months, and the total mean improvement was significantly higher in the overall study groups than in the controls (SMD, 1.06; 95% CI, 1.19-2.02; $I^2$ = 85%; $Z$ = 7.62;

TABLE 2
Details of Studies on Osteoarthritis Treatment Using Autologous Adipose Tissue[a]

| Characteristics | Freitag[10] (2019) | Garza[11] (2020) | Hong[16] (2019) | Lee[26] (2019) | Lu[30] (2019) |
|---|---|---|---|---|---|
| Country | Australia | USA | China | South Korea | China |
| Level of evidence | 1 | 1 | 1 | 1 | 1 |
| Sample size, n | | | | | |
| Study | 20 | 26 | 16 | 12 | 26 |
| Control | 10 | 13 | 16 | 12 | 26 |
| Age, y, mean ± SD | | | | | |
| Study | 54.7 ± 10.2 | 60.0 ± 9.8 | 51.0 ± 6.0 | 62.2 ± 6.5 | 55.0 ± 9.2 |
| Control | 51.5 ± 6.1 | 57.1 ± 9.1 | 53.0 ± 11.0 | 63.2 ± 4.2 | 59.6 ± 6.0 |
| Sex, male:female, n | | | | | |
| Study | 11:9 | 15:11 | 3:13 | 3:9 | 3:23 |
| Control | 1:9 | 7:6 | 3:13 | 3:9 | 3:23 |
| Body mass index, mean ± SD | | | | | |
| Study | 31.0 ± 5.6 | 28.2 ± 4.2 | 26.3 ± 1.8 | 25.3 ± 4.9 | 24.3 ± 3.0 |
| Control | 25.2 ± 3.4 | 27.1 ± 2.7 | | 25.4 ± 3.0 | 24.3 ± 2.6 |
| Lower limb alignment | <5° varus or valgus for inclusion criteria | NR | NR | NR | Mean varus 1.4° for ASC Mean varus 0.4° for control group |
| Follow-up, mo | 1, 3, 6, 12 | 1.5, 3, 6, 12 | 1, 3, 6, 12 | 3, 6 | 6, 12 |
| Kellgren-Lawrence grade | II, III | II, III | II, III | II, III, IV[b] | I, II, III |
| Entity of cells | ASC | ADSVF | ADSVF | ASC | ASC |
| Control | No injection | Placebo (lactated Ringer solution) | HA | Placebo (normal saline) | HA |
| Delivery method | IA ± second IA under US at 6 mo | IA under US | IA under arthroscopy | IA under US | Direct IA twice at 0 and 3 wk |
| Culture and cell expansion | Passage 2 | No | No | Passage 3 | Passage 4 |
| No. of cells ($\times 10^7$) | 10 | 1.5, 3.0 | 0.8 | 10 | 5 |
| Adipose donor site | Abdomen | Abdomen | Abdomen | Abdomen | Abdomen |
| Modified Coleman Methodology Score | 55 | 70 | 67 | 73 | 75 |

[a]ADSVF, adipose-derived stromal vascular fraction; ASC, adipose-derived stem cell; HA, hyaluronic acid; IA, intra-articular injection; NR, not reported; US, ultrasonography.
[b]One patient with Kellgren-Lawrence grade IV was included in the control group.

$P < .0001$) (Figure 3A). Furthermore, in the subgroup analysis for study groups, significantly larger improvements in the 100-mm VAS were also noted in the ASC (SMD, 1.32; 95% CI, 0.88-1.76; $I^2 = 72\%$; $Z = 5.87$; $P < .0001$) and ADSVF (SMD, 3.64; 95% CI, 2.47-4.82; $Z = 6.06$; $P < .0001$) groups than in the controls.

A total of 3 studies reported a 100-mm VAS improvement at 12 months, and the total mean improvement was significantly higher in the overall study groups than in the controls (SMD, 1.65; 95% CI, 1.20-2.10; $I^2 = 82\%$; $Z = 7.21$; $P < .0001$) (Figure 3B). Furthermore, in the subgroup analysis for study groups, significantly higher improvements in the 100-mm VAS score were noted in the ASC (SMD, 1.37; 95% CI, 0.87-1.87; $I^2 = 80\%$; $Z = 5.35$; $P < .0001$) and ADSVF (SMD, 2.81; 95% CI, 1.80-3.82; $Z = 5.45$; $P < .0001$) groups than in the controls.

## Function Improvement (Total WOMAC Score)

In total, 4 studies reported a total WOMAC score at 6 months, and the total mean improvement was significantly higher in the overall study groups than in the controls (SMD, 0.75; 95% CI, 0.39-1.11; $I^2 = 64\%$; $Z = 4.13$; $P < .0001$) (Figure 4A). Furthermore, in the subgroup analysis for study groups, significantly higher improvements in the total WOMAC score were noted in the ASC (SMD, 0.65; 95% CI, 0.24-1.05; $I^2 = 72\%$; $Z = 3.12$; $P = .002$) and ADSVF (SMD, 1.09; 95% CI, 0.35-1.83; $Z = 2.90$; $P = .004$) groups than in the controls at 6 months.

We found that 3 studies reported total WOMAC score improvement, and the total mean improvement was significantly larger in the overall study groups than in the controls (SMD, 0.83; 95% CI, 0.40-1.26; $I^2 = 87\%$; $Z = 3.79$; $P = .0002$) (Figure 4B). Furthermore, in the subgroup analysis for study groups, significantly higher improvements in the total WOMAC score were noted in the ASC (SMD, 0.67; 95% CI, 0.19-1.14; $I^2 = 92\%$; $Z = 2.76$; $P = .006$) and ADSVF (SMD, 1.60; 95% CI, 0.57-2.63; $Z = 3.05$; $P = .002$) groups than in the controls at 12 months.

## MRI Outcome (Cartilage or Structural Change)

All included studies reported MRI outcomes in terms of cartilage or structural changes after IA injection of ASCs or ADSVFs. A meta-analysis could not be performed owing to heterogeneity in the methods of assessment and a lack of studies. Details of the MRI assessment are shown in Table 3.

Among the 5 studies, 3 studies reported significantly better changes in cartilage status in the ASC or ADSVF

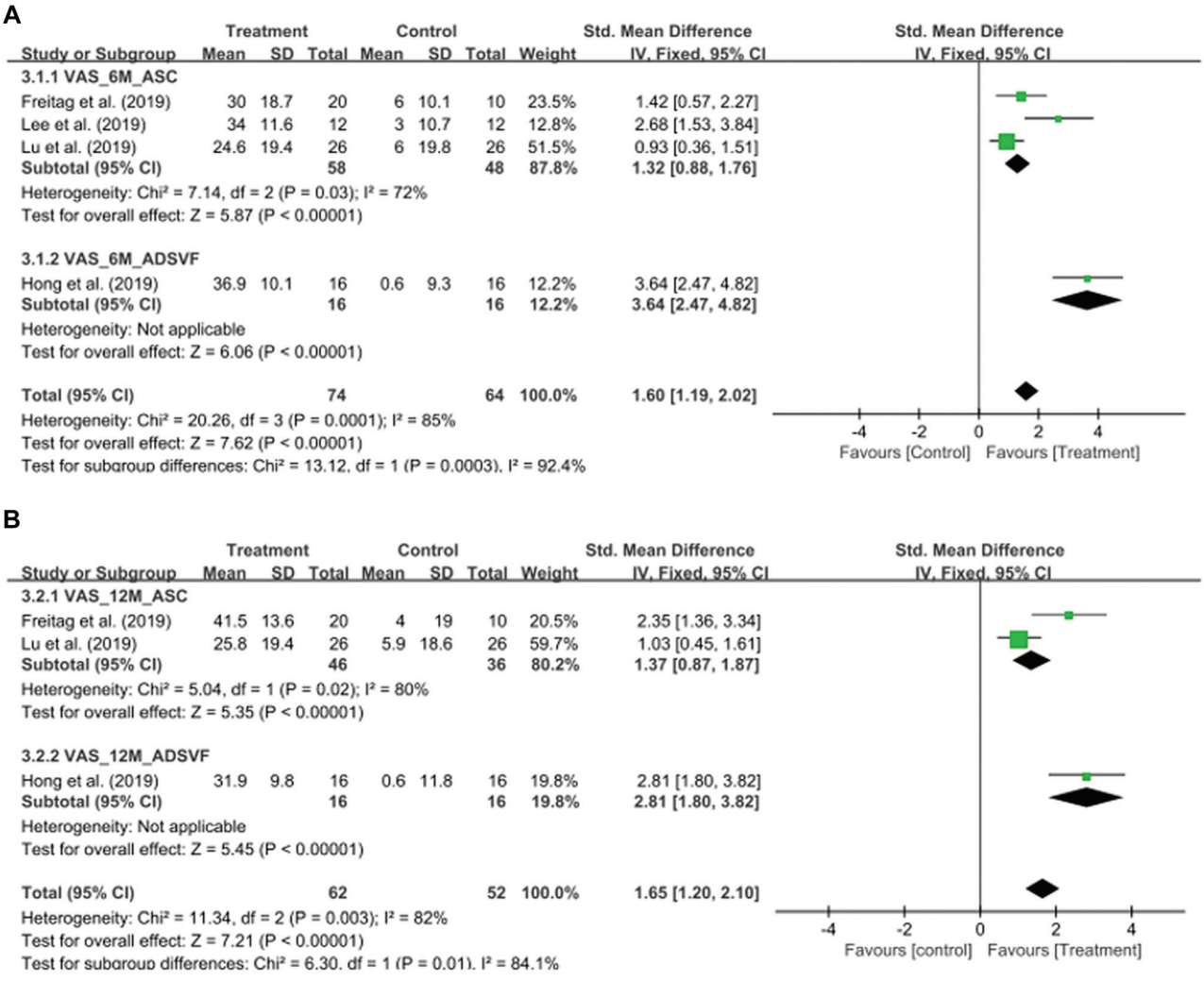


**Figure 3.** Forest plots of the included studies showing improvement in the 100-mm visual analog scale (VAS) score at (A) 6 months and (B) 12 months after intra-articular injection of adipose-derived stem cells (ASCs) or adipose-derived stromal vascular fractions (ADSVFs) compared with controls. Squares represent the mean difference in outcomes, with the size of the square being proportional to the sample size. IV, inverse variance; Std, standard.

groups than in the controls,[16,26,30] whereas 2 studies reported no significant change.[10,11]

Among the 3 studies on ASC treatment, 1 study reported no significant difference compared with the control based on MRI osteoarthritis knee score (MOAKS).[10] Another study reported a significantly increased cartilage defect size in the control group compared with no significant change in the ASC group at 6 months,[26] and yet another reported significantly increased cartilage volume change at 6 and 12 months in the ASC group compared with the control group.[30]

Among 2 studies of ADSVF treatment, 1 study reported that ADSVFs significantly improved the Whole-Organ Magnetic Resonance Imaging Score (WORMS) and magnetic resonance observation of cartilage repair tissue (MOCART) score at 6 and 12 months compared with the control group,[16] whereas no difference was reported in the change of cartilage thickness and Outerbridge classification between the ADSVF and control groups after treatment at 6 or 12 months.[11]

## Safety

Procedure-related knee pain or swelling was reported in all included studies at 46% and 46.7% in the treatment and control groups, respectively. The pooled estimate of risk ratio was 1.02 (95% CI, 0.77-1.33; $I^2$ = 52%; Z = 0.11), with no significant difference ($P$ = .91) (Figure 5).

Details of AEs in the included studies are shown in Table 4; no SAEs of ASC or ADSVF treatment were reported in the included studies. Only 1 patient in the HA group had an infection at 2 months and consequently underwent arthroscopic debridement.[30]

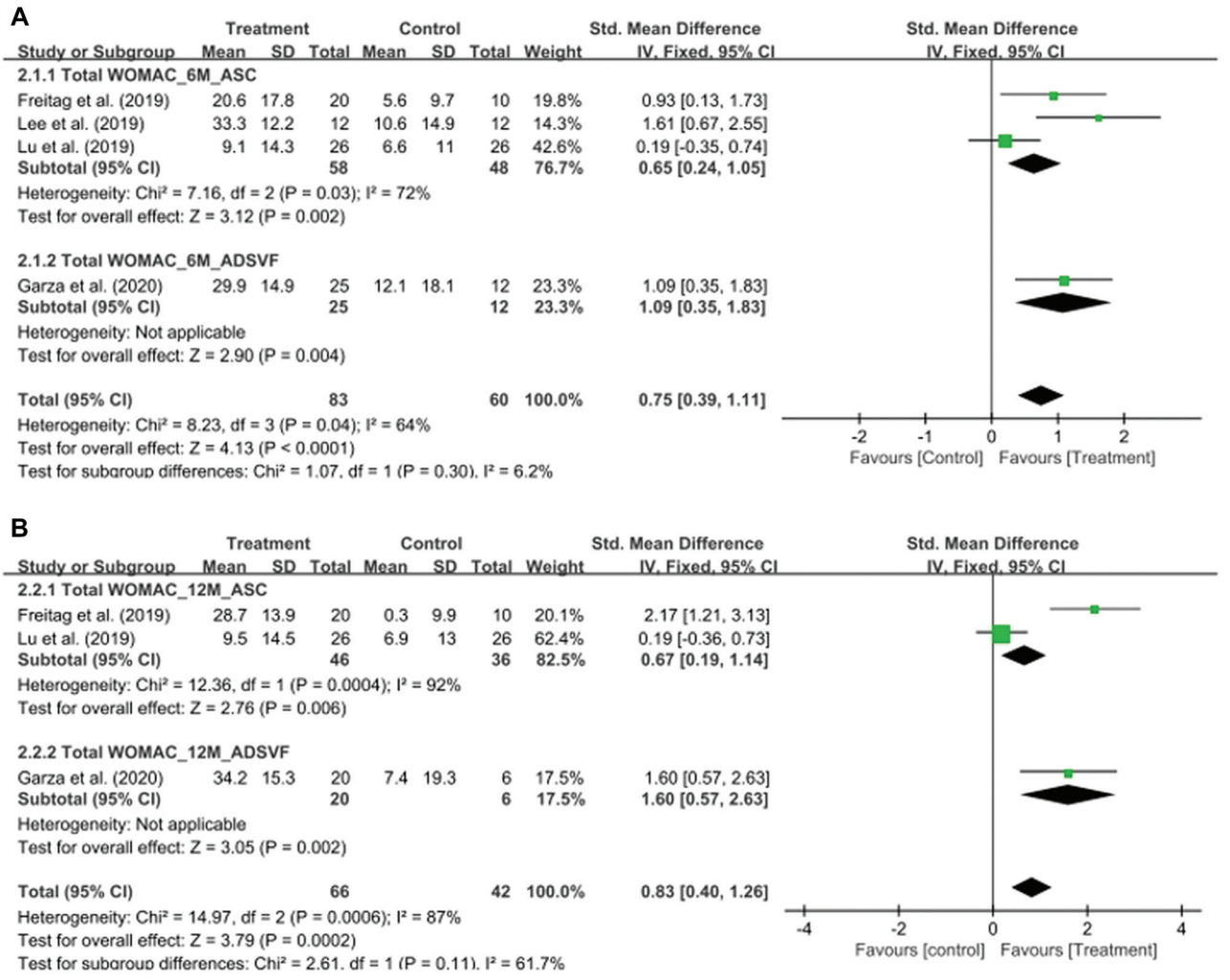


**Figure 4.** Forest plots of the included studies showing improvement in total Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score at (A) 6 months and (B) 12 months after intra-articular injection of adipose-derived stem cells (ASCs) or adipose-derived stromal vascular fractions (ADSVFs) compared with controls. Squares represent the mean difference in outcomes, with the size of the square being proportional to the sample size. IV, inverse variance; Std, standard.

### Subgroup Analysis (ASC vs ADSVF)

No significant differences were found between ASC and ADSVF treatments regarding improvement of VAS or total WOMAC scores at 6 and 12 months (Table 5). However, limited evidence remains owing to heterogeneous individual conditions and low statistical power.

## DISCUSSION

The principal findings of this meta-analysis were that ASC or ADSVF treatments had advantages over placebo or HA with respect to pain and functional improvement at 6 and 12 months without a significant difference in procedure-related knee pain or swelling. However, ASCs or ADSVFs had limited evidence for cartilage repair using MRI evaluation in the current review. No SAEs were reported after ASC or ADSVF IA injection. In addition, a subgroup analysis revealed similar efficacy in pain and functional improvement between ASCs and ADSVFs, although a direct comparison is necessary for the future.

This meta-analysis revealed that autologous ADMSC injection induced significant pain relief at 6 and 12 months compared with placebo or HA injection. All ASC and ADSVF groups in the included studies showed significant differences in pain improvement after treatment and between the treatment and control groups. The mean improvement of VAS ranged from 24.6 to 36.9 at 6 months and from 25.8 to 41.5 at 12 months in the ASC or ADSVF groups, whereas the mean improvement of VAS ranged from 0.6 to 6 at 6 months and from 0.6 to 5.9 at 12 months in the control groups. Freitag et al[10] showed that pain improvement above the minimal clinically important difference (MCID) on VAS at 12 months was 94.4% in the ASC group but 40% in the control group. Recent meta-

TABLE 3
MRI Assessment of Cartilage Regeneration on Osteoarthritis[a]

| Lead Author (Year) Cell Type | Assessment | MRI Protocol | F/U, mo | Cartilage Pathology | | Overall Results of Cell Therapy |
|---|---|---|---|---|---|---|
| | | | | Study | Control | |
| Freitag[10] (2019) ASC | MOAKS | 1.5-T or 3.0-T standard sequence (PDFS) | 12 | Improved: 1/19 (5.3%)<br>No change: 14/19 (73.7%)<br>Progression: 4/19 (21.0%) | Improved: 0/9 (0%)<br>No change: 3/9 (33%)<br>Progression:6/9 (67%) | Tended to be better in the ASC group than in the control group, but not significant<br>Modified disease progression |
| Garza[11] (2020) ADSVF | Cartilage thickness (change) | 1.5-T or 3.0-T with standard sequence | 6 | –0.2 mm | + 0.5 mm | No difference |
| | | | 12 | –0.1 mm | + 0.8 mm | No change after treatment |
| | Outerbridge grade (change) | | 6 | 0 | 0 | |
| | | | 12 | 0 | 0 | |
| Hong[16] (2019) ADSVF | WORMS | 3.0-T with standard sequence (PDFS) | 6 | Improved: 7.8 (cartilage), 11.4 (total) | Deteriorated: 2.6 (cartilage), 12.8 (total) | Significant improvement in the study group at 6 and 12 months |
| | | | 12 | Improved: 12.0 (cartilage), 15.4 (total) | Deteriorated: 4.1 (cartilage), 15.5 (total) | Significant deterioration in the control group at 6 and 12 months |
| | MOCART score | | 6 | Complete: 12.5%<br>Hypertrophy: 31.25%<br>Incomplete:<br>>50%: 25%<br><50%: 18.65%<br>SB exposure: 12.5%<br>Total: 54.1 ± 11.6 | Complete: 0%<br>Hypertrophy: 6.25%<br>Incomplete:<br>>50%: 12.5%<br><50%: 25.0%<br>SB exposure: 56.25%<br>Total: 19.4 ± 9.6 | Significant improvement in the study group at 12 months<br>Deterioration in the control group at 6 and 12 months |
| | | | 12 | Complete: 31.25%<br>Hypertrophy: 37.5%<br>Incomplete:<br>>50%: 12.5%<br><50%: 12.5%<br>SB exposure: 6.25%<br>Total: 62.8 ± 8.2 | Complete: 0%<br>Hypertrophy: 6.25%<br>Incomplete:<br>>50%: 12.5%<br><50%: 18.75%<br>SB exposure: 62.5%<br>Total: 19.1 ± 7.8 | |
| Lee[26] (2019) ASC | Cartilage defect size (change) | 3.0-T with standard sequence (PDFS) | 6 | + 2.4 ± 14.5 $mm^2$ | + 35.6 ± 58.8 $mm^2$ | No significant change in the ASC group; the defect size was significantly increased in the control group<br>Significant difference in the degree of change between the 2 groups<br>ASC treatment was better than placebo |
| Lu[30] (2019) ASC | Cartilage volume (change) | 3.0-T with standard sequence (PDFS) | 6 | Significantly increased in the right knee | Significantly decreased in the left tibia | Significant difference in the left tibia and right femur (ASC treatment was better than HA) |
| | | | 12 | Significantly increased in both femurs | No significant change | Significant difference in both femurs<br>ASC treatment was better than HA |

[a]ADSVF, adipose-derived stromal vascular fraction; ASC, adipose-derived stem cell; F/U, follow-up; HA, hyaluronic acid; MOCART, magnetic resonance observation of cartilage repair tissue; MOAKS, MRI Osteoarthritis Knee Score; MRI, magnetic resonance imaging; PDFS, proton density fat saturated; SB, subchondral bone; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

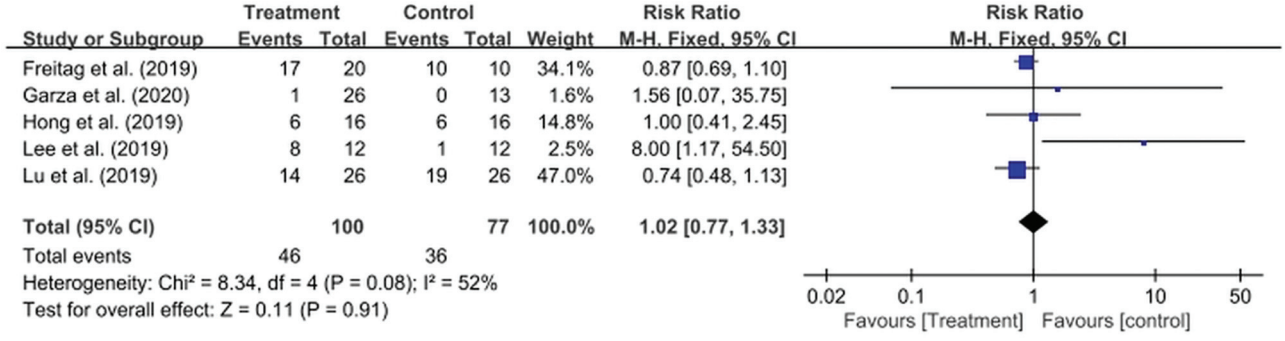


**Figure 5.** Forest plots of the included studies showing procedure-related knee pain or swelling after intra-articular injection of adipose-derived stem cells (ASCs) or adipose-derived stromal vascular fractions (ADSVFs) compared with controls. Squares represent the mean difference in outcomes, with the size of the square being proportional to the sample size. IV, inverse variance; M-H, Mantel-Haenszel.

TABLE 4
Adverse and Serious Adverse Events in the Included Studies[a]

| Lead Author (Year) Cell Type | Study Sample Size | Adverse Events | Serious Adverse Events |
|---|---|---|---|
| Freitag[10] (2019) ASC | 20 | None: 15%.<br>Mild: 55%.<br>Moderate: 20%.<br>Severe: 10% had pain and swelling for 4 weeks and observed an effect on their usual daily activity. | None |
| Garza[11] (2020) ADSVF | 26 | 3 patients (11.5%) had minor adverse events.<br>1 patient reported knee swelling, and 2 patients reported possible bacterial growth; however, none was due to an infection. | None |
| Hong[16] (2019) ADSVF | 16 | 4 patients (25%) had abdominal pain.<br>6 patients (37.5%) had pain and swelling in knee joints.<br>All of these events were resolved by pain medication. | None |
| Lee[26] (2019) ASC | 12 | 10 patients (83%) in the ASC group and 7 patients (58%) in the control group had adverse events.<br>8 patients (66.75%) in the ASC group had treatment-related adverse events, including arthralgia in 6 patients and joint effusion in 2 patients.<br>All of these events were resolved by pain medication. | None |
| Lu[30] (2019) ASC | 26 | Similar proportion between the ASC (73.1%) and the HA (55.9%) groups.<br>The most common symptoms were pain and swelling of the injection site.<br>Spontaneous relief within 7 days without special treatment. | None for the ASC group<br>1 for the HA group (infection 2 months after injection) |

[a]ADSVF, adipose-derived stromal vascular fraction; ASC, adipose-derived stem cell; HA, hyaluronic acid.

TABLE 5
Weighted Standard Mean Differences of Outcomes After Subgroup Analysis Comparing ASC and ADSVF Treatment[a]

| Outcome or Subgroup | No. of Studies | Standardized Mean Difference (Standardized Variance) | 95% CI | Significance |
|---|---|---|---|---|
| Improvement of 100-mm VAS score at 6 mo (ASC vs ADSVF) | 4 | –0.325 (0.080) | –0.881 to 0.231 | No significance |
| Improvement of 100-mm VAS score at 12 mo (ASC vs ADSVF) | 3 | 0.042 (0.085) | –0.527 to 0.611 | No significance |
| Improvement of total WOMAC score at 6 mo (ASC vs ADSVF) | 4 | –0.200 (0.057) | –0.067 to 0.270 | No significance |
| Improvement of total WOMAC score at 12 mo (ASC vs ADSVF) | 3 | –0.277 (0.072) | –0.804 to 0.250 | No significance |

[a]ADSVF, adipose-derived stromal vascular fraction; ASC, adipose-derived stem cell; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

analyses showed a significant pain reduction at 6 and 12 months after the administration of MSCs from adipose tissue,[8,14,19,48] although Jeyaraman et al[19] reported that no difference was noted in pain improvement at 12 months between the treatment and control groups. The inconsistent results and differences compared with our meta-analysis may be because previous studies included allogenic MSCs and adjuvant surgeries for HTO and microfracture, which could not fully reflect the efficacy in pain reduction by ASCs or ADSVFs alone. As mentioned above, we demonstrated that ASCs or ADSVFs significantly improved 100-mm VAS scores at 6 and 12 months compared with placebo or HA. With these points in mind, IA autologous ASC or ADSVF injections without any additional treatments would be an attractive option for pain relief in knee OA at 12 months.

The results of this review revealed that ASC or ADSVF injections led to a significant functional improvement in the total WOMAC score at 6 and 12 months compared with placebo or HA injection. Of the 5 included studies, 4 studies reported a significant improvement after treatments and better improvement compared with controls in the total WOMAC score at 6 and 12 months. Lu et al[30] showed that the difference in the improvement at 6 and 12 months did not reach statistical significance between ASCs (31.7% and 28.5%, respectively) and HA (20.2% and 20.7%, respectively). However, those investigators reported that ASC treatment was superior to HA in terms

of improvement in quality of life because ASC treatment showed a significantly better improvement than HA on the 36-Item Short Form Health Survey. In total, 2 of the included studies reported improvement above MCID in the total WOMAC score: Garza et al[11] showed that 62% of their ADSVF group and 38% of their placebo group had a WOMAC score above MCID at 6 months, whereas Freitag et al[10] showed that 94.4% of their ASC group and 20% of their placebo group were above MCID at 12 months. Further, 4 included studies showed that a significant difference started to appear after 3 months because the control groups had worsened or experienced no change.[10,11,16,26] According to recent systematic reviews and meta-analyses, controversy remains regarding functional efficacy of ASCs or ADSVFs,[8,13,14,17,19,35,48] which results from heterogeneity of the inclusion criteria of these reviews. Adjuvant treatments, such as cartilage repair, HTO, or other adjuvant cell therapies, including PRP, may affect the results because these procedures also enhance functional scores in addition to the MSC treatments.[1,13,20,23] Based on our findings, this review suggests that IA autologous ASC or ADSVF injections can be a viable therapeutic option to achieve functional improvement in patients with knee OA.

The efficacy of ASCs or ADSVFs in cartilage regeneration remains unclear in this systematic review. A quantitative meta-analysis could not be performed because the included studies evaluated cartilage change using different methods, such as the WORMS, MOAKS, and MOCART scores; the Outerbridge classification; and cartilage thickness, cartilage defect area, and cartilage volume using MRI. Thus, we describe the qualitative MRI results of cartilage changes in Table 3, which shows that among the 5 studies, 3 studies[16,26,30] showed that cartilage change was significantly better in the ASC or ADSVF groups compared with controls, 1 study[10] showed a tendency for better cartilage changes in ASC treatment compared with placebo without statistical significance, and 1 study[11] showed no significant differences between the 2 study groups. Recent systematic reviews have reported that the efficacy of MSCs for cartilage repair has limited evidence, which is consistent with our results.[8,13,17,33,48] Despite the limited evidence on cartilage regeneration, many clinical studies have suggested that MSCs, including ASCs and ADSVFs, have potential efficacy for cartilage regeneration in patients with knee OA.[16,18,24,26,30,49] Therefore, we believe that more high-quality, well-designed studies with long-term follow-up and no adjuvant treatments are necessary to draw a conclusion concerning the efficacy of ASC or ADSVF treatments for cartilage regeneration in patients with knee OA.

Safety has been a concern for clinicians regarding the administration of MSCs.[5,38,49] Procedure-related knee pain and swelling are the most common side effects after IA injection therapies.[5,33,38] Our meta-analysis showed no difference in procedure-related pain or swelling between ASC or ADSVF groups and their controls, which is consistent with recent meta-analyses.[19,48] Minor discomfort and bruising were commonly noted at the lipoharvested site, although liposuction has shown a very low complication rate of approximately 0.1%.[45] Fortunately, all of th AEs resolved spontaneously or with pain relievers a few days, and no SAEs were reported after ASC or ADSVF injection, although 1 patient had an infection with consequent surgery after HA injection. The results of recent systematic reviews were in accordance with our result that no SAEs, such as death, malignancy, or systemic reactions, that were definitely related to MSC injection were identified.[5,19,33,38,48] Based on this review, autologous IA ASC or ADSVF injection is a safe therapeutic option for patients with knee OA; however, as this evidence is limited to $\leq$1 year, long-term studies are warranted to guarantee confidence in the safety of ASC or ADSVF treatments.

ASCs and ADSVFs are commonly used types of MSC-based therapy from adipose tissue, but the terms used in previous clinical studies have been inconsistent and confusing.[13,17,50] Theoretically, ASCs are assumed to have higher potential efficacy than ADSVFs, but ASCs require time and costs for culture with cell expansion.[2,28,50] In contrast, ADSVFs are convenient because they are injected directly after tissue digestion and lavage of liberated cells, without cell-expansion culture; however, ADSVFs inevitably contain heterogeneous cells, including approximately only 9.2% MSCs, as well as hematopoietic, vascular, and stromal cells.[2,17,19,28,50] The current study also showed different cell concentrations, such as 0.8-3.0 $\times 10^7$ cells in the ADSVF group and 5-10 $\times 10^7$ cells in the ASC group. Recent reviews have reported comparable efficacy between the 2 methods,[17,19,29] whereas the only study that directly compared the 2 methods showed that ASCs outperformed ADSVFs in early improvement with less comorbidity.[50] Although our subgroup analyses were consistent with most reviews, these studies do not allow us to draw a conclusion about the efficacy between ASCs and ADSVFs because the indirect comparison had inherent statistical limitations. Rather, a direct comparison study may have stronger evidence than indirect comparisons suggesting the potential superiority of ASCs.[50] In addition, a higher number of MSCs tended to show advantageous long-term effects according to recent meta-analyses,[8,19] and the counted ADSVF cells in groups of the included studies were not only pure MSCs but also stromal vascular fraction (SVF) cells.[11,16] Thus, the current study has limited evidence to show the clinical efficacy of ASCs and ADSVFs. Further studies with direct comparison, longer-term follow-up, higher cell qualities, and identical cell counts are required to select the best strategy for this application.

This study has several limitations that need to be addressed. First, the number of studies and the sample sizes were small because we included studies that entailed strict designs (such as RCTs), autologous cells, patients without adjuvant treatments, and direct injections without transplantation to avoid heterogeneity. To the best of our knowledge, only 5 RCTs satisfying these inclusion strategies existed in 2020. Second, the heterogeneity in cell concentrations, passage of cell expansion, and control groups may have produced a potential risk of bias despite the strict inclusion criteria. Third, we were not able to perform a quantitative analysis of cartilage repair on MRI

essment owing to the variety of imaging modalities; s, we described a qualitative analysis, although this limited the evidence. Fourth, the short-term follow-up of the included studies does not guarantee the safety of cell-based therapy from adipose tissue; thus, high-quality studies with long-term follow-up are necessary to demonstrate the long-term efficacy and safety of this treatment. However, this meta-analysis included studies with strict and homogeneous conditions because we excluded possible confounders, such as allogenic sources, biologic adjuvants, and adjuvant treatments. This contributed to the strength of this study, which attempted to demonstrate the differential efficacy of ASCs or ADSVFs for the management of patients with knee OA. The current review demonstrates the interest in the scientific field for this nonoperative therapeutic approach, which may potentially contribute to the introduction of a new paradigm for the treatment of knee OA.

## CONCLUSION

For patients with knee OA, intra-articular injection of autologous ASCs or ADSVFs without adjuvant treatment showed remarkable clinical efficacy and safety at a short-term follow-up. Some efficacy has been shown for cartilage regeneration in knee OA, although the evidence remains limited. Further RCTs that directly compare ASCs and ADSVFs are needed.

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cells

MDPI

*Systematic Review*

# Meta-Analysis of Adipose Tissue Derived Cell-Based Therapy for the Treatment of Knee Osteoarthritis

**Nikhil Agarwal [1], Christopher Mak [2], Christine Bojanic [2], Kendrick To [2] and Wasim Khan [2,*]**

[1] MBChB Office, University of Aberdeen College of Life Sciences and Medicine, Foresterhill Rd, Aberdeen AB25 2ZD, UK; nikagarwal@live.co.uk
[2] Division of Trauma & Orthopaedic Surgery, Addenbrooke's Hospital, University of Cambridge, Cambridge CB2 0QQ, UK; chcm2@cam.ac.uk (C.M.); cbojanic@doctors.org.uk (C.B.); kendrick.to@doctors.org.uk (K.T.)
* Correspondence: wasimkhan@doctors.org.uk

**Abstract:** Osteoarthritis (OA) is a degenerative disorder associated with cartilage loss and is a leading cause of disability around the world. In old age, the capacity of cartilage to regenerate is diminished. With an aging population, the burden of OA is set to rise. Currently, there is no definitive treatment for OA. However, cell-based therapies derived from adipose tissue are promising. A PRISMA systematic review was conducted employing four databases (MEDLINE, EMBASE, Cochrane, Web of Science) to identify all clinical studies that utilized adipose tissue derived mesenchymal stem cells (AMSCs) or stromal vascular fraction (SVF) for the treatment of knee OA. Eighteen studies were included, which met the inclusion criteria. Meta-analyses were conducted on fourteen of these studies, which all documented WOMAC scores after the administration of AMSCs. Pooled analysis revealed that cell-based treatments definitively improve WOMAC scores, post treatment. These improvements increased with time. The studies in this meta-analysis have established the safety and efficacy of both AMSC therapy and SVF therapy for knee OA in old adults and show that they reduce pain and improve knee function in symptomatic knee OA suggesting that they may be effective therapies to improve mobility in an aging population.

**Keywords:** osteoarthritis; degenerative changes; knee; adipose tissue; mesenchymal stem cells; stromal vascular factor



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## 1. Introduction

### *1.1. The Burden of Osteoarthritis*

Osteoarthritis (OA) is a progressive degenerative joint disorder associated with aging. It is a leading cause of disability around the world. In 2019, the Global Burden of Disease Study reported that musculoskeletal disorders account for over 5% of worldwide disability adjusted life years (DALY) [1]. The World Health Organization (WHO) estimate that approximately 10% of all men and 18% of all women aged over 60 have OA [2]. Out of these individuals, they estimate that 80% have limitations in movement and 25% cannot perform major daily activities of life [2].

In addition to physical symptoms, there is evidence to suggest that OA is associated with mental health problems as well. A longitudinal cohort study, conducted by the Osteoarthritis Initiative, found that there was a greater risk of developing depressive symptoms in patients with hip or knee OA than those without [3]. Another observational study found that OA was associated with 1.27 times increase odds of suicidal ideation [4]. There is also evidence that OA increases the risk for myocardial infarction, with one meta-analysis reporting a 1.31 times increased risk for myocardial infarction [5].

These, among many other studies have highlighted the burden OA has on the individual. In addition to this, OA carries significant economic burden on societies across the world. When adjusted for age, sufferers are shown to be at high risk of sick leave and

disability pension due to knee OA. This was especially the case for those working in health care, childcare and in cleaning [6]. In the United States, annual healthcare costs from OA exceed $45 billion [7]. In France, annual costs from OA can be as high as €2 billion [8]. In Spain costs may be as high as €4.738 billion annually [9]. A significant proportion of these costs are associated with joint replacement surgeries.

Economic, societal, and individual burdens caused by OA are set to rise. The prevalence of OA is increasing because of an aging population and an increased incidence of obesity. The United Nations estimates that by 2050, 1 in 6 people in the world will be over 65 years of age [10]. As such incidence rates of OA will naturally also increase. Several projection studies have been performed in different countries. In Australia, the number of people with OA is estimated to increase from 2.2 million in 2015, to 3.1 million by 2030 [11]. In Sweden it is estimated that from between 2012 and 2032, the percentage of people aged over 45 with OA will rise from 26.6% to 29.5% [12]. In the United States, this number is set to grow from 47.8 million in 2005 to 67 million by 2030 [13]. These figures show there is a global rising prevalence of this disease. Coupled with the debilitating nature of OA, this must be addressed before the disease overburdens healthcare systems worldwide.

There is currently no cure to prevent or slow the progression of OA. Presently, it is first managed via conservative means through exercises, weight loss and occupational therapy. When this is inadequate, paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) are used for symptom control [14]. Intra articular corticosteroid injections are then used if the aforementioned therapies do not provide relief. For end-stage OA, joint replacement surgery (total knee arthroplasty) is the gold standard of treatment. Despite being a highly successful operation, joint replacement surgery carries significant risk, and nearly one in five knee replacements will not last beyond 25 years [15].

OA pathogenesis is predominantly driven by inflammatory mediators such as interleukin 1 (IL-1) and tumour necrosis factor alpha (TNF-α) [16]. Both are present in the synovial fluid of patients with OA [17,18]. IL-1 has been shown to encourage the production of molecules such as nitric oxide, cytokines and prostaglandin E2 [19]. Furthermore, these inflammatory molecules promote the release of matrix metalloproteinases. These encourage the catabolism of articular cartilage [20]. This process of cartilage catabolism and loss is associated with aging, [21] alongside the diminishing ability of cartilage to repair itself [22]. While these mechanisms are central to cartilage depletion and eventually OA and pain, blockade of these mediators have failed to demonstrate efficacy in clinical trials [23,24].

### *1.2. Mesenchymal Stem Cells*

MSCs are becoming increasingly popular in tissue engineering due to their multipotent potential to differentiate into different lineages of mesenchymal tissue types [25]. These include bone, fat, cartilage, tendon, and muscle. MSCs are of interest as an OA therapy due to their immunoregulatory function and potential to repair cartilage. This is particularly useful as OA predominantly affects individuals in old age, who have limited ability to repair cartilage.

MSC-based therapies can result in promotion of macrophage polarization from an M1 to M2 phenotype [20,26,27]. This enables macrophages in the cartilage to display anti-inflammatory properties which leads to down-regulation of the inflammatory milieu mentioned above in their role in triggering and sustaining osteoarthritic changes [28]. MSCs have also been shown to be capable of suppressing T-cell proliferation. MSCs do not express HLA class II on their surface and only express low levels of HLA class I and have demonstrated safety and low immunogenicity through various routes of administration [29–31]. Although laboratory studies have suggested that MSCs, both intrinsic and transplanted may promote cancer cell activity, in-human clinical trials of transplantation have yet to show evidence of carcinogenic effect [32,33].

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy recommends a minimal criterion to define human MSCs. There are three

elements to these standards. Firstly, MSCs must be adherent to plastic when they are maintained in standard culture conditions. Secondly, MSCs must express the following markers: CD105, CD73 and CD90. In addition, they must not express the following: CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. Thirdly MSCs must be able to differentiate into the following cell types in vitro: osteoblasts, adipocytes and chondroblasts [34].

MSCs can be derived from a variety of locations including skeletal muscle, synovium [35,36] and periosteum [37,38]. However, the most popular cell sources for MSC harvest, are bone marrow [39–42] and adipose tissue [43,44]. MSCs were first isolated from bone marrow before any other source [45,46] and bone marrow derived MSCs remain one of the top choices for MSCs due to their high cell-yield and proliferative capacity in vitro [47]. However, despite its advantages, extraction of bone marrow to acquire MSCs for autologous use is a highly invasive and painful procedure that can cause long term pain at the donor site. Thus, such a procedure is not always ideal. Hence, other sources of MSCs have been sought out, the most popular being adipose tissue derived MSCs. These are accessible as a surgical waste tissue and are associated with lower donor site morbidity than bone marrow [48]. The low rejection rates coupled with the anti-inflammatory properties of MSCs makes them an appealing therapeutic solution for OA.

### *1.3. Adipose Tissue Derived MSCs*

The most common harvest location for Adipose tissue derived MSCs (AMSCs) is the abdomen due to the high tissue fat content. The harvesting process by which AMSCs are collected have been described extensively in the past [44–50].

AMSCs have a greater regenerative profile than bone marrow derived MSCs [51]. AMSCs were also found to promote greater neovascularisation, display greater resistance to hypoxia induced apoptosis and higher telomerase activity [52]. Unlike bone marrow derived MSCs, which lose differentiation capacity with age, AMSCs do not [53]. AMSCs maintain their chondrogenic potential and their expansion properties [54]. This is very important to consider in MSC therapies targeted for OA, since these are directed at older patients.

AMSCs have been found to have greater anti-inflammatory properties compared to bone marrow derived MSCs and produce much higher levels of IL-1 receptor antagonist and tissue protective protein tumour-necrosis factor stimulated gene-6 (TSG-6) [55]. When assessed in their role for OA, AMSCs were able to adapt the environment and exerted anti-inflammatory effects on chondrocytes and synoviocytes via prostaglandin E2 [56]. The AMSCs caused polarization of Mo non-polarized macrophages and mature dendritic cells, towards anti-inflammatory and phagocytic phenotypes [57].

Adipose tissue derived stem cells can also be derived from the stromal vascular fraction (SVF) which has the advantage of greater ease of harvest. However, these cells are not plated to select for cells which are plastic adherent [58,59]. As such, cells from the SVF cannot strictly be considered MSCs according to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy criteria [34].

The aim of this systematic review and meta-analysis was to determine the effectiveness of AMSCs and SVF for the use of treatment in osteoarthritis.

## 2. Materials and Methods

### *2.1. Database and Inclusion Criteria*

A systematic review was conducted, based on the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist [60]. Using the PICOS model, (patient, intervention, control, outcome, study), inclusion and exclusion criteria were created.

A comprehensive literature search was conducted using four databases: Medline (1946 to 4 June 2020), EMBASE (1974 to 29 June 2020), Cochrane library (1946 to June 2020), and Web of Science (1900 to 2020).

The following was used as inclusion criteria for all studies screened:

1. Any studies which investigated use of AMSCs on humans for the treatment of knee joint osteoarthritis
2. Any study that included the use of stromal vascular fraction (SVF) or microfragmented adipose tissue
3. Any study which was clinical in nature

Consequently, the following was used as exclusion criteria for all studies screened.

1. Any study not conducted on human
2. Studies which investigated use of MSCs which were not of adipose origin
3. Any case studies and reviews
4. Studies in which the data sets were either incomplete or inaccessible such as conference abstracts and ongoing randomised controlled trials (RCTs)

A search strategy was created, on the basis of the Cochrane Highly Sensitive Search Strategy. This included but was not limited to the following terms: 'mesenchymal', or 'stem cell' and 'osteoarthritis, knee' and 'adipose'. Full search strings can be found in the Appendix A. Restrictions were applied to the search to only include studies conducted on humans and in the English language. Study selection was carried out by two reviewers independently. The titles of the articles were reviewed for relevance. Abstracts were then screened to check if they met inclusion criteria. Full-text manuscripts were then retrieved and analysed. A manual search was also performed on associated review articles to identify any articles that could have been missed by the search. The combined results of the comprehensive search strategy are shown in Figure 1.

### 2.2. Quality Assessment

Each study was critically appraised to ensure relevance. Studies were appraised by two independent reviewers using either the Risk of Bias in Nonrandomised Studies 1 (ROBINS-1) or the Risk of Bias 2 (RoB2) for randomised studies tools. Upon completion of the appraisal, data was stratified according to the tools used and was collated into tables (Supplementary Tables S1 and S2). Any uncertainty was solved through discussion between the reviewers.

### 2.3. Statistical Analysis

Pre and post treatment Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores were extracted from each study for each of the follow-up times. Percentage changes in these scores were then calculated for each follow-up time. Forest plots were created for each of the follow-up time periods. A summary forest plot was created to determine the overall statistical significance of treatment on WOMAC scores. We sought to elucidate whether the pooled effect of treatment resulted in minimum clinically important differences in WOMAC scores, which has been defined as 12, [61] to this end, we do not compare this against the various control groups used across the studies. All statistical analysis was conducted on R software through the 'metafor' package. The $I^2$ test was used to test for heterogeneity. To account for heterogeneity (all-cause) between studies, assuming effects assuming that effects between studies are either similar or not, Fixed effect models were used for analyses with $I^2 < 25\%$. Random effect models were used for analyses with $I^2 > 25\%$. All WOMAC percentage changes were calculated with a 95% confidence interval.

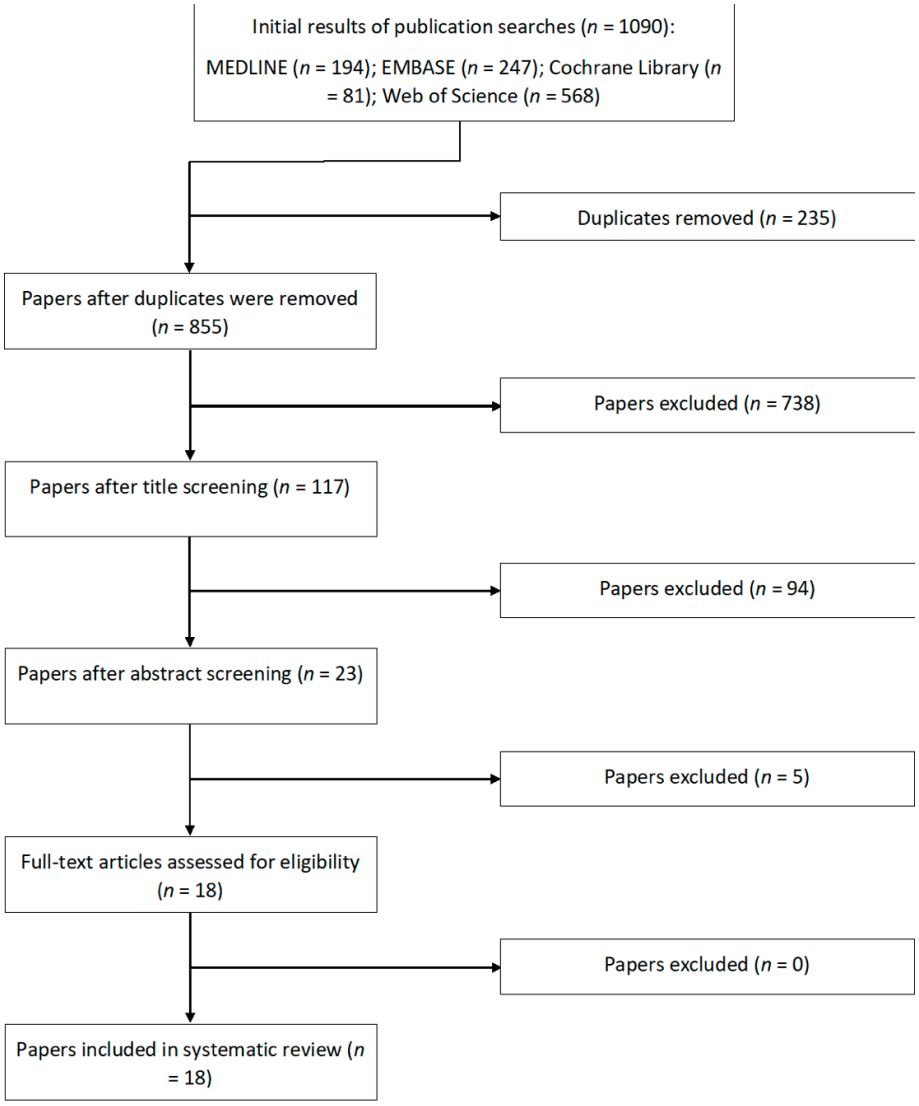


**Figure 1.** Overview of the screening and selection process of studies for the systematic review.

**Figure 1.** Overview of the screening and selection process of studies for the systematic review.

## 3. Results

The search conducted on Medline (1946 to 4 June 2020), EMBASE (1974 to 29 June 2020), Cochrane library (1946 to June 2020), and Web of Science (1900 to 2020) included 117 potentially relevant articles after title screening, and 94 studies were excluded after abstract screening as these were non-human, preclinical studies or did not assess the treatment of interest. After full textual analysis, five studies were removed as these did not include quantitative treatment outcomes relevant to our study, only eighteen studies were then included in this review. One of the 18 studies was a two-year follow-up of another included study [62]. The original study was a prospective cohort study which investigated the effects of three different AMSC dose injections [63]. Out of the studies, 16 were prospective [43,49,62–73] and two were retrospective [74,75]. Only five were randomised controlled trials [48,49,64,69–72]. The general characteristics of each AMSC study can be seen in Table 1. All studies investigated autologous treatments. Characteristics for SVF studies can be found in Table 2. Cellular characteristics for AMSC studies and SVF studies can be found in Tables 3 and 4, respectively. Outcomes measured and complications documented have been collated for each study in Table 5. The overall bias determined in all studies included in this systematic review were found to be low, indicating high quality studies.

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**Table 1.** Study characteristics for AMSC studies, $n = 11$.

| Authors | Type of Study | Treatment vs. Control | No. of Patients in Control Group (Gender) | No. of Patients in Treatment Group (Gender) | Age, Mean | Location of Defect | Grade of OA (Grading Classification) |
|---|---|---|---|---|---|---|---|
| Lee et al. (2019) [64] | Prospective double blinded RCT | AMSC at 1 dose, control | 12 (3M, 9F) | 12 (3M, 9F) | 62.7 | Knee | II–IV (K-L) |
| Freitag et al. (2019) [76] | Prospective non blinded RCT | AMSC at 2 doses, control | 10 (5M, 5F) | 10 (7M, 3F) for 1 injection. 10 (4M, 6F) for 2 injections | 53.6 | Knee | II–III (K-L) |
| Song et al. (2018) [48] | Prospective double blinded RCT | AMSC at 3 doses, no control | N/A | 18 (4M, 14F) | 54.8 | Knee | II–III (K-L) |
| Roato et al. (2019) [73] | Prospective single arm study | AMSC at 1 dose, no control | N/A | 20 (9M, 11F) | 59.6 | Knee | I–III (K-L) |
| Hudetz et al. (2019) [63] | Prospective non-randomised trial | AMSC injection, no control | N/A | 20 (15M, 5F) | Not specified | Knee | III–IV (K-L) |
| Spasovski et al. (2018) [65] | Prospective single arm study | AMSC at 1 dose, no control | N/A | 9 (3M, 6F) | 63 | Knee | B-D (IKDC) |
| Jo et al. (2017) [62] | Prospective cohort study | AMSC at 3 doses, no control | N/A | 18 (3M, 15F) | 61.8 | Knee | III–IV (K-L) |
| Bansal et al. (2017) [77] | Prospective interventional | AMSC injection, no control | N/A | 10 (6M, 4F) | 58.4 | Knee | I–II (BS) |
| Pers et al. (2016) [72] | Prospective single arm study | AMSC at 3 doses, no control | N/A | 18 (8M, 10F) | 64.6 | Knee | III–IV (K-L) |
| Jo et al. (2014) [63] | Prospective cohort study | AMSC at 3 doses, no control | N/A | 18 (3M, 15F) | 61.8 | Knee | III–IV (K-L) |
| Yokota et al. (2019) [75] | Retrospective cohort study | AMSC vs SVF, no control | N/A | 80 (16M, 64F) | 71.4 | Knee | II–IV (K-L) |

AMSC = Adipose Tissue Derived Mesenchymal Stem Cell, F = Female, IKDC = International Knee Documentation Committee, K-L = Kellgren-Lawrence, M= Male, N/A = Not Applicable, OA = Osteoarthritis, RCT = Randomised Controlled Trial.

**Table 2.** Study characteristics for SVF studies, $n = 7$.

| Authors | Type of Study | Treatment vs. Control | No. of Patients in Control Group (Gender) | No. of Patients in Treatment Group (Gender) | Age, Mean | Location of Defect | Grade of OA (Grading Classification) |
|---|---|---|---|---|---|---|---|
| Garza et al. (2020) [69] | Prospective double blinded RCT | High dose SVF vs low dose SVF vs placebo | 13 (6M, 7F) | 13 (4M, 9F) for low dose SVF. 13 (7M, 6F) for high dose SVF. | 59.0 | Knee | II–III (K-L) |

**Table 2.** *Cont.*

| Authors | Type of Study | Treatment vs. Control | No. of Patients in Control Group (Gender) | No. of Patients in Treatment Group (Gender) | Age, Mean | Location of Defect | Grade of OA (Grading Classification) |
|---|---|---|---|---|---|---|---|
| Hong et al. (2019) [70] | Prospective RCT | One knee with SVF and the other with hyaluronic acid placebo | 16 (3M, 13F) | 16 (3M, 13F) | Not specified | Knee | II-III (K-L) |
| Tran et al. (2019) [78] | Prospective non-randomised trial | Arthroscopic microfracture vs arthroscopic microfracture and injection of SVF | 15 (3M, 12F) | 18 (5M, 13F) | 58.64 | Knee | II–III (K-L) |
| Yokota et al. (2017) [68] | Prospective uncontrolled | Injection of SVF, no control | N/A | 13 (2M, 11F) | 74.5 | Knee | III–IV (K-L) |
| Nguyen et al. (2016) [67] | Prospective unblinded, non-randomised trial | Arthroscopic fracture vs arthroscopic fracture and injection of SVF and PRP. | 15 (3M, 12F) | 15 (3M, 12F) | 58.4 | Knee | II–III (K-L) |
| Koh et al. (2013) [66] | Prospective cohort study | SVF at 1 dose with PRP, no control | N/A | 18 (6M, 12F) | 54.6 | Knee | III–IV (K-L) |
| Panni et al. (2019) [74] | Retrospective single arm study | SVF at 1 dose following arthroscopy (for chondral shaving/abrasion and/or meniscal regularization), no control | N/A | 52 (22M, 30F) | 57.3 | Knee | 0–II (K-L) |

BS = Brandt Radiographic Grading Scale for Osteoarthritis, F = Female, K-L = Kellgren-Lawrence, M= Male, N/A = Not Applicable, OA = Osteoarthritis, RCT = Randomised Controlled Trial, SVF = Stromal Vascular Fraction.

**Table 3.** Cellular characteristics for AMSC studies, $n = 11$.

| Authors | Number of Cells Used/Multiple Injections | Method of Delivery of Cells | MSC Pre-Treatment | Follow-Up Period (Weeks) | Harvest Site | Method of Harvest | MSC Surface Marker Validation Via Flow Cytometry |
|---|---|---|---|---|---|---|---|
| Lee et al. (2019) [64] | $1 \times 10^8$ cells | Intra articular injection under USS guidance into unspecified joint space | Adipose tissues were treated with collagenase I and were centrifuged to obtain a pellet which was resuspended in culture media. The cells were cultured for up to 5 days in media until confluent and were then harvested at passage 3 | 26 | Abdomen | Liposuction | AMSCs were positive for CD73, CD90. AMSCs were negative for CD31, CD34, CD45 |
| Freitag et al. (2019) [49] | $100 \times 10^6$ cells, single and double injection | Intra articular injection under USS guidance into unspecified location in joint space | Lipoaspirate was digestion followed by centrifugation. MSCs were cultured under hypoxic conditions with standard growth media until 80% confluency and was expanded to passage 2. | 52 | Abdomen | Liposuction | AMSCs were positive for CD73, CD90, CD105, AMSCs were negative for CD14, CD19, CD34, CD45 |
| Song et al. (2018) [48] | $1 \times 10^7$, $2 \times 10^7$ and $5 \times 10^7$ cells, three injections | Intra articular injection under USS guidance into unspecified location in joint space | Lipoaspirated suspensions were digested and centrifuged, then cells were culture-expanded to passage 4. | 96 | Not specified | Liposuction | AMSCs were positive for CD29, CD49d, CD70, CD90 and were negative for actin, CD13, CD34, CD45, HLA-DR |
| Roato et al. (2019) [73] | Not specified | Intra articular injection under arthroscopic guidance into chondral defect site | Lipoaspirate was treated with Collagenase. The resulting cell pellet was then resuspended into culture media and counted. | 78 | Abdomen | Liposuction | AMSCs were positive for CD73, CD90, CD105, IgG1, IgG2a AMSCs were negative for CD44, CD45 |
| Hudetz et al. (2019) [71] | Unspecified | Intra articular injection into unspecified location in joint space | Samples were digested with collagenase and samples were filtered through a 100 µm cell strainer and centrifuged. The cell pellet was resuspended in DMEM. | 48 | Abdomen | Liposuction | AMSCs were positive for CD70, CD90, CD105, CD146. AMSCs were negative for CD31, CD34, CD45. |
| Spasovski et al. (2018) [65] | $0.5–1 \times 10^7$ cells | Intra articular injection into unspecified location in joint space | MSCs were digested using collagenase, expanded in standard culture media and harvested between passage 2 and 4. | 78 | Abdomen | Liposuction | AMSCs were positive for CD73, CD90, CD105. AMSCs were negative for CD34, CD45 |

**Table 3.** *Cont.*

| Authors | Number of Cells Used/Multiple Injections | Method of Delivery of Cells | MSC Pre-Treatment | Follow-Up Period (Weeks) | Harvest Site | Method of Harvest | MSC Surface Marker Validation Via Flow Cytometry |
|---|---|---|---|---|---|---|---|
| Jo et al. (2017) [62] | $1 \times 10^7$, $5 \times 10^7$ and $1 \times 10^8$ cells | Intra articular injection under arthroscopic guidance into unspecified location in joint space | Aspirated tissues were digested with collagenase I. Cells were cultured for 4-5 days until confluent. All AMSCs used in this study were collected at passage 3. | 104 | Abdomen | Liposuction | AMSCs were positive for CD73, CD90. AMSCs were negative for CD14, CD34, CD45 |
| Bansal et al. (2017) [77] | $1 \times 10^6$ cells | Intra articular injection into unspecified location in joint space | The adipose tissues was filtered and centrifuged. The cell pellet was re-suspended in culture medium and the media was changed every 3-4 days until the cells achieved 90% confluency. | 96 | Abdomen | Liposuction | AMSCs were positive for CD70, CD90, CD105. AMSCs were negative for CD34, CD45, HLA-DR |
| Pers et al. (2016) [72] | $2 \times 10^6$, $10 \times 10^6$ and $50 \times 10^6$ cells | Intra articular injection under USS guidance into unspecified location in joint space | Adipose tissue was digested with collagenase solution and plated in culture medium. Cells were passaged and then cultured in CCM for 14 days with media changes every 3–4 days until confluence. | 26 | Abdomen | Liposuction | AMSCs were positive for CD73, CD90, CD105, IgG1 AMSCs were negative for CD31, CD34, CD45 |
| Jo et al. (2014) [63] | $1 \times 10^7$, $5 \times 10^7$ and $1 \times 10^8$ cells | Intra articular injection under arthroscopic guidance into unspecified location in joint space | Aspirated tissues were digested with collagenase and cells were resuspended in media until confluent. AMSCs used were collected at passage 3. | 26 | Abdomen | Liposuction | AMSCs were positive for CD73, CD90. AMSCs were negative for CD31, CD34, CD45 |
| Nakamura et al. (2019) [75] | $12.75 \times 10^6$ cells, unknown for SVF | Intra articular injection into unspecified location in joint space | The collected aspirate was digested with collagenase. Cells were cultured in medium that was replaced every 3 days thereafter. When cells reached 80% confluency they were passaged up to four times. SVF cells were produced without culture in a sterile single-use functionally-closed system, requiring approximately 2–2.5 h from lipoaspirate. | 26 | Abdomen | Liposuction | Not specified |

AMSC = Adipose Tissue Derived Mesenchymal Stem Cell, BSA = Bovine Serum Albumin, CDU = Collagen Digestion Units, DPBS = Dulbecco's Phosphate-Buffered Saline, DMEM = Dulbecco's Modified Eagle Medium, EDTA = Ethylenediaminetetraacetic acid, FBS = Foetal Bovine Serum, MSC = Mesenchymal Stem Cell, PBS = Phosphate-Buffered Saline, SVF = Stromal Vascular fraction, USS = Ultrasound.

**Table 4.** Cellular characteristics for SVF studies, $n = 7$.

| Authors | Number of Cells Used/Multiple Injections | Method of Delivery of Cells | SVF Pre-Treatment | Follow-Up Period (Weeks) | Harvest Site | Method of Harvest | MSC Surface Marker Validation Via Flow Cytometry |
|---|---|---|---|---|---|---|---|
| Garza et al. (2020) [69] | $3.0 \times 10^7$, $1.5 \times 10^7$, 0 cells | Intra articular injection under USS guidance into unspecified joint space | SVF from dissociated tissue was centrifuged and the SVF cell pellet was extracted, resuspended for injection. | 48 | Abdomen | Liposuction | Not specified |
| Hong et al. (2019) [70] | $7.45 \times 10^6$ cells | Intra articular injection under arthroscopic guidance into unspecified location in joint space | The SVF from the lipoaspirate was isolated by means of collagenase digestion. The SVF was then washed twice with PBS to remove collagenase. | 48 | Abdomen | Liposuction | Not specified |
| Tran et al. (2019) [78] | $9–12 \times 10^7$ cells | Intra articular injection under arthroscopic guidance into chondral defect site | The SVF from the lipoaspirate was isolated through collagenase treatment. The SVF was then diluted with normal saline 0.9% to obtain 6 mL of solution containing 90–120 million cells to administer via injection. | 96 | Abdomen | Liposuction | Not specified |
| Yokota et al. (2017) [68] | Unknown, however estimated to be $3 \times 10^7$ cells | Intra articular injection into unspecified location in joint space | Autologous SVF cells were collected in a sterile single-use functionally-closed system, requiring approximately 2–2.5 h. | 4 | Abdomen | Liposuction | Not specified |
| Nguyen et al. (2016) [67] | $1 \times 10^7$ cells | Intra articular injection under arthroscopic guidance into chondral defect site | The adipose tissue was digested using collagenase and centrifuged, the pellet was suspended in PBS for cell counting before injection. | 72 | Abdomen | Liposuction | Not specified |

**Table 4.** *Cont.*

| Authors | Number of Cells Used/Multiple Injections | Method of Delivery of Cells | SVF Pre-Treatment | Follow-Up Period (Weeks) | Harvest Site | Method of Harvest | MSC Surface Marker Validation Via Flow Cytometry |
|---|---|---|---|---|---|---|---|
| Koh et al. (2013) [66] | $1.18 \times 10^6$ cells | Intra articular injection into unspecified location in joint space | SVF was derived from fat pad tissue and mixed with 3.0 mL of platelet-rich plasma for injection. | 97.2 | Infrapatellar fat pad | Surgical excision of infrapatellar fat pad | Not specified |
| Panni et al. (2019) [74] | Not specified | Intra articular injection under arthroscopic guidance into unspecified location in joint space | The harvested fat was processed with the Lipogems® ortho kit. The final product was transferred directly to syringes for injection. | 61.2 | Abdomen | Liposuction | Not specified |

AMSC = Adipose Tissue Derived Mesenchymal Stem Cell, CDU = Collagen Digestion Units, DPBS = Dulbecco's Phosphate-Buffered Saline, DMEM = Dulbecco's Modified Eagle Medium, FBS = Foetal Bovine Serum, MSC = Mesenchymal Stem Cell, PBS = Phosphate-Buffered Saline, SVF = Stromal Vascular fraction, USS = Ultrasound.

**Table 5.** Outcomes and complications, $n = 18$.

| Authors | Outcome Measures | Pre-Treatment WOMAC Scores | Post Treatment WOMAC Scores | Conclusions Based on Outcomes | Adverse Events | Nature of Complications |
|---|---|---|---|---|---|---|
| Lee et al. (2019) [64] | WOMAC, VAS, KOOS, ROM, K-L, Joint space width of medial and lateral compartment and HKA angle | Baseline WOMAC score was 60.0 (±17.0 SD) | At 6 months post procedure WOMAC scores were 26.7 (±13.3 SD) | Single injection of AD-MSCs led to a 55% reduction in the WOMAC total score, 59% in the pain score, 54% in the stiffness score, and 54% in the physical function score at 6 months. Significant improvements in the VAS, KOOS, ROM scores were also seen. K-L grade, joint space width of medial and lateral compartment, and HKA angle did not change significantly over 6 months in either groups. No evidence of significant cartilage regeneration in MRI at 6 months after the injection. | 8AEs | 6 cases of arthralgia and 2 cases of joint swelling after the procedure. |

**Table 5.** *Cont.*

| Authors | Outcome Measures | Pre-Treatment WOMAC Scores | Post Treatment WOMAC Scores | Conclusions Based on Outcomes | Adverse Events | Nature of Complications |
|---|---|---|---|---|---|---|
| Freitag et al. (2019) [49] | NPRS-11, WOMAC, KOOS, MOAKS | WOMAC scores were 59.6 (±17.9 SD) for the one injection group and 54.4 (±18.2 SD) for the two-injection group | WOMAC scores were 84 (±9.4 SD) for the one injection group and 87.3 (±8 SD) for the two-injection group at 12 months. | NPRS-11 scores were greater when compared with baseline (< 0.05) throughout all time points in all treatment groups. There was no difference however between treatment groups. KOOS and WOMAC improved in all subscales during follow-up to 12 months. Two-thirds of the control group showed cartilage loss. 30% of the one-injection group had further cartilage loss, 50% had progression of osteophyte formation at 12 months. 89% in the two-injection group had either no progression or improvement in cartilage loss. | 7 AEs and 1 SAE in the one injection group. 8AEs and 1 SAE in the first injection of the two-injection group. 10 AEs in the second injection in the two-injection group. | Mild AEs: minor discomfort, bruising and/or swelling after the injection. SAEs were classified as pain and sweeling for 4 weeks after injection which impacted the daily activities of life for the patient. |
| Song et al. (2018) [48] | WOMAC, NPRS-11, SF-36, | WOMAC scores were 34.75 (±17.05 SD) at baseline | WOMAC scores were 25.94 (±16.09 SD), 20.38 (±19.89 SD), 22.77 (±22.72 SD), 15.00 (±11.36 SD) and 12.44 (±8.99 SD) in the 12th, 24th, 48th, 72nd and 96th week. | WOMAC scores improved with time leading up to follow-up in all groups. Significant improvements in the NPRS-11 scores in the low- and high-dose groups were first observed at three months following treatment. A statistically significant reduction in SF-36 scores were only found in the 12th and 96th week of follow-up The volume of knee cartilage increased over the course of follow-up. This was more apparent in the high-dose group | 8 AEs in the low dose group (66.67%). 7 AEs in the middle dose group (58.33%). 6 AEs in the high dose group (50%). | No SAEs or deaths. All complications were AEs. These were most commonly transient pain and swelling of joints, which were mild to moderate and were spontaneously relieved within 7 days without special treatment. One patient experienced mild oedema and cramps of bilateral lower extremities, which were relieved in 21 days without treatment and not related to the MSC treatment. |
| Roato et al. (2019) [73] | WOMAC, VAS, K-L | WOMAC score was 45.91 (±2.8) pre procedure. (NO SE OR SD GIVEN) | WOMAC scores were 27.47 (±3.02), 15.84 (±2.5) and 12.97 (±2.3) at 3 months, 6 months and 18 months post procedure. (NO SE OR SD GIVEN) | Significant improvement of VAS and WOMAC scores, with a significant pain reduction and increased mobility at 3, 6, and 12 months follow-up. No increase in the thickness of cartilage at 18 months. | 1 SAE | Swelling persisted two months after surgery |
| Hudetz et al. (2019) [71] | KOOS, WOMAC, VAS | WOMAC baseline score was 55.38 (±18.8 SD) | WOMAC scores after 12 months was 32.25 (±14.6 SD) | All scores significantly improved after treatment. | 0 AEs or SAEs | N/A |
| Spasovski et al. (2018) [65] | KSS, HSS, Lysholm score, VAS, MOCART | N/A | N/A | All outcomes significantly improved at 3 and 6 months. However, there was no further improvement beyond 12 or 18 months after treatment. | N/A | N/A |

**Table 5.** *Cont.*

| Authors | Outcome Measures | Pre-Treatment WOMAC Scores | Post Treatment WOMAC Scores | Conclusions Based on Outcomes | Adverse Events | Nature of Complications |
|---|---|---|---|---|---|---|
| Jo et al. (2017) [62] | WOMAC, VAS, KSS, KOOS, K-L, Joint space width of the medial compartment, mechanical axis with weight bearing line, and anatomical axis | WOMAC scores were 43.3 (±12.7 SE) for the low dose group, 69.0 (±5.9 SE) for the mid dose group and 54.2 (±5.2 SE) for the high dose group. | WOMAC scores were 25.3 (±19.5 SE), 14.7 ± (12.7 SE) and 17.0 (±9.8 SE) at 6 months, 1 year and 2 years respectively for the low dose group. WOMAC scores were 48.5 (±9.5 SE), 13.1 (±10.0 SE) and 25.1 (±11.0 SE) at 6 months, 1 year and 2 years respectively for the middle dose group. WOMAC scores were 32.8 (±6.3 SE), 16.0 (±4.4 SE) and 19.0 (±5.5 SE) at 6 months, 1 year and 2 years respectively for the high dose group. | The WOMAC, VAS and KSS scores improved in the high-dose group at 6 months and 1 year. Non-significant trends in the low and middle dose groups. Significant improvement in KSS scores in the low dose groups up to one year. The sports subscore of the KOOS improved until 2 years for the high-dose group. No statistically significant improvements were found in the quality-of-life subscore of the KOOS for any of the dose groups. | None | None |
| Bansal et al. (2017) [77] | WOMAC, 6MWD, cartilage thickness | WOMAC score was 64 at baseline (NO SE OR SD GIVEN) | WOMAC scores were 52, 46, 42, 38 and 41 at 3 months, 6 months, 12 months, 18 months and 24 months respectively. (NO SE OR SD GIVEN) | Significant changes in the WOMAC and 6MWD scores were noted in both the subsets and the total after 2 years as compared to the baseline. MRI evaluation demonstrated that cartilage thickness improved. | 1 AE | Pain and swelling which resolved. |
| Pers et al. (2016) [72] | WOMAC, VAS, PGA, SAS, KOOS, OARSI, SF-36 | WOMAC scores were 63.2 (±4.1 SD) for the low dose group, 65.5 (±8.1 SD) for the mid dose group and 65.2 (±2.3 SD) for the high dose group. | WOMAC scores were 24.6 (±8.6 SD), 22.0 (±8.5 SD) and 30.1 (±8.9 SD) at 1 week, 3 months and 6 months respectively for the low dose group. WOMAC scores were 45.8 (±9.1 SD), 52.8 (±9.6 SD) and 42.6 (±9.1 SD) at 1 week, 3 months and 6 months respectively for the middle dose group. WOMAC scores were 61.1 (±15.3 SD), 38.4 (±16.0 SD) and 42.6 (±16.0 SD) at 1 week, 3 months and 6 months respectively for the high dose group. | Statistically significant improvements in WOMAC, VAS, KOOS and SAS scores were only found in the low dose group at 1 week, 3 months and 6 months. No improvements in the SF-36 in any groups. | 1 SAE and 5 AEs. | The SAE was unstable angina pectoris without increased cardiac markers, which was reported in 1 patient 3 months after ASC injection. The patient's risk factors included hypertension and hyperlipidemia. Five AEs reported by four patients. There was slight knee pain/joint effusion occurred during the first week after ASC injection that resolved with nonsteroidal anti-inflammatory drugs in three patients and spontaneously in one patient. |

**Table 5.** *Cont.*

| Authors | Outcome Measures | Pre-Treatment WOMAC Scores | Post Treatment WOMAC Scores | Conclusions Based on Outcomes | Adverse Events | Nature of Complications |
|---|---|---|---|---|---|---|
| Jo et al. (2014) [63] | WOMAC, VAS, KSS, K-L, Joint space width of the medial compartment, mechanical axis with weight bearing line, and anatomical axis, ICRS | WOMAC scores were 43.3 (±12.7 SE) for the low dose group, 69.0 (±5.9 SE) for the mid dose group and 54.2 (±5.2 SE) for the high dose group. | WOMAC scores were 44.0 (±4.4 SE), 30.0 (±12.0 SE), 38.7 (±24.7 SE) and 25.3 (±19.5 SE) at 1, 2, 3 and 6 months respectively for the low dose group. WOMAC scores were 72.3 (±4.3 SE), 51.3 (±6.5 SE), 51.3 (±6.7 SE) and 48.5 (±11.0 SE) at 1, 2, 3 and 6 months respectively for the mid dose group. WOMAC scores were 45.5 (±4.5 SE), 40.1 (±6.0 SE), 37.0 (±6.8 SE) and 32.8 (±6.3 SE) at 1, 2, 3 and 6 months respectively for the high dose group. | Significant improvement of the WOMAC and VAS at 6 months compared with baseline in the high-dose groups. This was not seen in the other treatment groups. Knee subsection of KSS significantly increased in the low-dose and the high-dose groups, but improvements in the function subsection of seen in the low-dose group only. Other parameters did not change significantly at 6 months in any groups. 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. No significant change was found in the lateral parts of the joint. | 1 AE and 1 SAE in the low dose group (66.6%). 2 AEs in the mid dose group (66.6%). 5 AEs in the high dose group (41.66%). | In the low dose group, the AE was an individual case of nasopharyngitis, and the SAE was a urinary calculus. In the mid dose group AEs were individual cases of nasopharyngitis, arthralgia and chest pain. In the high dose group AEs were individual cases of nasopharyngitis, arthralgia, back pain, cough and hypertriglyceridemia. |
| Yokota et al. (2019) [75] | KOOS, VAS, OARSI, K-L | N/A | N/A | Change in KOOS symptoms occurred earlier in the AMSC group than the SVF group, with significant improvement detected at 3 months follow-up. The extent of VAS improvement after injection was greatest in patients with mildest. Patients in the AMSC group had a greater improvement in VAS than patients in the SVF group, regardless of the extent of OA at baseline. The proportion of patients who responded to treatment as determined by the OMERACT-OARSI responder criteria was greater in the AMSC group than the SVF. | 3AEs in the ASC group and 26 AEs in the SVF group. | In the ASC group, there was 1 case of joint swelling after the injection and 2 cases of abdominal induration after harvest. These were all self-limiting. In the SVF group, there were 3 cases of joint swelling after the injection. There were 6 cases of abdominal pain, 5 cases of abdominal swelling and 12 cases of abdominal induration after harvest. These were all self-limiting. |

**Table 5.** *Cont.*

| Authors | Outcome Measures | Pre-Treatment WOMAC Scores | Post Treatment WOMAC Scores | Conclusions Based on Outcomes | Adverse Events | Nature of Complications |
|---|---|---|---|---|---|---|
| Garza et al. (2020) [69] | WOMAC, OS | Baseline WOMAC scores were 49.3 for the placebo group, 56.2 for the low dose group and 47.1 for the high dose group (THIS WAS THE MEAN. NO SE OR SD WAS GIVEN) Median was 49.8 (37.4–57.0), 51.6 (46.3–62.3) and 49.8 (35.6–55.2) for the placebo, low dose and high dose groups. | WOMAC scores for the placebo was 26.0, 22.9, 37.2 and 41.9 at 6 weeks, 3 months, 6 months and 1 year respectively. Median values were 23.0(14.2–37.4), 20.0 (16.0–32.0), 30.2 (21.4–55.2), 41.0 (19.5–55.2). WOMAC scores for the low dose group was 24.8, 19.7, 23.7 and 21.8 at 6 weeks, 3 months, 6 months and 1 year respectively. Median values were 20.0 (10.7–37.4), 14.0 (5.3–35.6), 26.7 (8.9–32.0), 12.5 (7.1–35.6) WOMAC scores for the high dose group was 25.7, 26.5, 20.0 and 13.2 at 6 weeks, 3 months, 6 months and 1 year respectively. Median values were 27.0 (14.2–36.0), 27.0 (10.7–34.7), 8.9 (3.6–32.0), 3.6 (0.0–26.7) | All groups displayed a reduction in total WOMAC score from baseline at 6 month follow-up. All treated groups continued to demonstrate lower total WOMAC scores 1 year after injection as compared with baseline scores and sixth month scores. There was no change in cartilage thickness detected at six month follow-up. | 0 AEs or SAEs | N/A |
| Hong et al. (2019) [70] | VAS, WOMAC, ROM, WORMS, MOCART | Baseline WOMAC pain score was 9.50 (±3.92 SD) for the control group and was 9.44 (±3.90 SD) for the treatment group. Baseline WOMAC stiffness score was 3.00 (±1.55 SD) for the control group and was 3.31 (±1.82 SD) for the treatment group. | WOMAC pain scores were 8.94 (±4.98 SD), 11.56 (±6.84 SD), 12.88 (±5.73 SD) and 15.19 (±4.29 SD) at 1 month, 3 months, 6 months and 12 months respectively for the control group. WOMAC stiffness scores were 4.38 (±2.22 SD), 4.94 (±2.49 SD), 5.44 (±2.56 SD) and 5.69 (±2.57 SD) at 1 month, 3 months, 6 months and 12 months respectively for the control group. WOMAC pain scores were 6.25 (±3.02 SD), 2.13 (±3.52 SD), 1.5 (±3.84 SD) and 1.44 (±4.77 SD) at 1 month, 3 months, 6 months and 12 months respectively for the treatment group. WOMAC stiffness scores were 1.75 (±1.59 SD), 1.12 (±1.80 SD), 0.81 (±1.59 SD) and 1.06 (±2.11 SD) at 1 month, 3 months, 6 months and 12 months respectively for the treatment group. | In the treated group, all scores including VAS, WOMAC pain, WOMAC stiffness, and knee ROM was founded to be significantly improved at one, three, six, and 12-months follow-up as compared with baseline scores within the treated groups and against control groups. Both WORMS and MOCART MRI scores showed a statistically significant improvement in the treatment group, while a deterioration in the control group. | 10 AEs | 4 AEs relating to abdominal pain after harvest which resolved after 1 week. 6 cases of pain and swelling in both knees after surgery. These all resolved after 2 weeks with analgesia. |

**Table 5.** *Cont.*

| Authors | Outcome Measures | Pre-Treatment WOMAC Scores | Post Treatment WOMAC Scores | Conclusions Based on Outcomes | Adverse Events | Nature of Complications |
|---|---|---|---|---|---|---|
| Tran et al. (2019) [78] | VAS, WOMAC, OS, BME, K-L | WOMAC scores were 52.0 (±18.26 SD) and 42.64 (±12.51 SD) at baseline for patients with KL OA grade 2 and 3 respectively. | For KL OA grade 2 patients, WOMAC scores were 24.25 (±19.77 SD) and 18.25 (±20.07 SD) at 12 and 24 months respectively. For KL OA grade 3 patients, WOMAC scores were 18.21 (±8.20 SD) and 9.00 (±8.46 SD) at 12 and 24 months respectively. | No significant difference was found between the VAS scores of the treatment and placebo groups at 12 months.A decreasing trend in the VAS and WOMAC scores of the treatment group was observed up to 24 months compared to controls. Between 12 and 24 months, the VAS scores increased in the placebo group. MRI results showed that after 24 months of treatment, bone marrow oedema was decreased in both the placebo and the SVF treatment groups, the latter demonstrated a greater effect. The Outbridge score also decreased in the SVF-treated group. | N/A | N/A |
| Yokota et al. (2017) [68] | JKOM, WOMAC, VAS | Baseline WOMAC scores were 49.6 (±20.4 SD) | WOMAC scores were 43.0 (±17.4 SD) and 36.5 (±21.9 SD) at 1 month and 6 months after treatment respectively | JKOM, WOMAC, and VAS scores were significantly improved compared to baseline one month following treatment. This effect was also observed at the six-month visit. JKOM scores improved by an average of 35% over baseline compared to a 32% improvement in WOMAC, and 40% for VAS. | 26 AEs | All patients experienced pain and swelling at the fat harvest and injection sites. These however resolved after a few days with analgesia. |
| Nguyen et al. (2016) [67] | WOMAC, Lysholm score, VAS, OS, BME, JMA, | WOMAC scores for the placebo group was 47.27 (±17.13 SD). WOMAC scores for the treatment group was 42.87 (±16.29 SD) | WOMAC scores for the placebo group was 23.27 (±15.61 SD) and 25.60 (±19.69 SD) at 6 and 12 months respectively. WOMAC scores for the treatment group were 19.27 (±14.87 SD) and 17.33 (±14.91 SD) at 6 and 12 months respectively. | WOMAC scores significantly decreased compared with baseline scores at 6 and 12 months.WOMAC scores between the treatment and placebo groups were not significantly different at 12 months, but a significant difference was seen at 18 months. VAS and Lysholm scores improved in the treatment group compared to pre treatment scores at all follow-up timepoints. | 0 AEs or SAEs | N/A |
| Koh et al. (2013) [66] | WOMAC, lysholm score, VAS, WORMS | Baseline WOMAC score was 49.9 (±12.6 SD) | After final follow-up post procedure WOMAC scores were 30.3 (±9.2 SD) | WOMAC scores decreased in the treatment group over the follow-up period. Greater changes in WOMAC score were seen in subjects injected with greater cell numbers. Lysholm and VAS scores also significantly improved over the follow-up period. Significant reduction was observed in the WORMS cartilage subscore. | 1 AE | Notable pain and swelling after injection for 2 weeks. This was self-limiting. |

**Table 5.** *Cont.*

| Authors | Outcome Measures | Pre-Treatment WOMAC Scores | Post Treatment WOMAC Scores | Conclusions Based on Outcomes | Adverse Events | Nature of Complications |
|---|---|---|---|---|---|---|
| Panni et al. (2019) [74] | IKS, VAS, | N/A | N/A | 96.2% of treated subjects reported improvements in knee function and/or pain. A subset (62%) achieved complete or near-complete function recovery and/or pain relief. Two (3.9%) patients reported slight reduction of the pain. | 3AEs | Transient haematoma after harvest |

6MWD = 6 Minute Walk Distance, BMA = Bone Marrow Oedema, HKA = hip-knee-ankle, HSS = Hospital for Special Surgery Knee score, ICRS = International Cartilage Repair Society, IKS = International Knee Society, JKOM = Japanese Knee Osteoarthritis Measure, JMA = Joint Motion Amplitude, KOOS = Knee Injury and Osteoarthritis Outcome Score, K-L = Kellgren-Lawrence, KSS = Knee Society Score, MOAKs = MRI Osteoarthritis Knee Score, MOCART = 2D Magnetic Resonance Observation of Cartilage Repair Tissue, NPRS = Numeric Pain Rating Scale, OARSI = Osteoarthritis Research Society International, OS = Outerbridge Classification System, PGA = Patient Global Assessment score, ROM = range of motion, SAS = Short Arthritis Assessment scale, SF-36 = Short Form-36, WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index, WORMS = Whole-Organ MRI Score, VAS = visual analogue scale.

Forest plots were created, according to the follow-up time periods utilised, to analyse changes in WOMAC scores post AMSC and SVF treatment (Figure 2) in the treatment group.

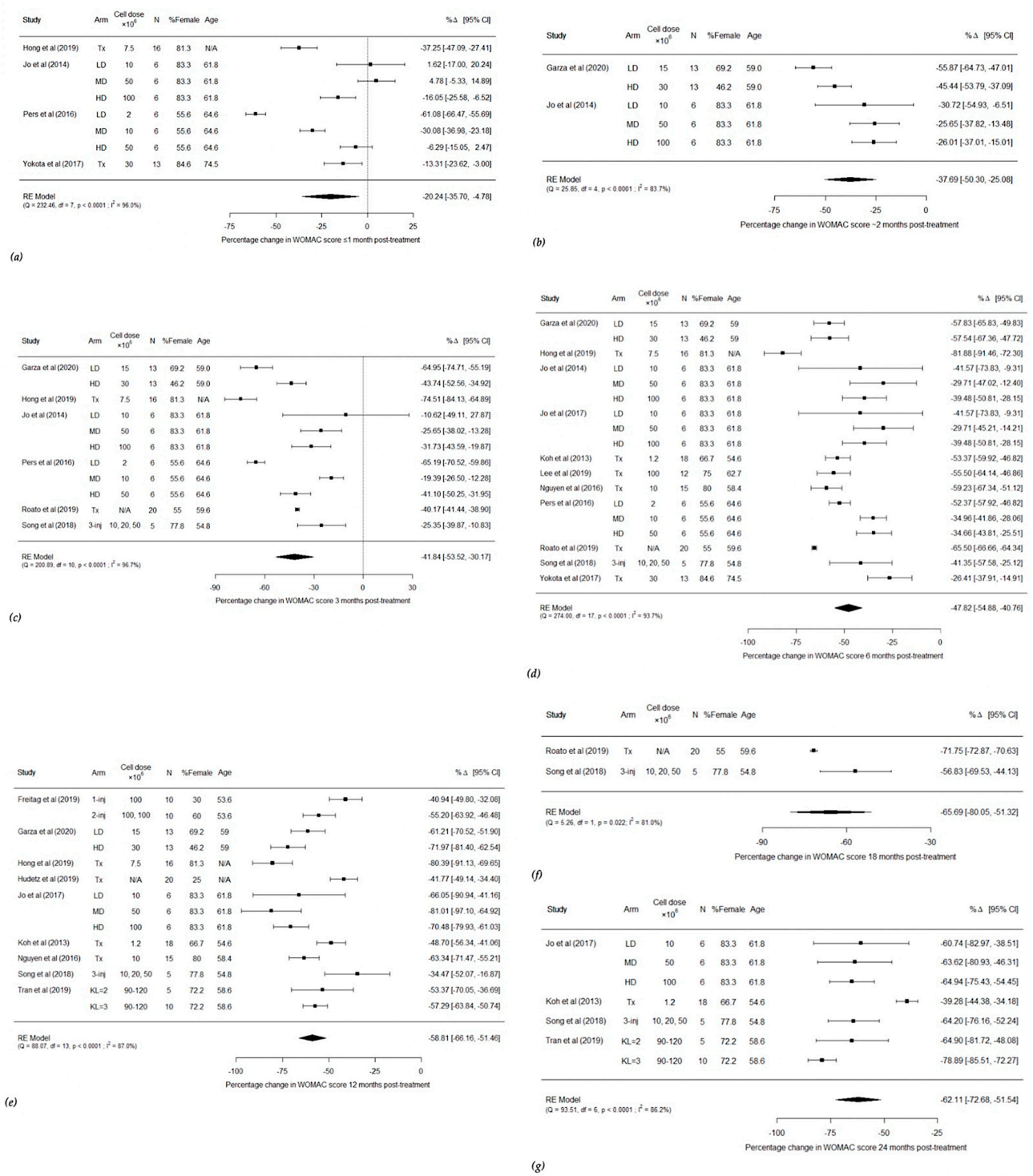


**Figure 2.** Forest plots showing percentage changes in WOMAC score less than one month after treatment (**a**), approximately two months after treatment (**b**), three months after treatment (**c**), six months after treatment (**d**), twelve months after treatment (**e**), eighteen months after treatment (**f**) and twenty-four months after treatment (**g**) (1-inj = 1 injection, 2-inj = 2 injections, 3-inj = 3 injections, CI = confidence intervals, df = degrees of freedom, HD = high dose, LD = low dose, MD = middle dose, N = number, RE = random effects, Tx = treatment group).

**Figure 2.** Forest plots showing percentage changes in WOMAC score less than one month after treatment (**a**), approximately two months after treatment (**b**), three months after treatment (**c**), six months after treatment (**d**), twelve months after treatment (**e**), eighteen months after treatment (**f**) and twenty-four months after treatment (**g**) (1-inj = 1 injection, 2-inj = 2 injections, 3-inj = 3 injections, CI = confidence intervals, df = degrees of freedom, HD = high dose, LD = low dose, MD = middle dose, N = number, RE = random effects, Tx = treatment group).

Figure 2a shows all four studies which documented WOMAC scores less than 1 month after treatment [63,68,70,72,78]. There was a statistically significant improvement in WOMAC scores in the individual arm studies, and in the high dose groups of the other studies. The pooled analysis showed a −20.24% change [95 CI −35.70, −4.78] suggesting that use of AMSCs and SVF resulted in a statistically significant improvement in WOMAC scores less than a month after treatment [Q = 232.46, $p < 0.0001$]. Figure 2b–g show all studies which evaluated WOMAC scores at approximately two months, three months, six months, twelve months, eighteen months and twenty-four months after treatment, respectively [48,49,62–64,66–73,78] The plots show that there are statistically significant improvements in WOMAC scores in all arms of all treatment groups across the follow-up

after treatment [63,68,70,72,78]. There was a statistically significant improvement in

[95% CI −50.30, −25.08, Q = 25.85, $p < 0.0001$] post two month treatment, −41.84% [95% CI −53.52, −30.17, Q = 200.89, $p < 0.0001$] post three month treatment, −40.07% [95% CI −54.44, −39.65, Q = 273.47, $p < 0.0001$] post six month treatment, −58.44% [95% CI −66.41, −50.47, Q = 85.71, $p < 0.0001$] post twelve month treatment, −65.69% [−80.05, −51.32, Q = 5.26, $p$ 0.022] post eighteen month treatment and −62.11% [95% CI −72.68, −51.54, Q = 93.51, $p <$ 0.0001] post twenty-four month treatment.

A forest plot was created to show a summary of the pooled analyses for the respective follow-up times (Figure 3).

Q = 93.51, $p < 0.0001$] post twenty-four month treatment.

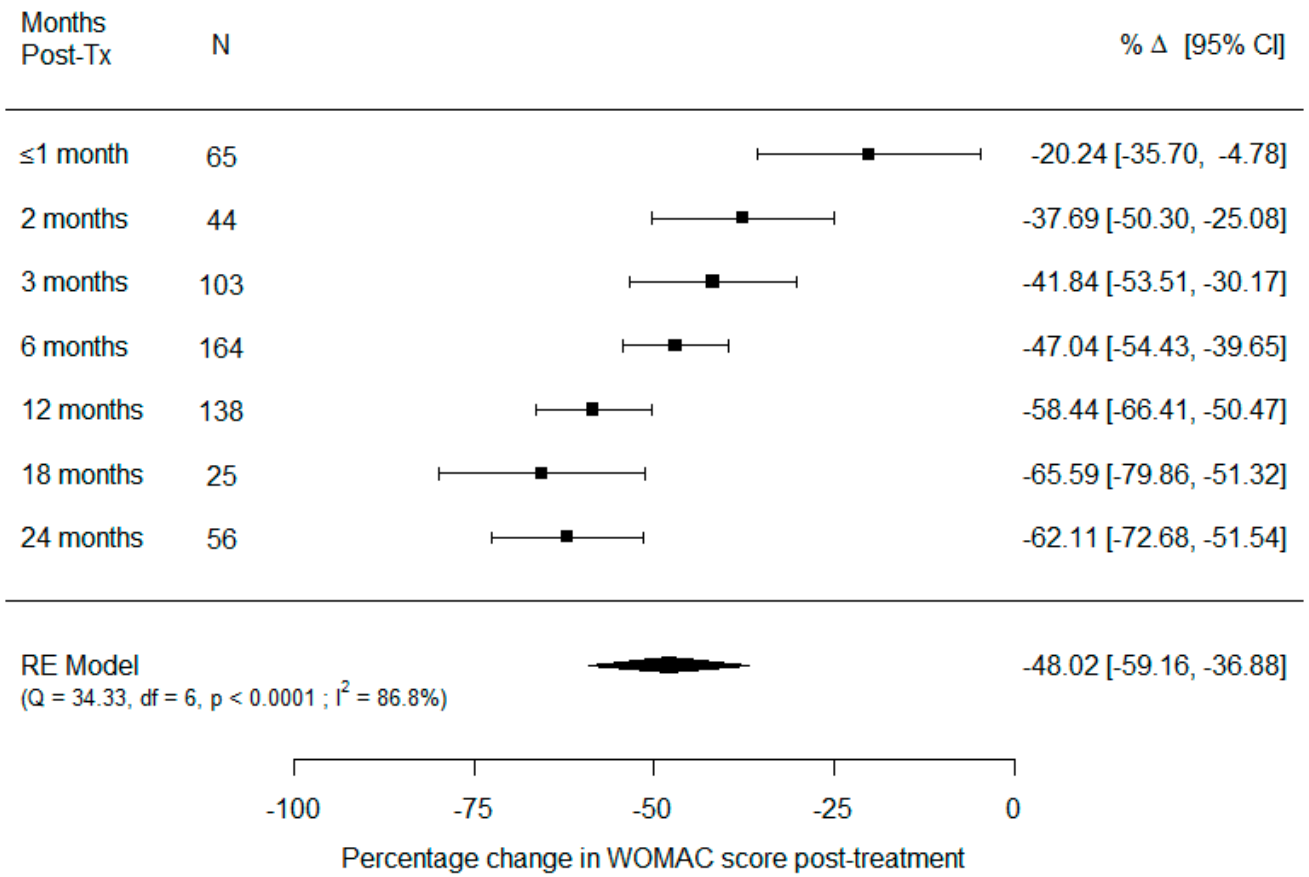


**Figure 3.** Forest plot showing pooled analyses of percentage changes in WOMAC scores across the different follow-up times (CI = confidence intervals, df = degrees of freedom, N = number, RE = random effects, Tx = treatment group).

Forest plot showing pooled analyses of percentage changes in WOMAC scores across the different follow-up
= confidence intervals, df = degrees of freedom, N = number, RE = random effects, Tx = treatment group).

Figure 3 shows the pooled analyses for all the follow-up time periods. In every follow-up, post-treatment, there was an improvement in WOMAC scores. This improvement increased as time went on, between less than month and up to 18 months post-treatment. There was a slight reduction in improvement at 24 months, compared to 18 months. These analyses were further pooled showing −48.02% [−59.16, −36.88, Q = 34.33, $p < 0.0001$]. This suggests that overall, there was a statistically significant improvement in WOMAC scores post treatment.

Figure 3 shows the pooled analyses for all the follow-up time periods. In every follow-up, post-treatment, there was an improvement in WOMAC scores. This improvement increased as time went on, between less than month and up to 18 months post-treatment. There was a slight reduction in improvement at 24 months, compared to 18 months. These analyses were further pooled showing −48.02% [−59.16, −36.88, Q = 34.33, $p < 0.0001$]. This suggests that overall, there was a statistically significant improvement in WOMAC scores post treatment.

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### 3.1. Classification of Osteoarthritis

Most studies in the literature used the Kellgren-Lawrence (KL) radiological classification of osteoarthritis to grade the severity of OA in patients [48,49,62–64,66–71,73–75,78]. Due to the subjective nature of clinical diagnoses of OA, this was not used in any studies. Several of the included studies used this classification in their inclusion and exclusion criteria where for example, patients with a KL grade of one would not be included [48,49,63,64,66–69,71,72,75,78] in the study. Some studies also only included patients who had an average pain intensity of four or more on the 10-point visual analogue scale for at least four months [48,63,64,70]. One study used the IKDC classification to grade OA [65] and another used the Brandt Radiographic Grading Scale for Osteoarthritis [77].

### 3.2. Follow-Up

The most common follow-up period was 6.5 months [63,64,72,74]. Three studies had a follow-up of 12 months [69–71]. Three studies had a follow-up of 24 months [38,60,61]. One study had a follow-up of one month [68]. Five studies had a follow-up between 12 and 24 months [49,65,67,73,74]. Two studies had a follow-up greater than 24 months [62,66].

### 3.3. Adverse Events

Only two studies have been found which did not report whether adverse events (AEs) or severe adverse events (SAEs) occurred during the clinical study [65,78] The rest of the studies all reported AEs. Two studies observed no AEs or SAEs during the study [67,71]. One of these studies however had four cases of complications which were deemed unrelated to the treatment regimen. These complications were high blood pressure, chest pain, dyspnoea, and urinary retention [67]. Eleven studies reported that subjects commonly experienced either transient pain or swelling of the joint after injection of the AMSCs or SVF [48,49,64–70,72–74,77]. In most patients this resolved spontaneously. In some patients, paracetamol was administered after which this resolved. Some studies also reported that subjects experienced discomfort at the site of lipoharvest [49,68,74]. However, this was resolved on further follow-up. Three studies reported patients experienced internal haematomas at the site of lipoharvest [49,74]. Four studies reported SAEs [49,63–66,72]. One subject in one study had a urinary stone. The subject had a past medical history of stones, and this was subsequently treated [63]. In another study, a subject experienced angina pectoris. However, they had risk factors of hypertension and hyperlipidaemia which predisposed them to the condition. In the remaining two studies, two patients and one patient respectively experienced severe pain and swelling following the procedure [49,66]. The two patients recovered after four weeks, while the single patient recovered after two weeks.

### 3.4. Outcome Measures

Several studies recorded two primary outcomes: clinical and radiological outcomes. Studies utilised patient reported outcome measures (PROMs) to document clinical outcomes and the PROMs used varied greatly between studies (Tables 4 and 5). The most widely employed scoring systems were the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and the visual analogue scale (VAS). The former was used by fifteen studies, while the latter was used by fourteen studies. The Knee Injury and Osteoarthritis Outcome Score (KOOS) was used by six studies. The Knee Society Score (KSS) was used by three studies. The Lysholm score was also employed by three studies. The Numeric Pain Rating Scale (NPRS), range of motion (ROM) and bone marrow oedema (BME) scoring were utilized by two studies. The Short Form-36 (SF-36), Hospital for Special Surgery Knee score (HSS), Patient Global Assessment score (PGA), Short Arthritis Assessment scale (SAS), International Knee Society (IKS) score, Japanese Knee Osteoarthritis

Measure (JKOM), Joint Motion Amplitude (JMA) and the six minute walking distance score (6MWD) were each utilized by one study.

Fewer papers assessed radiological outcomes. Six studies used radiographs to assess KL grades of patients after the said treatment was given. Several studies utilised Magnetic Resonance Imaging (MRI) to assess cartilage defects in the knee of patients. However, only seven of these used standardised radiological scoring systems. Freitag et al., (2019) used the MRI Osteoarthritis Knee Score (MOAKs). Koh et al., (2013) utilised the Whole-Organ MRI Score (WORMS) [66]. Spasovski et al. (2018) used the 2D Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score [65]. Hong et al., (2019) used both WORMS and MOCART [70]. Nguyen et al., (2016), Garza et al., (2020) and Tran et al., (2019), all used the Outerbridge Classification System (OS) [67,69,78].

Only two studies conducted a second look arthroscopy and subsequent histological analysis of the cartilage. Jo et al., (2014) used the International Cartilage Repair Society (ICRS) score for histological grading [63], while Pers et al. (2016) used Osteoarthritis Research Society International (OARSI) for histological grading [72].

## 4. Discussion

End-stage knee OA is currently managed with joint replacement surgery. This, however, does not target the underlying disease process of OA, but rather the end stage symptoms. Treatment options such as cartilage repair and osteotomy can delay the progression of OA, but do not modify the disease [79,80]. Recently, use of AMSCs has sprung into the clinical purview. AMSCs have the potential to regenerate new healthy articular cartilage and thus alleviate the symptoms of knee OA. The results of this systematic review and meta-analysis demonstrate that use of both AMSC and SVF treatments significantly reduce WOMAC pain scores. This suggests that these treatments provide improved function and a reduction in pain.

Numerous advantages of AMSCs have been described in the literature [76,81]. However, despite this, there is limited information on this topic in the literature, especially with regards to human studies. Animal studies are more widespread since the safety of use of AMSCs had to first be established, this was first conducted in mice. ter Huurne et al. (2012) conducted such a study (C57BL/6 mice), with early-stage collagenase induced OA. They found that injection of AMSCs into the knee joints of these mice, led to a reduction and inhibition of cartilage destruction and formation of enthesophytes. In addition, there was reduced synovial thickening and the treatments were safe.

Use of mice studies, have allowed the evaluation of safety of AMSC transplantation to treat knee OA. Song et al., (2018) conducted a human clinical trial using adipose tissue derived stem cells. However, they first conducted preclinical safety tests in vitro and on BALB/c-nu nude mice. After confirming the safety of administering AMSCs isolated through their methodologies, they enrolled 18 patients in their clinical study. These patients were separated into three groups: low-dose ($1 \times 107$), mid-dose ($2 \times 107$), and high-dose ($3 \times 107$). Each patient was injected three times and followed up over a course of 96 weeks. They found that use of AMSCs is safe for human use.

This study and several others documented AEs and SAEs. However, despite these occurrences, they did not cause long term detriments to the patients' quality of life and in most cases spontaneously resolved. As such several studies deemed AMSC therapy to be safe [48,49,63,65,72–74].

AMSC or SVF post treatment outcomes were determined by changes in WOMAC scores in fourteen of the eighteen studies included in this systematic review. One study conducted by Bansal et al. (2017) also used WOMAC scores, however since standard deviation or error figures were not provided, this was excluded from quantitative meta-analysis [77].

In the main pooled analysis, it was demonstrated that use of AMSCs and SVF in knee osteoarthritic joints improved WOMAC scores. All studies that documented WOMAC scores between two months and 24 months after treatment found that there was a sta-

tistically significant improvement in WOMAC scores after treatment. This was the case, regardless of the dose of AMSC or SVF used, or number of injections administered, suggesting that the less laborious preparation of SVF compared to MSCs may be an advantage as both therapies achieve good clinical outcomes. Furthermore, the pooled analyses in Figure 3 illustrates that there is a statistically significant improvement in WOMAC scores across all follow-up times, suggesting disease modification that persists and long-term efficacy without the need for repeat administration of treatment. These improvements increased as time from initial treatment increased between less than one month and eighteen months post treatment. After this, at twenty-four months there was a slight decrease in improvements in WOMAC scores. Overall, this suggests that the therapies act beyond short-term analgesia, and lead to changes in the disease process. Improvements in these scores suggest that these AMSC and SVF treatments reduce pain and improve knee function in patients with knee OA. Due to the low number of studies that compared AMSC and SVF therapies, we were unable to conduct a meta-analysis to directly compare these therapies. As there were also significant heterogeneity in the studies, subgroup analyses of AMSC and SVF separately could not be conducted to yield meaningful results. The pooled analysis in this review offers findings that are generalizable to multiple adipose derived cell-based therapies.

Out of the eighteen studies included in this review, five investigated the effects dose of AMSCs, had on outcomes [48,62,63,72]. In four of the studies, patients were divided into three groups: a low dose group, medium dose group and high dose group. Three of these studies found that patients in the high dose group gained greater clinical improvements than those in the low and medium dose groups. This suggests that there is a relationship between the number of AMSCs administered and the therapeutic effect gained. This seems intuitive, as joint-native MSCs in OA patients may have diminished capacity to proliferate and repair cartilage [82]. Thus, provision of healthy, functional MSCs could prevent further cartilage loss and repair existing defects. This is in line with conclusions made by two studies which investigated radiological outcomes. They discovered greatest reduction in cartilage defects were found in the high dose groups [62,63]. One can posit that this suggests that increasing dose is correlated with greater regenerative potential. Conclusions drawn by Pers et al., (2016) differed with the aforementioned studies, finding that the lowest dose group had the best improvements in clinical outcomes. This may have arisen, since the authors documented that the patients with the highest levels of inflammation were those in the low dose group [72]. AMSCs could have been primed to exert their immunoregulatory functions more efficiently in this more prominent inflammatory background. However other studies in the literature show similar findings, implying that lower dose administration of AMSCs may be more effective than high dose [83,84]. If low doses of AMSCs, are just as or more effective than high doses, then clinical and radiological improvements do not correlate with the number of cells used. Using less cells may reduce the laborious preparation required. Moreover, this may cut down on costs, making AMSCs more appealing for clinical use. Since very few studies investigated doses on outcomes, subgroup analyses were not conducted.

Freitag et al., (2019) compared the effect one injection of $100 \times 10^6$ cells, to two injections of $100 \times 10^6$ cells. Both were compared to a control group. They concluded that two injections of AMSCs achieved more consistent OA stabilisation than one injection [49]. They were the only study to do this, which represents a gap in the literature. This indicates that multiple low dose injections of AMSCs may provide superior clinical and radiological improvements than a singular dose. These results may be reflective of the overall increased number of cells used in the double injection group. On the other hand, this increased efficacy of the double injection treatment could be due to spaced out exposure to AMSCs. Repetitive low dose spaced out injections of AMSCs may prove to be more successful than a single large dose injection. Nevertheless, there is also a possibility that there is a ceiling on the correlation between numbers of injections and improvements seen. Moreover, Freitag et al., (2019) reported an increase in the number of moderate AEs in the second

injection group. This implies that increased numbers of injections are linked to increased AEs, which may affect the tolerate of the therapy in question.

Yakota et al. (2019) was the only study that compared the efficacy of AMSCs with SVF [74]. They found that both therapies improved osteoarthritic symptoms and pain. However, when they analysed the clinical scores, they found that the improvements were more significant and occurred earlier in the AMSC group. Earlier clinical improvements suggest that AMSCs have a faster mechanism of action. More significant clinical improvements suggest that AMSCs are superior to SVF for symptomatic control of knee OA. No radiological outcomes were investigated in this study. Therefore, no conclusions can be drawn regarding the cartilage regenerative potential of either treatment. However, other studies have shown that AMSCs have great cartilage regenerative potentials, and reduce cartilage defects [62,63]. Yakota et al., (2019) also discovered that there was a higher frequency of knee effusion and minor complications related to the harvesting of adipose tissue in the SVF patient group [74]. As such iatrogenic complications may be higher in SVF. This may affect tolerate and favourability of such treatment in the future. More clinical studies need to be conducted to make robust conclusions regarding which treatment is superior.

Garza et al., (2020) was the only study to compare the effects of different doses of SVF on clinical and radiological outcomes. They led a study in which they compared a placebo group using hyaluronic acid, with a low dose SVF group (1.5 × 107 cells) and a high dose SVF group (3.0 × 107 cells) [69]. They discovered both doses of SVF resulted in improved WOMAC scores compared to the placebo. Nevertheless, there was no statistical difference between the high and low dose groups. Therefore, this suggests that the benefits expressed by SVF is not dose dependent. Additionally, there was no visible quantifiable changes detected in cartilage thickness on the MRI scans between all three groups. The result from the radiological outcomes suggests that unlike AMSCs, which improve cartilage defects, SVF do not. Therefore, SVF treatment has no impact on the disease process of OA and only plays a role in symptomatic relief. This could be potentially explained by the fact that AMSCs have a higher number of colony forming fibroblast units (CFU-F) and greater differentiation potential [58]. This study also further illustrates the limitations of hyaluronic acid for the treatment of knee OA. This review has found a gap in the literature, which must be addressed with studies investigated SVF dose and outcomes, to further determine the role of SVF therapy in the treatment of knee OA.

Koh et al., (2013), Bansal et al. (2017) and Nyugen et al. (2016) were the only studies which used a growth factor alongside SVF [66,67,77]. They all used a platelet rich plasma (PRP) scaffold. All studies concluded that there was significant improvement in clinical scores long term post treatment. Use of PRP may have improved the efficacy of treatments. PRP is known to enhance MSC proliferation and chondrocyte differentiation, and as such could bolster cartilage degeneration [85,86]. However, none of these studies compared use of PRP alone against SVF and PRP. As such the efficacy of PRP cannot be quantified. Only with further studies, comparing AMSCs and SVF with and without PRP can we definitively determine the role of PRP in therapy for knee OA. If use of growth factor proves to increase efficacy, these could be applied to AMSC therapy as well.

Many of the studies included in this systematic review used K-L grading of OA as an inclusion and exclusion criterion in the recruitment of their patients. Out of all studies, only one included patients with a K-L grade of 1 [73]. As such most of the studies could not determine the efficacy of treatments on low grade knee OA. Since, there is reduced levels of inflammation in the earlier stages of OA than end stages, AMSC and SVF therapy may be more effective. Alternatively, these therapies may be more effective in end stage OA, since the high inflammatory environment could modulate cells to exercise their immunoregulatory function more effectively. In addition, only eight studies included patients with K-L grade 4 [48,62–64,68,71,72,74]. As such there is less data on the effects of AMSC treatment on end stage severe knee OA, and more on middle stage knee OA. Furthermore, most studies did not stratify patients according to the severity of the OA. As such they could not determine whether the severity of knee OA has any bearing on the

efficacy of the MSC treatment. Nonetheless, three of the studies did make observations based on this. Tran et al., (2019) inferred that treatment was more effective in patients with KL grade 3 than with grade 2 [78]. In the higher severities of OA, there is greater inflammation. On the other hand, Nyugen et al., (2016) and Yokota et al., (2019) found that lower K-L grades had greater clinical improvements, indicating that efficacy was greater in patients with less severe OA [67,74]. If this is the case, more studies need to be conducted to include K-L grade 1 patients.

In addition to K-L grading, studies did not stratify patient cohorts according to age or BMI. Thus, the impact these could have on the efficacy of outcomes is unknown. It is possible that in younger patients, who have superior regenerative potential, the quality and therefore efficacy of AMSC and SVF is superior to elderly patients [87]. Maredziak et al. (2016) illustrated that there is reduced CFU-F, proliferation rates, and quantified chondrogenic and osteogenic differentiation in aged AMSC cells. Furthermore, aged cells seem to shift more in favour of adipogenic differentiation [88]. In addition, it has been shown that there is a biological role of adipose inflammation in obese patients and OA [89]. As such, it is possible, this mechanism of OA may respond differently to AMSC and SVF treatments, compared to age related articular cartilage degeneration. Therefore, stratifying patients into different BMI groups, may be of benefit. However, this must be investigated further before definitive conclusions can be made.

The gold standard of evaluating new-born cartilage in the face of cartilage repair, is second look arthroscopy and histological biopsy. It is important to determine quantify the size of cartilage regeneration as well as the constitution of the cartilage. Nevertheless, only two studies performed such procedures [63,72]. To confidently determine the role of AMSC treatment in knee OA, we must understand the qualities and mechanism of cartilage repair involved.

Out of all the studies, six used arthroscopies prior to injection of the treatment. Roata et al. (2019) and Jo et al., (2017) only used arthroscopy as guidance for injection of the AMSCs into the osteoarthritic site [62,73]. However, the rest of the studies performed arthroscopic debridement prior to injection of the treatment [67,70,74]. As such it is possible that this led to bias of outcome. Due to the heterogeneity in treatment modality between the studies, and the low number of studies that examined arthroscopic delivery, sub-group analysis was not conducted. It should be noted that arthroscopy for the treatment of knee OA has been shown to be largely ineffective [90,91]. However, arthroscopic debridement removes inflammatory synovial fluid which can interfere with AMSC adhesion in vitro, and therefore increasing the effectiveness of said treatment [73]. As such, arthroscopic debridement may prime the joint to become more responsive to injections. Consequently, one cannot definitively rule out the effect arthroscopy has on the clinical and radiological outcomes of AMSC treatment.

Five of the studies included, utilised ultrasound to aid guidance of the AMSC or SVF injections. When the outcomes of these studies are compared to those which used arthroscopy or utilised neither, there is no difference in clinical outcomes. As mentioned previously, only two studies conducted second look arthroscopies. Hence, we cannot determine whether use of image guidance leads to greater cartilage regeneration. Future studies should directly compare use of image guidance against blind injection. In addition, studies should perform radiological and histological analysis on all patients to determine if imaging guidance has any bearing on cartilage regeneration.

There was a lack of long-term studies carried out, as shown in the results. The average follow-up across all studies was 60.1 weeks. The longest follow-up period was 104 weeks [62]. This would be considered a short-term follow-up. Thus, there is a gap in the literature for such long-term studies. As a result, the effect AMSC injections and SVF injections have on long term knee OA is unknown. The importance of this was made evident by Park et al., (2016). They conducted a study in which they investigated cartilage regeneration in OA patients, through use of umbilical cord derived MSCs. They discovered that three years after treatment, cartilage repairs persisted. This implied that use of such

MSCs could provide a long-term solution to OA. Conversely, Jo et al., (2017) found that two years post treatment, cartilage deterioration was apparent. As such, it is possible that the effect of AMSC and SVF treatment may be limiting, and further injections may be required for persistence of cartilage regeneration. Alternatively, it could be possible after several years, the knee joint becomes unresponsive to AMSC and SVF treatment injections. Only with long term studies, can the lasting implications of this treatment be determined. Understandably, the studies included in this review are very recent, as such long-term studies with 5–10-year follow-ups are not possible at this stage.

There are significant variations in the outcome measures utilised by studies. In terms of clinical outcomes, most studies using WOMAC and VAS. However, several did not. As a result, the clinical outcomes recorded are harder to compare between studies. Scoring systems such as WOMAC and VAS are non-specific. Perhaps creation of a PROM specifically for post intra-articular injection would be more beneficial. Use of a handful of scoring systems rather than a wide array may allow for better comparison between studies and therefore allow scientists to determine what the most effective treatment for knee OA.

SVF can be prepared through various methods. It has been shown that different methods have different compositions and properties [92]. This has not been addressed by any of the studies included in this review which investigated SVF. As a result, it may be unwise and inaccurate to compare the results of studies which used differing methods to produce their SVF.

The gold standard in medical research when testing the efficacy of a new treatment is a double blinded randomised controlled trial (RCT). However, use of AMSCs is very novel. Many of the studies included in this systematic review were carried out as pilot studies. Out of these studies, only six used a control group [49,64,67,69,70,78] As such the other studies are unable to truly define the efficacy of the treatments tested. Many of these studies recognised this, however their principal goal was to determine the safety of AMSCs rather than its effectiveness. They stated that future studies should include control arms in clinical trials. However, Freitag et al., (2019) and Pers et al., (2016) believed there may be ethical concerns with conducting an RCT [48–78]. All patients would have to undergo a lipoharvest procedure before randomisation. This procedure is not without complications. A study conducted by Comella et al. (2017) found that such complications are low in lipoharvest procedures [93]. Since these studies were pilot in nature, their sample sizes were also very small, with the largest being 80 patients, and the average being 30.5 across all studies.

## 5. Conclusions

The studies in this systematic review have established the safety and efficacy of both AMSC therapy and SVF therapy for knee OA in humans. In addition, the meta-analyses show that use of AMSC and SVF therapy for knee OA definitively improves WOMAC scores up to two-years, with improvements increasing with time. This suggest that AMSC and SVF treatments reduce pain and improve knee function in patients with severe knee OA. Future clinical studies must now incorporate control arms and have larger sample sizes to successfully determine the effectiveness of these treatments. Specifically, there is little to no literature on studies comparing use of single vs multiple AMSC injections, comparing AMSC treatments with SVF treatments and the effect of different doses of SVF on outcomes.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/cells10061365/s1, Table S1 Critical appraisal of the non-randomised studies included in this systematic review, using the ROBINS-1 tool $n$ = 13, Table S2. Critical appraisal of the randomised studies included in this systematic review, using the RoB-2 tool $n$ = 5

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All authors reviewed the data, evaluated, drafted and approved the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A

**Databases searched:**
OVID MEDLINE®: 1946 TO JUNE WEEK 4 2020
Date of search: 29/06/20
Date range searched: January 1946 to June 2020
**S**EARCH STRATEGY

1. exp cartilage/ or exp "bone and bones"/
2. exp Injections, Intra-Articular/
3. exp Osteoarthritis, Hip/ or exp Osteoarthritis/ or exp Osteoarthritis, Spine/ or exp Osteoarthritis, Knee/
4. exp Mesenchymal Stem Cells/ or exp Mesenchymal Stem Cell Transplantation/
5. exp Transplantation, Homologous/ or exp Stem Cell Transplantation/ or exp Transplantation, Autologous/ or exp Mesenchymal Stem Cell Transplantation/
6. exp Adipose Tissue/ or adipose.mp.
7. adipo*.tw.
8. exp Autografts/
9. exp Heterografts/
10. 4 or 5 or 8 or 9
11. 1 or 2 or 10
12. 6 or 7
13. 11 and 12
14. 3 and 13
15. Limit 14 to (English language and humans)

**EMBASE:** 1974 TO 2019 JUNE 26
Date of search: 29/06/20
Date range searched: January 1974 to June 2019
**S**EARCH STRATEGY

1. exp cartilage/
2. exp intraarticular drug administration/
3. exp knee osteoarthritis/
4. exp mesenchymal stem cell transplantation/ or exp mesenchymal stem cell/
5. exp autograft/
6. exp adipose tissue/
7. adipo*.tw.
8. 4 or 5
9. 1 or 2 or 8
10. 6 or 7
11. 9 and 10
12. 3 and 11
13. Limit 12 to (human and English language)

**COCHRANE LIBRARY:** 1946 TO JUNE 2020
Date of search: 29/06/20
Date range searched: January 1946 to June 2020
**SEARCH STRATEGY**
**#1 MeSH descriptor:** [Cartilage] explode all trees
#2 MeSH descriptor: [Injections] explode all trees
#3 MeSH descriptor: [Osteoarthritis] explode all trees
#4 MeSH descriptor: [Mesenchymal Stem Cells] explode all trees
#5 MeSH descriptor: [Stem Cells] explode all trees
#6 MeSH descriptor: [Adipose Tissue] explode all trees
#7 MeSH descriptor: [Autografts] in all MeSH products
#8 MeSH descriptor: [Heterografts] in all MeSH products
#9 #1 or #2 or #3
#10 #4 or #5 or #6 or #7 or #8
#11 #10 and #9
#12 #11 and #6
WEB OF SCIENCE: 1900 TO 2020
Date of search: 29/06/20
Date range searched: 1900 to June 2020
**SEARCH STRATEGY**
**#1:** (TS = (cartilage or inject*)) Indexes=SCI-EXPANDED, CPCI-S Timespan=1900–2020
#2: (TS=(osteoarthritis)) Indexes=SCI-EXPANDED, CPCI-S Timespan=1900–2020
#3: (TS=(mesenchymal stem cell or stem cell or homologous or autologous or autograft or heterograft)) Indexes=SCI-EXPANDED, CPCI-S Timespan=1900–2020
#4: (TS=(adipose)) Indexes=SCI-EXPANDED, CPCI-S Timespan=1900–2020
#5: #3 or #1 Indexes=SCI-EXPANDED, CPCI-S Timespan=1900–2020
#6: #5 and #2 Indexes=SCI-EXPANDED, CPCI-S Timespan=1900–2020
#7: #6 and #4 Indexes=SCI-EXPANDED, CPCI-S Timespan=1900–2020
**CLINICAL TRIALS.GOV:** 1900 TO 2020
Date of search: 29/06/20
Date range searched: 1900to July 2019
#1 Osteoarthritis, knee
#2 Adipose

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ORIGINAL PAPER

# Intra-articular injection of autologous adipose-derived stromal vascular fractions for knee osteoarthritis: a double-blind randomized self-controlled trial

Zheping Hong[1] & Jihang Chen[2] & Shuijun Zhang[2] & Chen Zhao[2] & Mingguang Bi[2] & Xinji Chen[1] & Qing Bi[1,2]

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## Abstract

**Objective** The purpose of this study was to compare the clinical and radiological efficacy of autologous adipose-derived stromal vascular fraction (SVF) versus hyaluronic acid in patients with bilateral knee osteoarthritis.

**Methods** Sixteen patients with bilateral symptomatic knee osteoarthritis (K-L grade II to III; initial pain evaluated at four or greater on a ten-point VAS score) were enrolled in this study, which were randomized into two groups. Each patient received 4-ml autologous adipose-derived SVF treatment (group test, $n=16$) in one side of knee joints and a single dose of 4-ml hyaluronic acid treatment (group control, $n=16$) in the other side. The clinical evaluations were performed pre-operatively and post-operatively at one month, three months, six months, and 12-months follow-up visit, using the ten-point visual analog scale (VAS), the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and the knee range of motion (ROM). The whole-organ assessment of the knees was performed with whole-organ magnetic resonance imaging score (WORMS) based on MRI at baseline, six months and 12-months follow-up. The articular repair tissue was assessed quantitatively and qualitatively by magnetic resonance observation of cartilage repair tissue (MOCART) score based on follow-up MRI at six months and 12 months.

**Results** No significant baseline differences were found between two groups. Safety was confirmed with no severe adverse events observed during 12-months follow-up. The SVF-treated knees showed significantly improvement in the mean VAS, WOMAC scores, and ROM at 12-months follow-up visit compared with the baseline. In contrast, the mean VAS, WOMAC scores, and ROM of the control group became even worse but not significant from baseline to the last follow-up visit. WORMS and MOCART measurements revealed a significant improvement of articular cartilage repair in SVF-treated knees compared with hyaluronic acid-treated knees.

**Conclusion** The results of this study improve function, and repair cartilage defects in patients with knee osteoarthritis.

**Keywords** Osteoarthritis · Adipose-derived stromal vascular fractions · Intra-articular injection · Articular cartilage

## Introduction

Osteoarthritis (OA) results from degeneration of joint cartilage and subchondral bone and is one of the leading causes of joint pain and disability [1, 2]. The knee is the most frequently involved weight-bearing joint [3]. As a Bwear to tear^ disease, OA is associated with significant morbidity and healthcare expenditure [4, 5]. Many treatment modalities for knee OA such as lifestyle modification, pharmaceutical, and surgery have been advocated [6]. Intra-articular injection of hyaluronic acid (HA) is effective in improving symptoms and slowing down the progression of OA [7, 8], but fail to reverse or repair the degenerative cartilage or bone [9].

Regenerative cell therapies for knee OA such as adipose-derived stromal vascular fraction (SVF) have been recently investigated [10–14]. Adipose-derived stromal cells (ADSC) included in SVF have the potential of differentiating into adipogenic, osteogenic, chondrogenic, and other mesenchymal lineages, and have been widely applied to knee OA

✉ Qing Bi
frankhong671101@163.com

[1] The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

[2] Department of Orthopedic Surgery, Zhejiang Provincial People's Hospital and People's Hospital of Hangzhou Medical College, No. 158 Shangtang Road, Hangzhou 310014, Zhejiang, China

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research for their immunomodulatory, anti-inflammatory and paracrine effects [15, 16]. Several recent studies showed the feasibility and safety of ADSC treatments, and it should be an ideal therapeutic option for knee OA [17–21]. However, cell expansion greatly increases the hospitalization costs. Unlike ADSC, SVF can be readily obtained from the lipoaspirate samples without the need for any cell separation or culturing conditions, which make it more cost efficient and convenient. There is a dearth of literature in the area of SVF treatments for knee OA, few clinical trials have been performed except several case reports. In addition, most of these published clinical trials failed to blind for both the participants and the outcome assessor because of the liposuction and other additional intervention procedures [10, 13, 18, 22, 23], which would lead to a high risk of performance bias. Finally, we designed a double-blind, randomized, self-controlled trial to compare the clinical and radiological efficacy of autologous adipose-derived SVF versus hyaluronic acid treatment among patients with grade II/III knee osteoarthritis of bilateral knee.

## Materials and methods

### Patients and study design

This trial's protocol was approved by Ethics Committees of Zhejiang Provincial People's Hospital before first patient's enrollment; all patients were provided a written informed consent voluntarily. Eligible patients were 18–70 years of age with bilateral symptomatic knee osteoarthritis of grade II to III according to Kellgren-Lawrence criteria [24] and had an initial pain evaluated at four or greater on a ten-point visual analog scale (VAS) in bilateral knee joints. More details of inclusion and exclusion criteria were listed in Table 1.

Before the study, the sample size was estimated on the basis of the results from our pilot study to obtain a power of 80% with α risk = 0.05. From January 2015 to June 2016, 16 patients (32 knees) were enrolled in this study. Three of them were male, and 13 of them were female. The completely randomization process was finished by an assistant accountant who was blinded to the patients' data using SPSS 20.0 software (IBM Corporation, NY, US). First, we listed 1–16 serial numbers (patient serial number) in accordance with the outpatient order. Second, 16 random numbers were generated by RV.UNIFORM (0, 1) in the computer that matched number-by-number with 16 patients' serial numbers. Third, the 16 random numbers were arrayed in ascending order; the corresponding patients of first eight random numbers were injected with 4-ml SVF in the left knee and 4-ml hyaluronic acid (SOFAST, Freda, china) in the right knee. The last eight patients were intervened with 4-ml hyaluronic acid (SOFAST, Freda, china) in the left knee and 4-ml SVF in the opposite. All SVF-treated knees formed the test group. By contrast, another 16 knees exposed with hyaluronic acid formed the control group. More details were shown in Fig. 1. All injections were done under the guidance of knee arthroscopy.

Table 1 Inclusion and exclusion criteria

| |
|---|
| Inclusion criteria |
| • Age 18–70 years old |
| • Bilateral knees with Grade II-III osteoarthritis, identified by two different observers, according to the Kellgren-Lawrence grading scale |
| • Bilateral knees with initial pain evaluated at four or greater on a ten-point visual analog scale (VAS) |
| • Patient is able to understand the instructions given by the doctors |
| • Signing informed consent form |
| Exclusion criteria |
| • Had secondary arthritis (related to rheumatoid arthritis, gouty arthritis, post-infectious arthritis, and previous articular fractures) |
| • Severe heart, lung, liver, and kidney disease that cannot tolerate general anesthesia |
| • Psychiatric disorders |
| • History of liposarcoma and other cancer |
| • Pregnancy |
| • Immunosuppression |
| • Coagulopathy |
| • Abdominal hernia |
| • Any knee joint operation or intra-articular injection of any drug within 6 months before the screening |
| • Sign of infection or serological positive of HIV, syphilis |
| • A low level of body fat content that may make liposuction difficult |

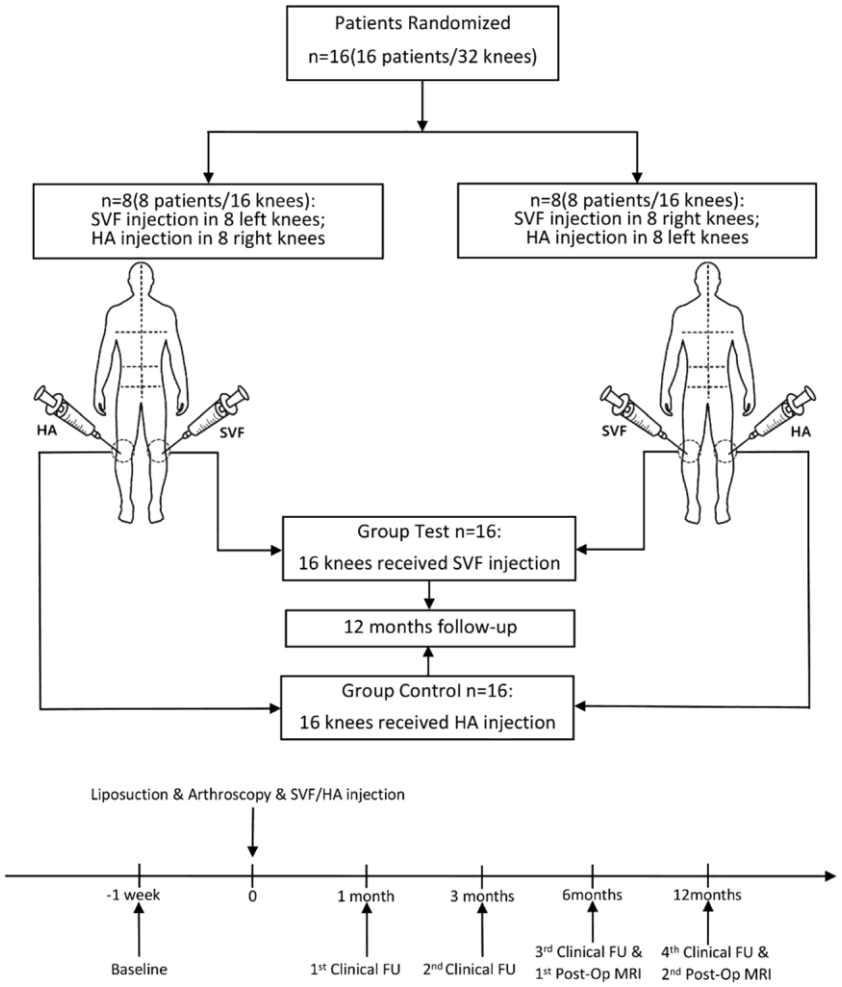
Five investigators were included in the protocol for clinical evaluation, corresponding to pre-operation (1 week before operation; baseline), and one, three, six and 12-months post-operation respectively. At each visit, patients were carefully evaluated using the visual analog scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), as well as range of motion (ROM) measurement, and magnetic resonance imaging (MRI) examination (1 week pre-operation, baseline; 6 months and 12-months post-operation).

Except for the orthopedic surgeon, all patients, radiologists, and investigators were blind to treatment allocation of the participants. The orthopaedic surgeon who delivered the intervention did not take outcome measurements.

### Preparation of SVF and cell counting

All patients were fasted of at least six hours and water deprivation of at least two hours before operation, general anaesthesia was performed in supine position after checking the patients' information by operator, anaesthetist, and circulating nurse. Liposuction was performed by one regular skilled plastic surgeon, who was blind to patients' information. After sterilizing on abdominal and both lower extremities skin, two small incisions about 5 mm were made around umbilicus,

Fig. 1 Study flowchart




and a target volume of approximately 100 to 150 cc of lipoaspirate was harvested through superwet technique from the subcutaneous layer around umbilicus. The incisions were closed with sutures but not tightened to allow more drainage of the blood-tinged tumescent fluid. Abdominal binder was used after operation to prevent bruising in the surgical area.

The harvest adipose tissue was immediately put into a sterile container which was packaged in a portable cryopreservation box on the way to the laboratory. The lipoaspirate was washed twice with 37 °C phosphate buffered saline (PBS), and the residual blood cells and tissue fragments were removed by the mesh filter. Equal volume of type I collagenase (Worthington, Lakewood, NJ, USA) was added into the washed adipose tissue for digestion. The mixture was then placed in a shaking incubator at 37 °C for 30 minutes. After enzymolysis, the tube was centrifuged at 1000 g for 10 min (Eppendorf 5810R, Germany). The supernatant was discarded, and the remnant SVF pellet at the bottom was resuspended in PBS reaching a volume of 4.5-ml SVF. A 0.5-mL sample of the final product was collected for cell counting, and the cell quantity and viability was measured through an automatic cell counter (Countstar IC1000, China).

## Surgical procedures and injection

While the adipose processing was going on, arthroscopic debridement was performed in bilateral knee joint by a single orthopaedic surgeon. After a standard arthroscopic examination, all unstable cartilage around the lesion was debrided to form a stabile circumstance of the cartilage. Once the SVF processing was accomplished, SVF and HA were injected under arthroscopic guidance, after the arthroscopic fluid was drained. In the test group, about 4 ml of SVF suspension was injected into the cartilage lesion surface. The contralateral knee received 4 ml of HA injection. Incisions subcuticular suture and pressure dressing after injection were confirmed. All the proce- dures were done under general anesthesia that the patients themselves were blind about the injection allocation.

## Post-operative protocol

All patients were instructed to be non-weight bearing for one day after operation and were discharged two days post-operation with the same health propaganda. Regular daily activities were allowed during follow-up period, and all participants should contact the doctor in charge immediately once there was any sign of adverse event, including fever; cutaneous pruritus, and erythra; swelling, pyorrhea, or fissuration of the incisions. Additionally, a dosage of 200-mg Celebrex twice daily for 2 days was applied as a discharge medication, when patients complained about incision pain with an evaluation over five on a VAS scale on the discharged day. These patients were followed via telephone until the incision pain was relieved.

## Clinical evaluation

Pain and functional limitation were evaluated using VAS and WOMAC questionnaire. The WOMAC measures five items for pain (score range 0–20), two for stiffness (score range 0–8), and 17 for functional limitation (score range 0–68) with a total score range from 0 (slightest) to 96 (worst). While functional limitation cannot be scored per joint, pain and stiffness were measured per joint separately by two copies of the questionnaires. In addition, ROM of bilateral knee joints was also recorded.

## MRI assessment

The protocol required three MRI scan: baseline (1 week before operation), six months, and 12 months of follow-up. Each MRI was performed using SIEMENS 3.0 T Skyra MRI device, with the 15-channel knee coil. The patients lay supine 30 mintes to reduce the influence of the knee motion and weight bearing to the results of scanning. The following sequences were applied: PDWI-FS images in the sagittal, coronal, and transverse planes; T1 W1 images in the sagittal planes. All data were transmitted to Siemens post-processing workstation, two trained radiologists blinded to each other completed the measurement and recording, and finally obtained a consensus conclusion. The whole-organ assessment of the knees was performed by whole-organ magnetic resonance imaging score (WORMS) [25]. The cartilage repair tissue was assessed by magnetic resonance observation of cartilage repair tissue (MOCART) score (include 9 variables) [26].

## Statistical analysis

All data are presented as means ± SD. We used SPSS software (version 20.0, IBM Corporation, NY, US) for all data calculation. Within group analysis of follow-up statistics (VAS, WOMAC score, ROM, and WORMS) were compared with baseline using the paired *t* test, and the independent *t* test was used to compare data at same follow-up time point between groups. The discrete data were analyzed by chi-square test. Differences with $P < 0.05$ were considered statistically significant.

# Results

## Patient characteristics

were randomly allocated to the group test (knee received SVF (Fig. 1). The patients characteristics showed no significant leg distribution between patients received SVF therapy in the (Table 2). No relevant baseline differences in symptom dura-WOMAC pain and stiffness, knee ROM, and WORMS between two groups were observed (Tables 3 and 5). In addition, there was no significant difference in preferred leg proportion between the group test, and group control showed ($P > .05$), which diminished the influence of preferred leg in the treatment and follow-up.

## Safety

Four patients (25%) complained about pain of the abdomen, like muscle soreness after strenuous exercise, sustained about one week after liposuction. Six patients (37.5%) reported pain and swelling in bilateral knee joints that continued for a few days after knee surgery and all resolved within two weeks. The pain reported above all responded well to Celebrex. There were (including infection, allergy, and poor wound healing) and adipose harvest (including deformity and severe ecchymosis).

## Clinical outcome

Mean changes of clinical scores from baseline to one month, three months, six months, and 12 months were summarized in Fig. 2 and Table 4. In the test group, all scores including VAS, cantly improved at one month, three months, six months, and (Fig. 2). The mean VAS, WOMAC pain, WOMAC stiffness, and ROM in the test group improved by 3.19 ± 0.98, 8.00 ± baseline and last follow-up (Table 4). In the control group, and three months after HA injection, but was amplified again

Table 2 Baseline characteristics of patients with different treatment of bilateral knees

| Patient characteristics | Patients with SVF therapy in the left knee<br>*N* =8 | Patients with SVF therapy in the right knee<br>*N* =8 | *P* value |
|---|---|---|---|
| Age, year | 53 ± 10.97 | 51 ± 5.95 | 0.561 |
| Sex, *n* | | | 0.522 |
| Female | 7 | 6 | |
| Male | 1 | 2 | |
| BMI, kg/m$^2$ | 25.98 ± 1.95 | 26.63 ± 1.62 | 0.480 |
| Preferred leg, *n* | | | |
| Left lower extremity | 2 | 3 | |
| Right lower extremity | 6 | 5 | |
| History of trauma, n | 3 | 2 | |

Values are expressed as mean ± SD unless otherwise indicated. *BMI* body mass index

at six and 12-months visits, from 5.75 ± 1.24 to 5.81 ± 1.33 ($P = 0.791$) and 5.81 ± 1.83 ($P = 0.835$) (Fig. 2a). Functional improvement of ROM was significant at one month after HA therapy ($P < 0.001$). However, this trend even took a turn for the worse after three months post-operation in the control group (decreased by 1.88 ± 6.40 from baseline to last follow-up, not significantly) (Fig. 2b). Unlike the SVF treated group, the general tendency of WOMAC pain and stiffness subscores towards worsening in the control group showed significant differences compared with the test group, as showed in Fig. 2c and Fig. 2d.

## Radiologic evaluation

The whole-organ assessment of the knees was performed with WORMS based on MRI at baseline, six months and 12-months follow-up (Tables 5 and 6). In the test group, WORMS showed an important improvement that the mean WORMS decreased by 11.38 ± 24.89 ($P = 0.088$) and 15.44 ± 21.95 ($P < 0.05$) from baseline to six and 12 months, respectively. By contrast the consequence in the control group was poor, WORMS deteriorated by 12.81 ± 12.66 ($P < 0.01$) and 15.50 ± 14.65 ($P < 0.01$) from baseline to six and 12 months, respectively. The repair of the articular cartilage defects was measured by MOCART system based on the MRI results at six and 12-months follow-up, details were shown in Table 7. In the test group, the mean MOCART score was 54.06 ± 11.58 at six months visit and was 62.81 ± 8.16 at 12-months follow-up, showing a significant improvement ($P < 0.01$). However, the mean MOCART, in the control group was poor in both six months (19.38 ± 9.64) and 12 months (19.06 ± 7.79), showed no improvement from six months to 12 months in the HA treated group ($P = 0.924$). It is remarkable that the MOCART in the test group was significantly better than that

Table 3 Baseline characteristics of the group test and group control

| | Group test (*N* = 16)<br>knee treated with SVF | Group control (*N* = 16)<br>Knee treated with HA | *P value* |
|---|---|---|---|
| SVF cell density, (× 10$^6$/ml) | 7.45 ± 3.73 | – | |
| SVF cell viability, (%) | 70.25 ± 5.04 | – | |
| Preferred leg, *n* (%) | 7 (43.75) | 9 (56.25) | |
| Symptom duration, mo | 6.88 ± 3.56 | 6.38 ± 2.68 | 0.230 |
| Kellgren-Lawrence Grade, *n* | | | 0.288 |
| Grade II | 10 | 7 | |
| Grade III | 6 | 9 | |
| Baseline VAS score | 5.38 ± 1.20 | 5.75 ± 1.24 | 0.392 |
| Baseline WOMAC pain | 9.44 ± 3.90 | 9.50 ± 3.92 | 0.964 |
| Baseline WOMAC stiffness | 3.00 ± 1.55 | 3.31 ± 1.82 | 0.604 |
| Baseline knee ROM | 120.13 ± 13.27 | 116.31 ± 14.65 | 0.446 |
| Baseline WORMS | 71.31 ± 24.2 | 69.81 ± 18.05 | 0.844 |

Values are expressed as mean ± SD unless otherwise indicated. *SVF*, stromal vascular fraction; *HA*, hyaluronic acid; *VAS*, visual analog scale; *WOMAC*, Western Ontario and McMaster Universities Osteoarthritis Index; *ROM*,

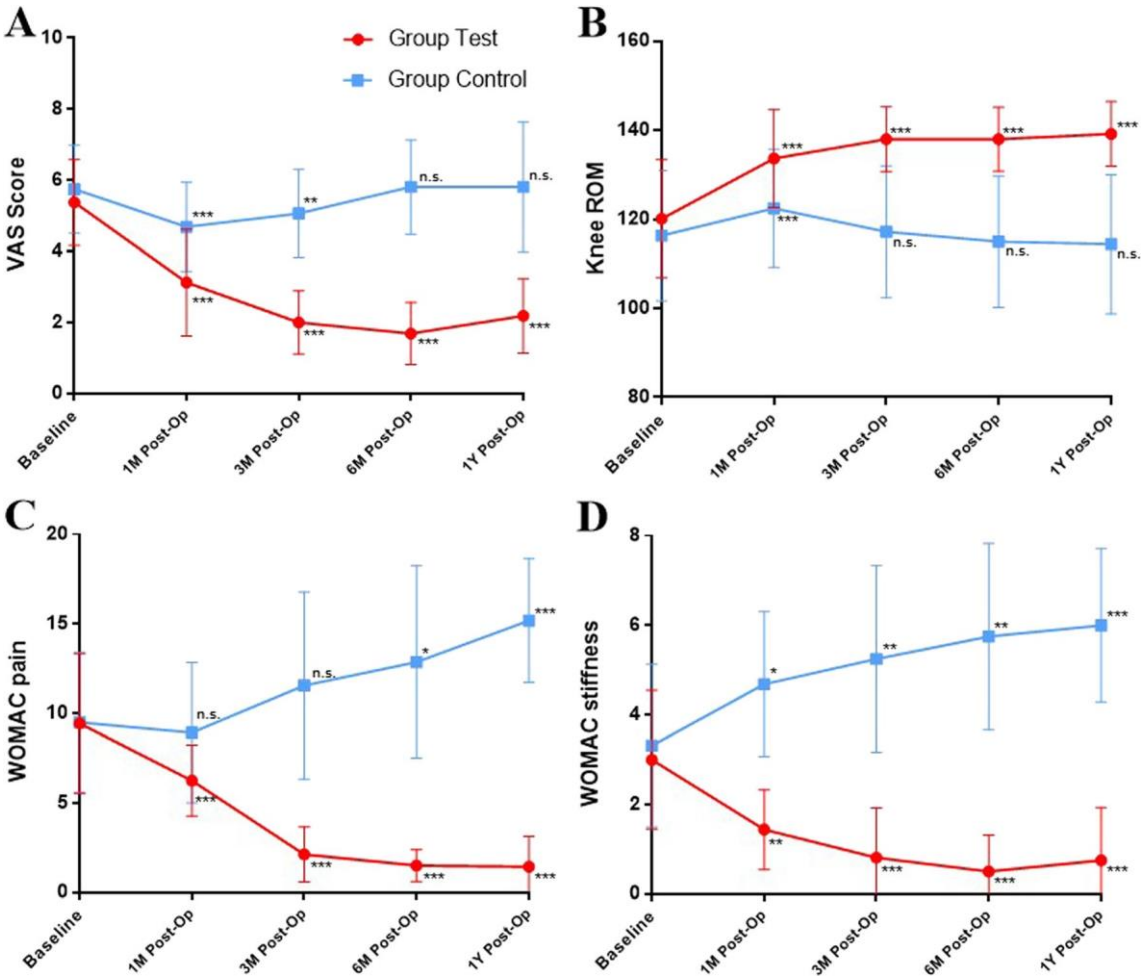



Fig. 2 Changes of VAS, WOMAC score, and knee ROM in two groups during 12-months follow-up. Values in graphs are expressed as mean ± SD in vertical bars, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, non-significant ($P > 0.05$). All values were compared with baseline. a VAS score. b Knee ROM. c WOMAC pain. d WOMAC stiffness

Table 4 Clinical and WORMS changes during 12 months follow-up

| | Δ.1 month | *p* value | Δ.3 month | *p* value | Δ.6 month | *p* value | Δ.12 month | *p* value |
|---|---|---|---|---|---|---|---|---|
| Group test | | | | | | | | |
| WOMAC pain | − 3.19 ± 3.02 | < 0.001 | − 7.31 ± 3.52 | < 0.001 | − 7.94 ± 3.84 | < 0.001 | − 8.00 ± 4.77 | < 0.001 |
| WOMAC stiffness | − 1.56 ± 1.59 | < 0.01 | − 2.19 ± 1.80 | < 0.001 | − 2.50 ± 1.59 | < 0.001 | − 2.25 ± 2.11 | < 0.001 |
| VAS score | − 2.25 ± 1.39 | < 0.001 | − 3.38 ± 1.09 | < 0.001 | − 3.69 ± 1.01 | < 0.001 | − 3.19 ± 0.98 | < 0.001 |
| ROM | 13.56 ± 8.52 | < 0.001 | 17.88 ± 7.82 | < 0.001 | 17.88 ± 7.82 | < 0.001 | 19.06 ± 7.76 | < 0.001 |
| WORMS | | | | | − 11.38 ± 24.89 | 0.088 | − 15.44 ± 21.95 | < 0.05 |
| Group control | | | | | | | | |
| WOMAC pain | − 0.56 ± 4.98 | .658 | 2.06 ± 6.84 | .246 | 3.38 ± 5.73 | < 0.05 | 5.69 ± 4.29 | < 0.001 |
| WOMAC stiffness | 1.38 ± 2.22 | < 0.05 | 1.94 ± 2.49 | < 0.01 | 2.44 ± 2.56 | < 0.01 | 2.69 ± 2.57 | < 0.001 |
| VAS score | − 1.06 ± 0.68 | < 0.001 | − 0.69 ± 0.70 | < 0.01 | 0.06 ± 0.93 | .791 | 0.06 ± 1.18 | 0.835 |
| ROM | 6.13 ± 4.21 | < 0.001 | 0.88 ± 5.80 | 0.556 | − 1.31 ± 4.76 | .287 | − 1.88 ± 6.40 | 0.259 |
| WORMS | | | | | 12.81 ± 12.66 | < 0.01 | 15.50 ± 14.65 | < 0.01 |

Values are expressed as mean ± SD. *VAS*, visual analog scale; *WOMAC*, Western Ontario and McMaster Universities Osteoarthritis Index; *ROM*, range of motion; *WORMS*, whole-organ magnetic resonance imaging score

Table 5 Baseline characteristics of two groups with WORMS

| Variables | Group test | Group control | *P* value |
|---|---|---|---|
| Cartilage | 32.94 ± 14.24 | 34.44 ± 11.61 | 0.746 |
| Marrow abnormality | 4.44 ± 1.71 | 3.5 ± 1.51 | 0.11 |
| Bone cysts | 3.94 ± 1.95 | 4.81 ± 2.71 | 0.30 |
| Bone attrition | 1.25 ± 1.13 | 1.31 ± 1.2 | 0.88 |
| Osteophytes | 24.38 ± 16.25 | 22.19 ± 12.02 | 0.668 |
| Menisci | 3.25 ± 2.41 | 2.81 ± 2.43 | 0.613 |
| Ligaments | 0.13 ± 0.34 | 0.06 ± 0.25 | 0.559 |
| Synovitis | 1 ± 0.97 | 0.69 ± 0.79 | 0.325 |
| WORMS total | 71.31 ± 24.2 | 69.81 ± 18.05 | 0.844 |

Values are expressed as mean ± SD. *WORMS*, whole-organ magnetic resonance imaging score

in the control group, both at six and 12-months MRI follow-up ($P < 0.001$). In addition, in the test group, there were 11(69%) knees that showed complete or hypertrophic repair tissue filling of the defect compared with only one (6%) knee in the control group, seven (44%) knees in the test group showed complete integration with adjacent cartilage, and the value in the control group is only one (6%) (Fig. 3).

## Discussion

In this paper, we reported our findings comparing SVF versus HA treatment for 16 pairs of knees with K-L grade II-III osteoarthritis, with 12-months follow-up. Our data demonstrated that SVF could provide effective improvements in both radiological (WORMS and MOCART), and clinical (include VAS, WOMAC pain and stiffness, knee ROM) outcomes which was significantly superior to HA treatment (single dose of 40 mg) for bilateral knee joints with osteoarthritis at II-III stage (K-L grade). In a multi-centre analysis among 2372 patients underwent MSC treatment, the major adverse event was pain post-procedure [27]. Except pain and swelling after liposuction and operation, there was no severe adverse event in the whole process of our study.

In the test group treated with SVF, the knee joints showed statistically significant improvements in the mean VAS, ROM, WOMAC pain, and stiffness compared with baseline after 12-months follow-up, but the mean VAS score of 12-months visit increased significantly ($p = 0.015$) compared with that of six months. We found these patients with increased VAS score of 12 months in the test group; all had a gradually aggravating the VAS score of the knee in the control group. When checking the history, we found that these patients were used to load more weight on the milder knee rather than the most severe knee, which may explain the worsening trend of the VAS score from six months to 12 months in the test group. From the previous literature, we knew that HA treatment was effective in ameliorating pain and symptoms for OA studied and often served as a control [28, 29]. In our study, we used a single dose of 40-mg hyaluronic acid (SOFAST, Freda) injection in the control group for a better blind and variable control, but the outcome indicated that the therapeutic effect of one-single dose of 40-mg HA injection (SOFAST, Freda) was not obvious in the intermediate and long-term follow-up. This result was different from the study of Vega et al. [28]. They used a single dose of hyaluronic acid (60 mg in 3 mL; Durolane) as control, and the VAS score was significantly improved at 12-months follow-up in the control group. More research comparing SVF and adequate course of HA treatment for knee OA is needed in the future.

The MRI follow-up showed a significant improvement of the WORMS in knees treated with SVF. Particularly notable was the reduction in the cartilage and marrow abnormality subscores, which decreased by 12 ± 21.55 ($P < 0.05$) and 2.50 ± 2.00 ($P < 0.001$) from baseline to 12-months MRI. The radiological outcome of MOCART proved that the test

Table 6 WORMS changes during 12-months follow-up

| | Group test | | | | Group control | | | |
|---|---|---|---|---|---|---|---|---|
| Variables | Δ.6 month | *P* value | Δ.12 month | *P* value | Δ.6 month | *P* value | Δ.12 month | *P* value |
| Cartilage | − 7.81 ± 23.42 | 0.20 | − 12.00 ± 21.55 | < 0.05 | 2.56 ± 5.93 | 0.105 | 4.13 ± 7.12 | < 0.05 |
| Marrow abnormality | − 2.13 ± 2.13 | < 0.01 | − 2.50 ± 2 | < 0.001 | 5.38 ± 6.79 | < 0.01 | 5.50 ± 7.17 | < 0.01 |
| Bone cysts | − 0.44 ± 2.45 | 0.486 | − 0.56 ± 2.28 | 0.339 | 0.25 ± 1.00 | 0.333 | 0.31 ± 1.01 | 0.237 |
| Bone attrition | − 0.19 ± 0.40 | 0.083 | − 0.19 ± 0.75 | 0.333 | 3.63 ± 4.87 | < 0.01 | 3.81 ± 5.22 | < 0.05 |
| Osteophytes | − 0.44 ± 0.73 | < 0.05 | 0 ± 1.63 | 1 | 0.38 ± 0.89 | 0.111 | 0.69 ± 1.66 | 0.119 |
| Menisci | − 0.19 ± 1.17 | 0.53 | − 0.13 ± 1.36 | 0.718 | 0.13 ± 0.72 | 0.497 | 0.25 ± 0.93 | 0.3 |
| Ligaments | − 0.06 ± 0.25 | 0.333 | 0.13 ± 0.89 | 0.58 | 0.06 ± 0.25 | 0.333 | 0.25 ± 0.68 | 0.164 |
| Synovitis | − 0.13 ± 0.81 | 0.544 | − 0.19 ± 0.75 | 0.333 | 0.44 ± 1.15 | 0.15 | 0.56 ± 1.15 | 0.07 |
| WORMS Total | − 11.38 ± 24.89 | 0.088 | − 15.44 ± 21.95 | < 0.05 | 12.81 ± 12.66 | < 0.01 | 15.50 ± 14.65 | < 0.01 |

Table 7 MOCART results during 12-months follow-up

| Variables | Maximum score | Group test, *n* (%) | | Group control, *n* (%) | |
|---|---|---|---|---|---|
| | | 6 months | 12 months | 6 months | 12 months |
| 1. Degree of defect repair and filling of the defect | | | | | |
| Complete | 20 | 2 (12.50) | 5 (31.25) | 0 (0) | 0 (0) |
| Hypertrophy | 15 | 5 (31.25) | 6 (37.50) | 1 (6.25) | 1 (6.25) |
| Incomplete | | | | | |
| > 50% of the adjacent cartilage | 10 | 4 (25.00) | 2 (12.50) | 2 (12.50) | 2 (12.50) |
| <50% of the adjacent cartilage | 5 | 3 (18.75) | 2 (12.50) | 4 (25.00) | 3 (18.75) |
| Subchondral bone exposed | 0 | 2 (12.50) | 1 (6.25) | 9 (56.25) | 10 (62.50) |
| 2. Integration to border zone | | | | | |
| Complete | 15 | 5 (31.25) | 7 (43.75) | 1 (6.25) | 1 (6.25) |
| Incomplete | | | | | |
| Demarcating border visible (split-like) | 10 | 6 (37.50) | 4 (25.00) | 1 (6.25) | 2 (12.50) |
| Defect visible | | | | | |
| <50% of length of the repair tissue | 5 | 3 (18.75) | 4 (25.00) | 5 (31.25) | 4 (25.00) |
| > 50% of length of the repair tissue | 0 | 2 (12.50) | 1 (6.25) | 9 (56.25) | 9 (56.25) |
| 3. Surface of the repair tissue | | | | | |
| Surface intact | 10 | 9 (56.25) | 10 (62.50) | 2 (12.50) | 1 (6.25) |
| Surface damaged | | | | | |
| < 50% of repair tissue depth | 5 | 6 (37.50) | 5 (31.25) | 2 (12.50) | 2 (12.50) |
| > 50% of repair tissue depth or total degeneration | 0 | 1 (6.25) | 1 (6.25) | 12 (75.00) | 13 (81.25) |
| 4. Structure of the repair tissue | | | | | |
| Homogeneous | 5 | 9 (56.25) | 10 (62.50) | 3 (18.75) | 2 (12.50) |
| Inhomogeneous or cleft formation | 0 | 7 (43.75) | 6 (37.50) | 13 (81.25) | 14 (87.50) |
| 5. Signal intensity of repair tissue | | | | | |
| Normal (identical to adjacent cartilage) | 30 | 3 (18.75) | 5 (31.25) | 1 (6.25) | 1 (6.25) |
| Nearly normal (slight areas of signal alteration) | 15 | 8 (50.00) | 8 (50.00) | 2 (12.50) | 3 (18.75) |
| Abnormal (large areas of signal alteration) | 0 | 5 (31.25) | 3 (18.75) | 13 (81.25) | 12 (75.00) |
| 6. Subchondral lamina | | | | | |
| Intact | 5 | 10 (62.50) | 9 (56.25) | 7 (43.75) | 5 (31.25) |
| Not intact | 0 | 6 (37.50) | 7 (43.75) | 9 (56.25) | 11 (68.75) |
| 7. Subchondral bone | | | | | |
| Intact | 5 | 4 (25.00) | 6 (37.50) | 5 (31.25) | 3 (18.75) |
| Not intact (edema, granulation tissue, cysts, sclerosis) | 0 | 12 (75.00) | 10 (62.50) | 11 (68.75) | 13 (81.25) |
| 8. Adhesions | | | | | |
| No | 5 | 11 (68.75) | 10 (62.50) | 3 (18.75) | 4 (25.00) |
| Yes | 0 | 5 (31.25) | 6 (37.50) | 13 (81.25) | 12 (75.00) |
| 9. Synovitis | | | | | |
| No synovitis | 5 | 9 (56.25) | 10 (62.50) | 5 (31.25) | 7 (43.75) |
| Synovitis | 0 | 7 (43.75) | 6 (37.50) | 11 (68.75) | 9 (56.25) |
| Mean ± SD | | 54.06 ± 11.58 | 62.81 ± 8.16 | 19.38 ± 9.64 | 19.06 ±7.79 |

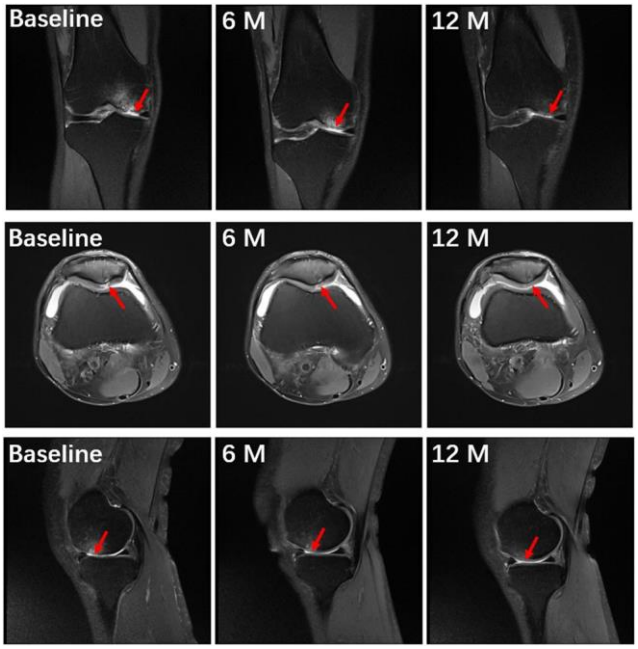



Fig. 3 Magnetic resonance imaging scans of three SVF-treated knees from baseline to 6 and 12-months follow-up showed complete repair and filling of the defects, as well as good integration with the adjacent cartilage and underlying bone in the coronal, transverse and sagittal planes (red arrows)

group had a statistically significant superior articular cartilage repair both at six months (mean MOCART 54.06 ± 11.58 in the test group and 19.38 ± 9.64 in the control group, $P < 0.001$) and 12-months (mean MOCART 62.81 ± 8.16 in the test group and 19.06 ± 7.79 in the control group, $P < 0.001$) MRI follow-up, compared with the control group (Table 7). In the group treated with SVF, four knees had a MOCART score of less than 60 at last follow-up; all accompanied with a poor subchondral lamina and bone as well as a large area of cartilage defect on baseline MRI, suggesting that SVF injection provided a less satisfactory outcome in relatively large cartilage defects. Different from the test group, the MRI outcome in the control group was poor, as the previous literature indicated that hyaluronic acid played a limited role in the repair of damaged cartilage. Furthermore, several other researches studied the relationship between cell dose and therapeutic efficacy of ADSC [18–21], but came to contradictory results. In the two year follow-up study of Jo CH et al.[18, 19], significant improvement was found mainly in the high-dose group ($1 \times 10^8$), and the outcomes in the low and medium dose groups tended to deteriorate after one year; whereas, those in the high-dose group plateaued until two years. Interestingly, in another clinical trial of ADIPOA [21], significant improvement was detected only in the low-dose ($2 \times 10^6$) ASCs-treated patients. In another pilot study treated with repeated injections of ADMSCs, the dosage of $5 \times 10^7$ showed the highest improvement [20]. In our study, we failed to find an actual association between SVF cell density, cell viability, and outcomes that we need more studies to explore the cell dose effect in the future. There are multiple sources of stem cells for orthopedic conditions [30–32]. Since adipose tissue-derived stem cells (ADSCs) were first characterized by Zuk et al. in 2001 [16], ADSCs have been widely studied for their regenerative and therapeutic potential. Recently, several researches indicated that the regenerative potential was also found in the SVF [33–35], a mixture of ADSCs, endothelial precursor cells (EPCs), endothelial cells (ECs), macrophages, smooth muscle cells, lymphocytes, pericytes, and pre-adipocytes [36, 37]. Traditionally, SVF is isolated by enzymatic processing from lipoaspirate. The advantages of SVF over ADSCs consist of the following parts. Firstly, unlike ADSCs, SVF is readily accessible from the lipoaspirate without the requirement for any cell separation or cell culture. Secondly, SVF therapy is much cheaper and faster than ADSCs because of the absence of culturing procedures. Thirdly, besides the similarities in immunomodulation, anti-inflammatory, and angiogenesis, the characteristic, heterogeneous cellular components of SVF may explain the better therapeutic effect observed in some animal studies [36, 38].

As far as we know, this was the first prospective, randomized, double-blind, and self-controlled clinical trial studying autologous adipose-derived stromal vascular fractions injection for bilateral human knee osteoarthritis. The study was designed according to the principle of completely random, minimizing the distinctions between two groups and reducing the interference of the preferred leg. The setting of self-control between bilateral knees ensured the consistency of sample size between groups during the follow-up process. All procedures were performed under general anaesthesia, minimizing the pain of the patients. Furthermore, adequate blinding was guaranteed in our study, all patients, radiologists, and investigators were blind of treatment allocation, and the orthopedic surgeon who delivered the intervention did not take outcome measurements, reducing the performance bias of the study.

In conclusion, our results indicates that autologous adipose-derived SVF treatment is safe and can effectively relief pain, improve function, and repair cartilage defects in patients with K-L grade II-III knee osteoarthritis. It is therefore believed that adipose tissue may be a good cell source for cartilage regenerative engineering.

## Limitations of the study

We must acknowledge that there were several limitations in this study. First, the follow-up period seemed short (12 months); we need more follow-up time to determine the long-term effects of SVF. Second, the sample size was small because the incidence of bilateral knee osteoarthritis was lower than unilateral knee OA. Third, second-look arthroscopy and pathological biopsy of newborn cartilage tissue is the gold standard for evaluating cartilage repair; however, arthroscopy

and biopsy are invasive and inconvenient for dynamic follow-up, and therefore difficult to carry out in China. Fourth, we could not find a clinical rating index aiming at unilateral knee joint that patients should complete two same questionnaires focusing on the individual characteristics with different sides of knees. Fifth, it is unknown, whether SVF injection in one knee could influence the contralateral knee. Sixth, we did not find an actual association between SVF cell density, cell viability, and outcomes, more studies are needed to explore the cell dose effect of SVF treatment.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Ethical approval** All procedures performed in the studies involving human participants were in accordance with the ethical standards of Ethics Committee of the Zhejiang Provincial People's Hospital and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was registered at Chinses Clinical Trial Registry with identifier ChiCTR1800015125.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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# Time- and Kellgren–Lawrence Grade-Dependent Changes in Intra-Articularly Transplanted Stromal Vascular Fraction in Osteoarthritic Patients

**Tung Dang Xuan Tran [1,2], Chi-Ming Wu [3], Navneet Kumar Dubey [3,4], Yue-Hua Deng [5], Chun-Wei Su [4], Tu Thanh Pham [2], Phuong Bich Thi Le [6], Piero Sestili [7] and Win-Ping Deng [1,4,*]**

1 School of Dentistry, Taipei Medical University, Taipei 11031, Taiwan; d204105004@tmu.edu.tw
2 Van Hanh Stem Cells Unit, Van Hanh Hospital, Ho Chi Minh City 700000, Vietnam; thanhtuvanhanh@gmail.com
3 Graduate Institute of Biomedical Materials and Tissue Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei 11031, Taiwan; chiming.wu@jade-dental.com.tw (C.-M.W.); bioengineer.nkd@gmail.com (N.K.D.)
4 Stem Cell Research Center, College of Oral Medicine, Taipei Medical University, Taipei 11031, Taiwan; q7s5w8a4@gmail.com
5 Department of Life Science, Fu Jen Catholic University, New Taipei City 242, Taiwan; yuehuahua828@gmail.com
6 Department of Pulmonary Medicine, Vietnam Military Medical Academy, Ha Noi 12108, Vietnam; drbphuong@gmail.com
7 Dipartimento di Scienze Biomolecolari, Università degli Studi di Urbino Carlo Bo Via "I Maggetti" 26, 61029 Urbino, Italy; piero.sestili@uniurb.it
* Correspondence: wpdeng@tmu.edu.tw; Tel.: +886-2-2739-0863 or +886-2-2736-1661 (ext.7169, 7172); Fax: +886-2-2739-5584

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**Abstract:** Knee osteoarthritis (OA) is one of the most prevalent disorders in elderly population. Among various therapeutic alternatives, we employed stromal vascular fraction (SVF), a heterogeneous cell population, to regenerate damaged knee cartilage. OA patients were classified on the basis of age, gender, body mass index (BMI), and x-ray-derived Kellgren–Lawrence (KL) grade. They were treated with SVF and followed-up for 24 months. Visual analogue scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis (WOMAC) Index were used to determine treatment efficacy. Cartilage healing was assessed using the MRI-based Outerbridge score (OS) and evaluation of bone marrow edema (BME) lesions, while a placebo group was used as a control. Time- and KL-dependent changes were also monitored. We observed a decreasing trend in VAS score and WOMAC index in the SVF-treated group up to 24 months, as compared with the placebo group. Besides, a significant increase and decrease in Lysholm and OS, respectively, were observed in the treatment group. Compared with the values before treatment, the greatly reduced WOMAC scores of KL3 than KL2 groups at 24 months, indicate more improvement in the KL3 group. Highly decreased BME in the treated group was also noted. In conclusion, the SVF therapy is effective in the recovery of OA patients of KL3 grade in 24 months.

**Keywords:** knee osteoarthritis (OA); KL grade; stromal vascular fraction (SVF); MRI; WOMAC; VAS; OS; BME

## 1. Introduction

Knee osteoarthritis (OA) is one of the most common progressive joint disorders, especially among elderly population in the United States and other developed countries [1–3]. Cartilage devolution, stiffness, loss of joint function, bone loss/rearrangement, and pain are primary

characteristics of OA [4,5]. In the clinics, OA patients are categorized on the basis of their Kellgren–Lawrence (KL) grades (1 to 4), whose range of symptomatic characteristics includes the narrowing of the joint space to definite deformity of bone ends [6]. Multiple risk factors for OA include age, gender, inflammation, genetics, mechanical wear and tear during exercise, sports, or any other stressful activity [7–10]. There is wide perception that obesity and increase in life expectancy are major causes of the increase in OA in the last decades; however, a recent study carried out by Wallace et al. suggests that life longevity and body mass index (BMI) are not the only factors for the increase in OA, and extensive research is needed to determine other factors associated with OA increase [11]. The self-renewal ability of chondrocytes is significantly lost in aged persons (>60 years), and this severely affects cartilage structure and maintainance [12]. Moreover, it has also been established that the secretion of proteolytic enzymes such as aggrecanases and metalloproteinases further degrades the damaged cartilage [13,14]. OA-related pain is treated by non-pharmacological approaches such as physical therapy, yoga, land- and water-based exercise, tai chi, and weight loss [15–20], as well as with pharmacological agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) [21,22], chondroprotective compounds, calcium, opioids [23,24], and hormones [25]. Hyaluronic acid (HA) is intra-articularly administered to restore the viscoelastic properties of injured cartilage [26,27]. Surgical treatments including arthroscopy, microfracture, subchondral drilling, and abrasion arthroplasty are used to treat late-stage OA; however, the limitations of these procedures include the formation of fibrocartilage, which has less ability to absorb shock, thereby compromising the functional characteristics of the native cartilage tissues [25].

An alternative surgical technique, the autologous chondrocyte implantation (ACI), has been recently used to overcome the limitations associated with the previously mentioned surgical techniques. ACI is a common surgical intervention to promote healing of cartilage injuries in OA [28,29]. However, the effectiveness of ACI is restricted because of the limited availability of chondrocytes and the compatibility between implanted chondrocytes and host site [30]. Cell-based regenerative therapies along with biomaterials, especially stem cells and hydrogels, are emerging and promising procedures to counter OA. Bone marrow-derived stem cells (BM-MSCs), peripheral blood-derived stem cells, adipose-derived stem cells (ADSCs), and synovial fluid-derived stem cells have been studied in the presence or absence of biomaterials [31]. The paracrine effects of stem cells have been widely associated with regeneration and repair activities [32]. The adipose tissue is considered a rich and preferable source of stem cells due to the feasibility of harvesting tissue and isolating stem cells.

Stromal vascular fraction (SVF) is a heterogeneous population of various immune, precursor, progenitor, and stem cells. SVF is considered to be as equal as or sometimes more effective than ADSCs; therefore, it provides other functional advantages, such as structural support, over ADSCs [33–36]. However, SVF is immunologically restricted because of the presence of various cells and only fit for autologous treatment [37], whereas, ADSCs are multipotent cells that can differentiate into chondrocytes, with capability of self-renewal, high plasticity, and immunomodulatory and anti-inflammatory properties [38,39]. SVF has been widely studied as an alternative therapeutic agent to treat sclerosis, myocardial and bone-related disorders, blood vessel regeneration, and pulmonary diseases [40–42]. Recent works have also been extensively focused on evaluating SVF potential in orthopedic ailments [41,42]. Various clinical studies combining SVF with plasma-rich protein (PRP) , hyaluronic acid (HA), ceramic and fibrin glue were carried out to assess the potential of SVF in the treatment of OA [43–45]. Considering the therapeutic significance of SVF, this study was carried out to assess the therapeutic efficacy of SVF in OA treatment through the regeneration of articular cartilage. During our study, we specifically investigated time- and KL grade-dependent changes up to 24 months.

## 2. Materials and Methods

### *2.1. Study Design and Participants*

This study was an open-label, single-center, non-randomized, placebo-controlled, phase I/II clinical trial to evaluate the improvement in knee pain and knee function, as well as cartilage restoration. The 33 patients enrolled in the study were deliberately allocated to two groups, which were designated arthroscopic microfracture treatment only and arthroscopic microfracture treatment combined with SVF injection. Observation and follow-up data were recorded after 12 and 24 months. The eligibility criteria included: osteoarthritic knee joint with KL grades 2–3 and age >38 years. Patients meeting the following criteria were excluded: autoimmune or inflammatory disease, infection requiring parenteral administration of antibiotics, serious internal disorders, corticosteroids or viscosupplements injection into the affected knee within the past 3 months, and stiffness due to previous severe injury. The protocol was approved by the Viet Nam Ministry of Health (No. 2288/QDBYT) and the Ethical Committee in Biomedical Research of Van Hanh General Hospital (No. 90-084/QD-BVVH). Patients participating in this research provided an informed consent, in accordance with the Declaration of Helsinki.

### *2.2. Fat Tissue Harvest and SVF Isolation*

Lipoaspirates were harvested from patients' lower abdomen by a standard liposuction technique. Briefly, through incision, a solution of tumescent lidocain, 250 mL of normal saline, 0.9% and 0.2 mL of 1:1000 epinephrine was injected in the subcutaneous fat. Thereafter, 50–100 mL of lipoaspirate was collected through Triport Harvester Cannula (Tulip Medical Product, CA 92117 USA), and a 60 mL Luer-lock syringe. The SVF from the lipoaspirate was isolated by means of collagenase digestion (Collagenase NB 6 GMP Grade, Nordmark Biochemicals, Ho Chi Minh City, Vietnam) and the ADSC Extraction Kit (Geneworld Co. Ltd., Ho Chi Minh City, Vietnam) approved by the Viet Nam Ministry of Health. The SVF was then washed thrice with sterile PBS to remove collagenase. Finally, the SVF was diluted with normal saline 0.9% to obtain 6 mL of solution containing 90–120 million cells to administer in each knee joint.

### *2.3. Arthroscopy Microfracture Procedure*

Spinal anesthesia for knee arthroscopy was done by using 2 mL (5 mg/mL) bupivacaine hydrochloride. The debris, crystal, and synovitis were removed, and microfracture holes were placed 3–4 mm apart by the arthroscopy microfracture technique, as described by Steadman et al [46]. After arthroscopy, the knee joint was drained for 6 hours, and the drainage tube was withdrawn before the injection of the SVF. The rehabilitation period of the patients under the guidance of a physician included three time points. In the first 6 weeks, walking with crutches, partial weight bearing, and passive motion of the joint up to 90° were allowed. During 6–12 weeks, normal walking in combination with the use of a knee protector and quadriceps and hamstring training were performed. After 12 weeks, balance and core training with unlimited knee joint movement was administered.

### *2.4. Follow-Up and Evaluation*

Patients were monitored in the hospital for one week post-arthroscopy. After this, patients were followed for 24 months. Clinical manifestations such as pain, stiffness, and functional mobility were substantially recorded. Western Ontario and McMaster Universities Arthritis Index (WOMAC) [47], Lysholm [48], and visual analogue scale (VAS) scores were assessed before treatment and at 12 and 24 months after surgery. Magnetic resonance imaging (MRI) was performed before treatment and at 12 and 24 months after treatment. Specifically, the MRI analysis was performed to assess the extent of cartilage damage according to the Modified Outerbridge Classification [49].

### *2.5. Statistical Analysis*

The data are expressed as the mean ± SD. The comparisons between groups were performed by one-way analysis of variance (ANOVA) and *t*-test, using SPSS-22 (IBM, New York, NY, USA), and $p$ values $<0.05$ were considered statistically significant.

## 3. Results

### *3.1. Patient Characteristics*

The study was conducted from September 2014 to June 2017 at Van Hanh Hospital, Ho Chi Minh city, Vietnam. The overall schematic is illustrated in Figure 1, which shows that the OA patients were identified on the basis of their clinical and MRI scores, in addition to x-ray-dependent KL grades.

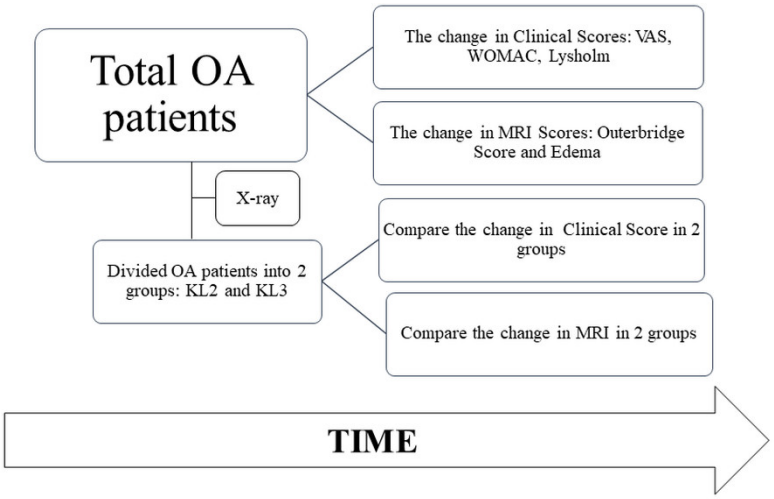


**Figure 1.** The schematic of the study, which shows that the osteoarthritis (OA) patients were identified on the basis of their clinical and MRI scores, in addition to x-ray-dependent Kellgren–Lawrence (KL) grades. These pateints were further treated with stromal vascular fraction (SVF), and all the outcome scores were assessed after 12 and 24 months.

Eighteen patients who satisfied the exclusive and inclusive criteria were selected to receive the treatment of SVF, a heterogeneous cell population containing mesenchymal progenitor/stem cells, preadipocytes, endothelial cells, pericytes, T cells, and M2 macrophages [50]. The demographic characteristics of the patients are shown in Table 1.

**Table 1.** Population characteristics of the patients. BMI: Body mass index.

| Characteristics | Placebo Group | SVF-Treated Group |
|---|---|---|
| Age | 58.2 ± 5.70 | 59 ± 6.04 |
| Sex | | |
| Male | 3 | 5 |
| Female | 12 | 13 |
| BMI | | |
| Normal: Overweight: Obese | 9:5:3 | 11:5:3 |
| KL grades | | |
| KL2 | 5 | 4 |
| KL3 | 10 | 14 |

The patients were classified on the basis of their age, gender, BMI, and KL grade (Table 1). In general, the two groups (SVF treatment and placebo) shared quite similar demographic characteristics.

### *3.2. Changes in VAS and Western Ontario and WOMAC Index after SVF Treatment*

VAS is a reliable scale for the assessment of pain in osteoarthritic condition [51], whereas WOMAC includes a questionnaire about pain, stiffness, and inability of conducting activities in daily

life [52]. In both scales, the lower score represents a better functional status of the patient. The effects of the SVF treatment on the VAS and WOMAC scores of KL2 and KL3 patients are represented in Figure 2A,B, respectively. The results revealed that after 12 months, no significant difference was found between the VAS scores of the SVF treatment and placebo groups (5.1 ± 2.5 vs. 4.9 ± 2.4). However, both scores were significantly decreased compared to that before the SVF treatment ($p < 0.05$). Further, as compared to the placebo group, a sharp decreasing trend in the VAS score of the treatment group was observed up to 24 months. The VAS score in the treated group continuously reduced after 12 and 24 months. Specifically, compared to the mean VAS score at 12 months, the score at 24 months was significantly reduced (5.1 ± 1.2 vs. 3.4 ± 1.8, $p < 0.05$). On the contrary, the score of the placebo group after 12 and 24 months increased from 4.9 ± 2 to 5.9 ± 2.47, but this difference was not significant. A similar trend was also observed for the WOMAC score in the placebo group, which was significantly decreased after 12 months of treatment (47.3 ± 17.1 vs. 28.6 ± 12.7, $p < 0.05$). However, a significant increase was observed thereafter at 24 months (36.5 ± 20.3 vs. 28.6 ± 12.7, $p < 0.05$). Meanwhile, the WOMAC score in the treated group decreased sharply after 12 months (44.7 ± 15.4 vs. 16.4 ± 12.1, $p < 0.05$) and further declined significantly to 11.1 ± 11.9 at 24 months (11.1 ± 11.9 vs. 16.4 ± 12.1, $p < 0.05$). Overall, at 24 months, both VAS and WOMAC scores in the placebo and treatment groups diminished compared with the scores before treatment. However, the decreasing trend in the treatment group was larger than in the placebo group, which is indicative of improvement after SVF therapy.

### 3.3. *Changes in Lysholm Score after SVF Treatment*

The Lysholm Knee Scale is another recommended measure of knee function [48]. As per Lysholm scale interpretation, a higher score represents better knee function. Before treatment, the Lysholm scores of the placebo and treatment groups showed a significant difference (64.1 ± 10.2, 52.8 ± 13.2; $p < 0.05$) (Figure 2C). The results showed that the score of the placebo group increased to 76.5 ± 12.4 after 12 months; thereafter, a notable decrease was recorded after 24 months (68.3 ± 15.0). However, the overall increase from the value before treatment to that at 24 months in the placebo group was found not to be significant (64.1 ± 10.2 vs. 68.3 ± 15.0). Similarly, the treatment group showed no statistically significant increase in Lysholm score after 24 months, compared to 12 months. However, compared to the value before treatment, this score was significantly increased at 24 months (52.8 ± 13.2 vs. 85.9 ± 9.9, $p < 0.05$), implying an improvement in knee function.

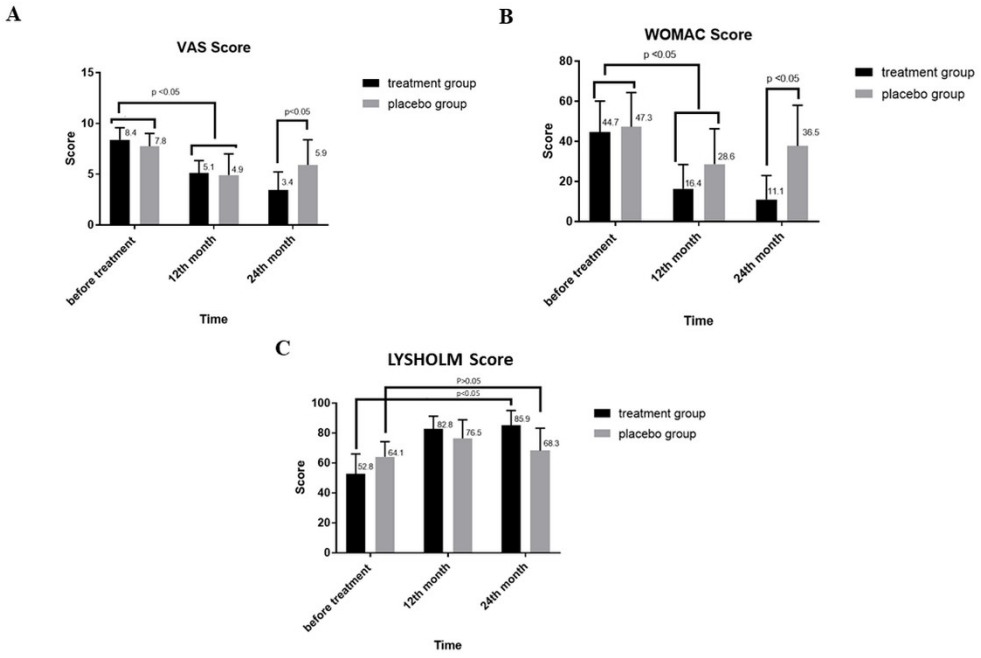

**Figure 2.** Assessment of clinical outcomes of OA patients treated with SVF at 12 and 24 months. (**A**) Visual analogue scale (VAS) score (**B**) Western Ontario and McMaster Universities Arthritis Index (WOMAC) index, and (**C**) Lysholm score of the SVF-treated group compared to the placebo group.

### 3.4. *MRI-Based Evaluation of Bone Edema and Cartilage Healing*

MRI results showed that after 24 months of treatment, bone marrow edema was decreased in both the placebo and the SVF treatment groups; however, the decrease in bone marrow edema in the SVF treatment group was larger (22 mm vs. 8 mm) than in the placebo group (20 mm vs. 12 mm) (Figure 3A). Similarly, the Outbridge score was decreased from 4 (at 0 months) to 3 (at 12 months) and 1 (at 24 months), implying a considerable improvement in cartilage generation in the SVF-treated group (Figure 3B).

### 3.5. *Cartilage Injury Evaluation by MRI-based Outerbridge Score*

The level of cartilage injury was measured by the Outerbridge score (OS) [53]. The OS of the study groups were recorded on the basis of MRI examination for assessment of cartilage lesions, particularly, depth of defect (Figure 3C) [54]. In the placebo group, the OS score increased slightly after 12 months (2.7 ± 1.3 vs. 2.9 ± 1.3), and this trend was maintained up to 24 months (3.2 ± 1.1). On the contrary, as compared to the values before treatment, the OS score in the treated group decreased after 12 and 24 months from 3.0 ± 0.8 to 2.7 ± 0.7 and 2.0 ± 0.7, respectively. The OS score pattern initially showed no significant difference between placebo and treatment groups (2.7 ± 1.3 vs. 3.0 ± 0.8); however, after 24 months, a significant difference between the OS scores of the two groups could be observed (3.2 ± 1.1 vs. 2.0 ± 0.7; $p < 0.05$). Taken together, the OS score of the treated group clearly decreased, while that of the placebo group displayed nearly no change.

### 3.6. *Bone Marrow Edema (BME)*

BME-like lesions are also associated with the pathogenesis of osteoarthritis and are characterized by histologic abnormalities such as bone marrow necrosis and fibrosis, in addition to trabecular abnormalities [55]. Therefore, MRI was also used to assess BME before and after 12 and 24 months of treatment (Figure 3D). Before the sham treatment, the length of BME in the placebo group was 1.9 ± 0.74 mm; an increase in BME length was observed at 12 and 24 months (2.0 ± 0.53 mm and 2.1 ± 0.64 mm, respectively $p < 0.05$). Interestingly, compared to the placebo, the BME length before SVF treatment (2.4 ± 0.34 mm) was significantly larger than after 12 and 24 months of treatment (1.5 ± 0.5 mm and 0.9 ± 0.73 mm, respectively ($p < 0.05$). On the whole, these results indicate a reduction in the formation of BME-like lesions after SVF treatment.

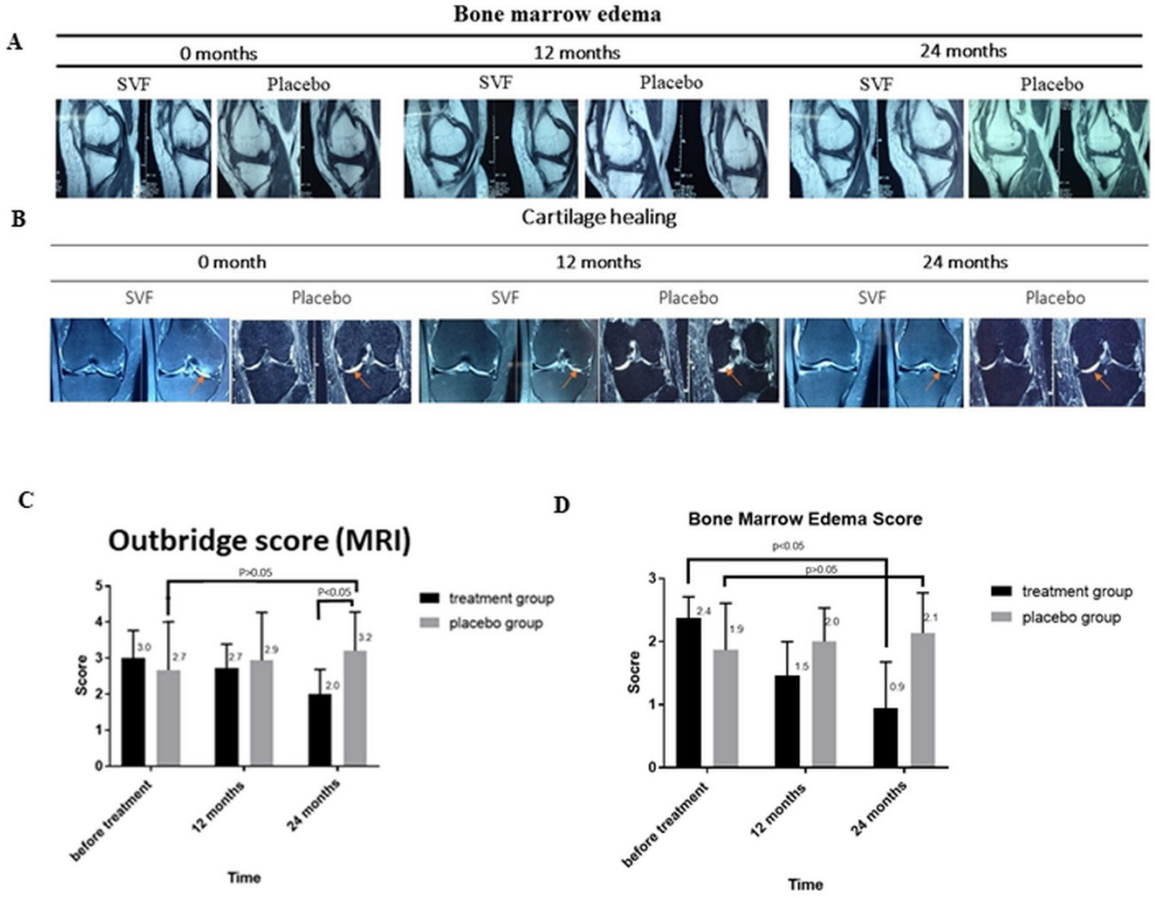


**Figure 3.** MRI analysis of OA knee-joints after SVF therapy. (**A**) Bone marrow edema (BME) and (**B**) (**B**) Cartilage healing and decrease in bone marrow edema (orange arrow) determined though the Outbridge score (OS) at 0, 12, and 24 month, respectively. (**C**) Cartilage injury evaluation by OS scores indicating the depth of defect in cartilage lesions before treatment and at 12 and 24 months after treatment in placebo and SVF-treated groups. (**D**) Length of BME lesions before and 12 and 24 months after treatment in placebo and treatment groups.

### 3.7. *Comparative Assessment of the VAS Score between KL2 and KL3 Groups*

The X-ray image-derived KL grading scale is a gold standard for determining the severity of OA, on the basis of which, the total OA patients were divided into KL2 and KL3 groups [6]. Further, we analyzed the relation between KL grading and VAS score in KL2 and KL3 treatment groups (Figure 4). Before treatment, the VAS score of the KL2 treatment group was 8.50 ± 1.92; it decreased to 4.50 ± 1 after 12 months. Notably, this score further decined to 3.00 ± 2 after 24 months of treatment, indicating a 57.2% decrease in the VAS score. Next, the effect of the placebo on VAS score of KL2 group was assessed. We found no considerable reduction in the VAS score of the KL2 placebo group before and after 24 months of placebo administration. Similarly, a reduction in the VAS score of the KL3 group was also observed post-treatment. Before treatment, the VAS score was 8.36 ± 1.00 and was reduced after 12 and 24 months of treatment to 5.29 ± 1.27 and 3.57 ± 1.79, respectively. This reduction in the VAS score was 64.7% after 24 months compared to the value before treatment. Taken together, the improvement in the pain status of KL3-grade patients was better than for KL2-grade patients.

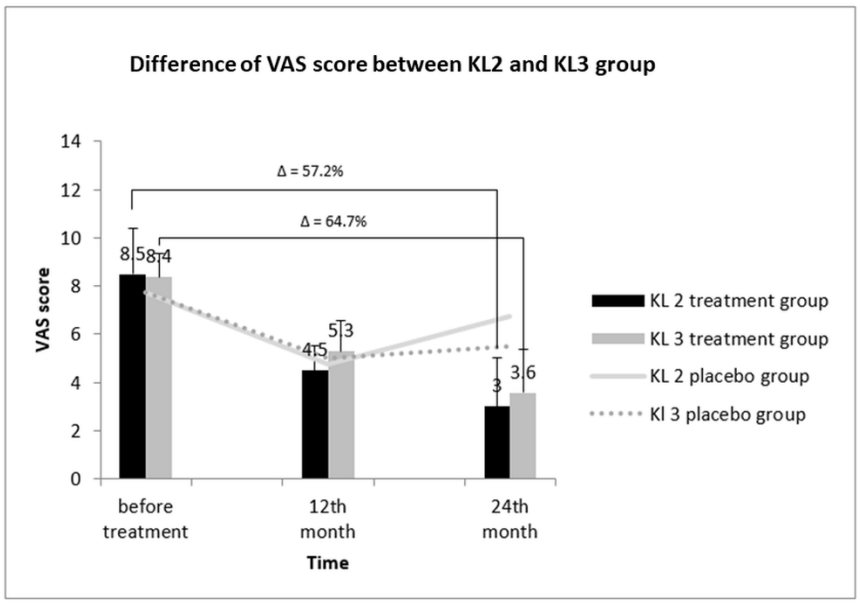


**Figure 4.** VAS scores of KL-grade 2 and 3 patients in SVF-treated OA groups at 12 and 24 months. After treatment, improvement was noted in patients with KL grade 2 and KL grade 3 (64.7% and 57.2%). Δ: percentage of reduction in VAS score.

### 3.8. *Correlation between WOMAC Score and KL Grades to Determine Treatment Efficacy*

Similarly, after treatment of KL2- and KL3-grade patients, differences in the WOMAC scores between the two groups were observed (Figure 5). The WOMAC scores before treatment in KL2 and KL3 patients were 52.00 ± 18.26 and 42.64 ± 14.51, respectively. After 12 and 24 months of treatment, the WOMAC score of the KL2 treatment group revealed a decreasing pattern, being 24.25 ± 19.77 and 18.25 ± 20.07, respectively. Similarly, the WOMAC score of the KL3 treatment group also dropped after 12 and 24 months of treatment to 18.21 ± 8.20 and 9.00 ± 8.46, respectively; however, this decline found to be not significant. Overall, compared with the value before treatment, at 24 months, the percentage of WOMAC score of the KL3 group was reduced with respect to that of the KL2 group (78.9% vs. 64.9%), indicating a greater extent of improvement in the KL3 group.

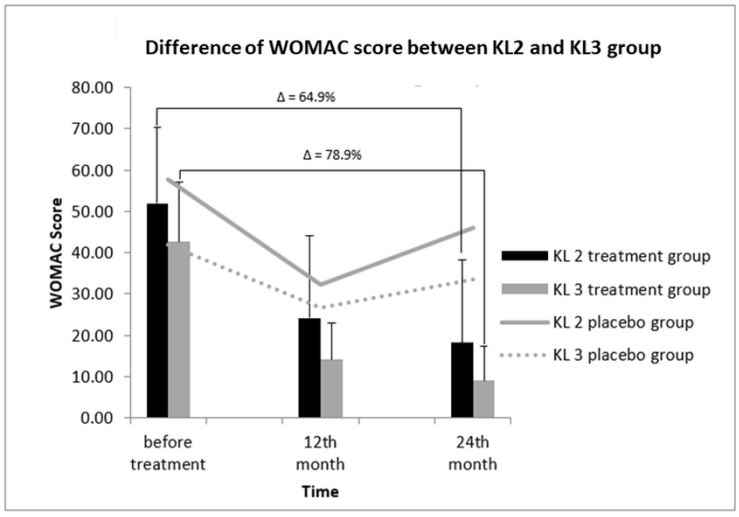


**Figure 5.** WOMAC scores in KL-grade 2 and 3 patients after SVF therapy at 12 and 24 months. After treatment, the reduction of the WOMAC score in KL-grade 3 patients was comparatively greater than that observed in KL-grade 2 patients (78.9% vs. 68.9%). The WOMAC scores of KL-grade 2 and 3 patients in the placebo group remained constant. Δ: percentage of reduction in WOMAC score.

### 3.9. *Relative KL Grading and Lysholm Score between KL2 and KL3 OA Groups*

The impact of KL-OA grades on the Lysholm score is represented in Figure 6. Before treatment, the Lysholm score of the KL2 treatment group was 40.25 ± 11.18; it increased rapidly to 82 ± 9.38 after 12 months of treatment. However, after 24 months, only a marginal increase in the Lysholm score in the KL 2-treated group to 86 ± 10.42 was observed, corresponding to a 33.6% increase compared to the value before treatment (40.25 ± 11.18 vs 86 ± 10.42). The Lysholm score of the KL3 treatment group followed almost a similar pattern as that of the KL2 group. The score before treatment was 56.4 ± 11.66 and increased to 83.1 ± 8.52 after 12 months of treatment, showing an increase of 53.1%. However, a slight increase to 85.0 ± 10.19 after 24 months of treatment was observed. These data showed that the improvement of the KL3 group were greater than that the KL2 group.

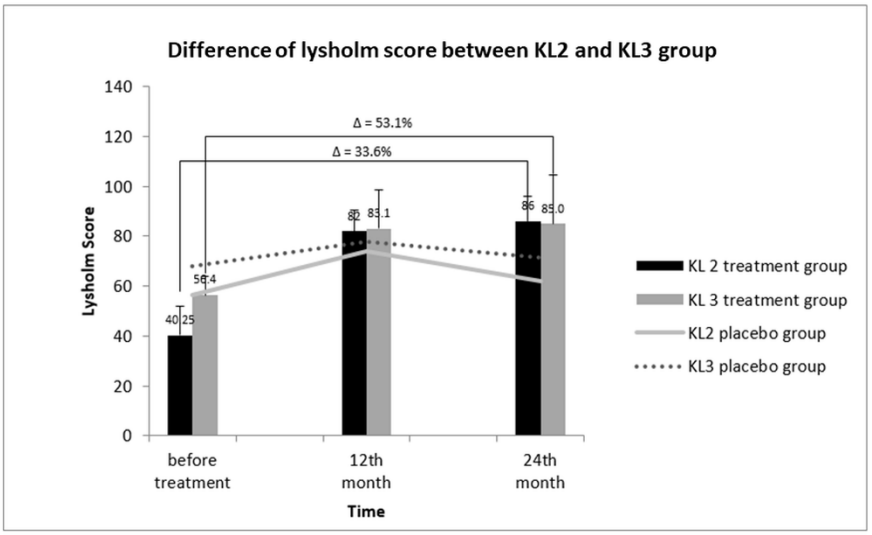


**Figure 6.** Lysholm scores of KL-grade 2 and 3 patients after SVF therapy at 12 and 24 months. After 24 months of treatment. The increase of the Lysholm score in KL-grade 3 patients was comparatively greater than that in KL-grade 2 patients (33.6%. vs. 53.1%). Δ: percentage of improvement in lysholm score.

### 3.10. *Comparative Outerbridge Score (OS) between KL2 and KL3 Groups*

The comparative profile of cartilage injury, as measured by the OS score in KL2 and KL3 patients after treatment, is represented in Figure 7. No significant improvement was observed in the OS of the KL 2 placebo group up to 24 months of treatment when compared to the scores before treatment. Specifically, the OS of the KL2 treatment group before treatment was 3.25 ± 0.55; however, it decreased to 2.58 ± 0.70 after 12 months of treatment and further reduced to 2.0 ± 1.19 after 24 months. The net decrease in OS score after 24 months of treatment was 38.5%. In accordance with the OS score pattern of the KL2 treatment group, the OS score of the KL3 treated group also decreased after 12 and 24 months of treatment to 2.8 ± 0.51 and 2.0 ± 0.61, respectively, compared to the value before treatment of 2.9 ± 0.51. The OS score of the KL3 placebo group showed a linear increase after 24 months of treatment. In contrast to the WOMAC and VAS scores, OS showed no difference in improvement between KL2 and KL3 groups.

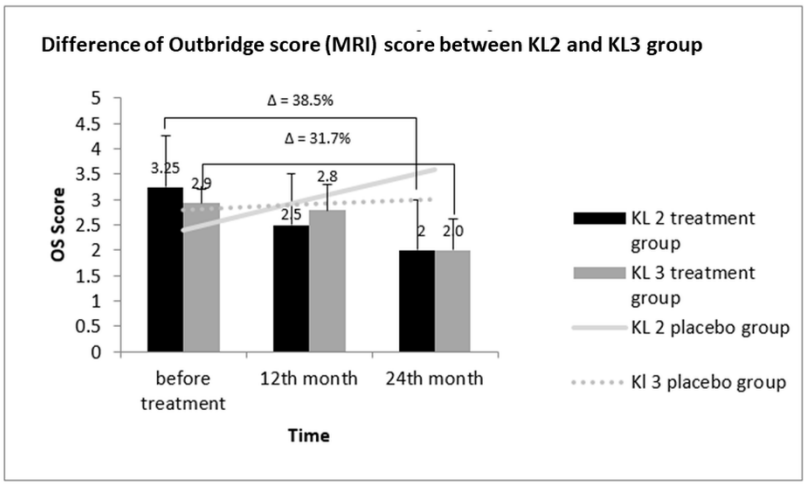


**Figure 7.** OS in SVF-treated and placebo groups of KL-grade 2 and 3 patients after at 12 and 24 months. After treatment, improvement was noted in KL-grade 2 and KL-grade 3 patients (38.5% and 31.7%). Δ: percentage of reduction in OS score.

## 4. Discussion

SVF contains a heterogeneous cell population of progenitor cells and ADSCs, which possess enhanced therapeutic potential against immune disorders, degenerative tissue pathologies, and other ischaemic conditions [37]. The complexity of knee OA related to pain, stiffness, muscle atrophy, and ligament damage has made its treatment difficult. Surgical procedures and drugs for controlling pain and inflammation have proven to be inadequate [56]. However, recent developments in regenerative therapy have provided the opportunity to address the bottlenecks associated with OA treatment. Similar to other MSCs, SVFs containing ADSCs are considered a better candidate at par with ADSCs and in some case better than pure ADSCs [35,36]. Therefore, this study assessed the efficacy of SVF treatment in OA therapy. In particular, the VAS, WOMAC, Lysholm, and MRI-based Outerbridge scores were evaluated to assess the improvement in OA status. VAS, WOMAC, and Lysholm score, closely represent the real-time status of OA; therefore, they are precise enough to evaluate the effectiveness of OA treatments [57]. The VAS score is directly measured through questionnaires [58]. The level of pain is established between two extreme points—no pain at all and worst pain imaginable [59]. This scale is simple, reliable, and valid to represent the level of pain [60]. As compared to the placebo group, a considerable reduction in the VAS score of the treatment group was observed after 24 months of treatment, reflecting an improvement of pain. On the contrary, no significant difference between the VAS scores of SVF and placebo groups after 12 months of treatment was found when an arthroscopic procedure was conducted prior to SVF administration. During this process, the inflamed tissues in both the groups were removed, which might have suppressed the pain symptom even in the placebo group, compared to the pain level before treatment. In coherence to our study, the SVF/PRP treatment has also been reported to improve the VAS score of OA patients [58]. A recent clinical study approved by the Japanese Regenerative Medicine Safety Act has documented a 40% decrease in VAS score after SVF treatment [61]. Furthermore, our study demonstrated that the WOMAC score was considerably decreased after 24 months of SVF treatment. These decreases in VAS (Figure 2A) and WOMAC scores (Figure 2B) compared to placebo groups were significant, which indicates improvement in the painful condition of OA patients. Following the pattern of VAS and WOMAC scores, the Lysholm score was also employed to assess the improvement in quality of life and status of instability post-surgery and post-treatment. The current modified Lysholm score is based on eight features, including limp, support, locking, instability, pain, swelling, stair climbing, and squatting [62]. Lysholm is mainly based on the opinion of a patient assessing function and stability of treatment; an increased score indicates improved quality of life. Our study indicates a

significant effect of SVF on the Lysholm score in OA patients 24 months post-treatment as compared to the placebo group. This increase in Lysholm score is an indication of patient relief to therapy. This result is in accordance with previous studies carried out to assess the efficacy of SVF therapy in OA treatment.

Further, the level of cartilage injury was assessed on the MRI-based OS score. An increase in OS score represents a loss of cartilage thickness. In this study, initially there was no significant difference between the OS scores of the treatment and placebo groups; however, a significant decrease in OS score was observed in the treatment group compared to the placebo group after 24 months of treatment ($p < 0.05$). These data establish the role of SVF in improving the BME score which is used as an indicator of knee OA progression and is characterized by increased accumulation of fluid [63]. A significant decrease in the BME score was observed in the SVF-treated group after 24 months of treatment with respect to the placebo which showed increased tendency. The comparison of the BME and OS scores of placebo and treatment groups at the end of 24 months of treatment indicated considerable improvements in the cartilage phenotype, particularly increased thickness.

KL classification is a five-grade scaling system in which the radiographs of eight joints are used to grade knee OA [64]. In this study, KL2- and KL3-grade patients were included to assess the effect of SVF treatment on the OA grade. On the basis of the decrease in WOMAC score and the increase in Lysholm score and considering the static response of the placebo groups during the 24 months of this study, it can be inferred that the SVF treatment was more effective in KL3-grade patients than in KL2-grade patients. The greater improvement of KL3-grade group patients might be attributed to the subjective assessment of the VAS score, WOMAC score, and Lysholm score, whereby patients with a severe condition tend to feel a greater improvement. In contrast, in the case of MRI scores (OS and BME scores) which are based on objective assessment, no differences between the two groups were witnessed. Inflammation plays a central role in pathogenesis of osteoarthritis and significantly contributes to joint pain [65]. Hence, the reduction of pain observed by us is likely to be related to the anti-inflammatory properties of SVF cells. As a corollary, it is also plausible that the better results obtained for KL3 patients, characterized by a higher level of inflammation before treatment compared to KL2 patients, depend on a better and more profitable exploitation of the anti-inflammatory activity of SVF. On the other hand, the degenerative properties of SVF will have the same effect on KL2 and KL3 patients.

The claim of SVF potential in improving clinical scores of OA patients might be attributed to SVF, which is a mixture of ADSCs, endothelial precursor cells (EPCs), endothelial cells (ECs), macrophages, smooth muscle cells, lymphocytes, pericytes, and pre-adipocytes [37,66]. The improvements in the clinical scores might be attributed to immuno-modulator and anti-inflammatory effects of SVF cells, which can lead to tissue remodeling. SVF cells secrete immunosuppressive and anti-inflammatory molecules like IL-10, IL-1, receptor antagonist (IL-1ra), indoleamine 2,3-dioxygenase, transforming growth factor (TGF)-β, and prostaglandin [67]. Further, the anti-fibrotic effect of ASDC might also play a role through the secretion of HGF or adrenomodullin, thereby reducing the fibrotic activity of overexpressed TGF-β1 and its target genes, such as collagen type I, type III, and $\alpha$-SMA in OA knee [68–70].

Besides these therapeutic activities, the regenerative ability of SVF may be due to ADSCs differentiation potential into chondrocytic and osteocytic cells lineages. EPCs may also induce angiogenesis by releasing growth factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1) [71]. Macrophages and monocytes have been demonstrated to mediate the immune response through secretion of various cytokines [72]. These macrophages are modulated by T regulatory cells, which may possess immunosuppressive characteristics [73]. In a mouse model, the pericytes found in SVF were able to regenerate the muscle tissue [74], which indicates their therapeutic potential role in knee joint. Eventually, stromal cells can secrete extracellular matrix components which improve cellular adhesion, migration, cell–matrix interactions, and regeneration [75,76]. To our knowledge, this is the first study reporting time- and KL grade-dependent changes of intra-articularly transplanted SVF in osteoarthritic patients over a period of two years. The main limitation of this study is the small sample size. However, even a small

sample might have some valid scientific merit with cost effectiveness [77,78]: on the basis of it we have inferred SVF-mediated therapeutic clinical outcomes in this study. To overcome this limitation, this study will be extended to a larger population and conducted for a longer time.

## 5. Conclusions

On the basis of the improvements observed in treated patients during follow-up and the behavior of the placebo group, our study revealed a trend toward a better efficacy of SVF with the microfracture method for OA treatment over a period of two years. We also inferred that the SVF therapy is more effective in KL 3-grade OA patients compared with KL 2-grade OA patients.

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# Comparative Clinical Observation of Arthroscopic Microfracture in the Presence and Absence of a Stromal Vascular Fraction Injection for Osteoarthritis

PHU DINH NGUYEN,[a] TUNG DANG-XUAN TRAN,[b] HUYNH TON-NGOC NGUYEN,[a] HIEU TRUNG VU,[a] PHUONG THI-BICH LE,[b] NHAN LU-CHINH PHAN,[c] NGOC BICH VU,[c] NGOC KIM PHAN,[c] PHUC VAN PHAM[c]

**Key Words.** Osteoarthritis • Stromal vascular fraction • Platelet-rich plasma • Arthroscopic microfracture



[a]115 Hospital, [b]Van Hanh General Hospital, and [c]Laboratory of Stem Cell Research and Application, University of Science, Vietnam National University, Ho Chi Minh City, Vietnam

Correspondence: Phuc Van Pham, Ph.D., Laboratory of Stem Cell Research and Application, University of Science, Vietnam National University, 227 Nguyen Van Cu, District 5, Ho Chi Minh City, Vietnam. Telephone: 84 903870153; e-mail: pvphuc@hcmuns.edu.vn

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## ABSTRACT

**Osteoarthritis (OA) is a degenerative cartilage disease that is characterized by a local inflammatory reaction. Consequently, many studies have been performed to identify suitable prevention and treatment interventions. In recent years, both arthroscopic microfracture (AM) and stem cell therapy have been used clinically to treat OA. This study aimed to evaluate the clinical effects of AM in the presence and absence of a stromal vascular fraction (SVF) injection in the management of patients with OA. Thirty patients with grade 2 or 3 (Lawrence scale) OA of the knee participated in this study. Placebo group patients ($n$ = 15) received AM alone; treatment group patients ($n$ = 15) received AM and an adipose tissue-derived SVF injection. The SVF was suspended in platelet-rich plasma (PRP) before injection into the joint. Patient groups were monitored and scored with the Western Ontario and McMaster Universities Arthritis Index (WOMAC), Lysholm, Visual Analog Pain Scale (VAS), and modified Outerbridge classifications before treatment and at 6, 12, and 18 months post-treatment. Bone marrow edema was also assessed at these time points. Patients were evaluated for knee activity (joint motion amplitude) and adverse effects relating to surgery and stem cell injection. Treatment efficacy was significantly different between placebo and treatment groups. All treatment group patients had significantly reduced pain and WOMAC scores, and increased Lysholm and VAS scores compared with the placebo group. These findings suggest that the SVF/PRP injection efficiently improved OA for 18 months after treatment. This study will be continuously monitored for additional 24 months.** STEM CELLS TRANSLATIONAL MEDICINE *2017;6:187–195*

## SIGNIFICANCE STATEMENT

Arthroscopic microfracture (AM) and stem cell therapy have been used clinically to treat osteoarthritis (OA). This study evaluated the clinical effects of AM in the presence (treatment group) and absence (placebo group) of a stromal vascular fraction (SVF) injection in the knee for OA. The SVF was suspended in platelet-rich plasma (PRP) before injection. Treatment efficacy differed significantly between placebo and treatment groups. All treatment group patients had significantly improved pain and arthritis index scores compared with the placebo group. These findings suggest that the SVF/PRP injection efficiently improved OA after 18 months. This study will be continuously monitored for 24 months.

## INTRODUCTION

Osteoarthritis (OA) is a chronic progressive disease characterized by cartilage degeneration, osteophyte formation, bone reorganization, and loss of joint function [1]. OA is the most frequent cause of disability among adults in the United States, and it occurred in >10% of the U.S. adult population in 2009. In 2009, 905,000 knee and hip replacements were carried out in OA patients, costing approximately $42.3 billion in total.

At present, OA is mainly treated with pharmaceuticals [2, 3], hyaluronic acid [4], and neridronate [5, 6]. However, these treatments only reduce symptoms and pain or control the inflammation process [7–9]; none of these drugs actually prevents the progression of OA [10, 11].

Arthroscopic microfracture (AM) has recently gained popularity as a therapy for OA [12–14], with some studies reporting significant symptom and functional improvement following the procedure [15]. Consequently, AM is indicated as a routine treatment for OA. However, meta- and systematic analyses indicate that although AM initially improves OA symptoms [16, 17], this effect is only short term [16]. In some cases, particularly among older people, AM can be harmful [16, 18, 19].

As an alternative approach, OA has been treated using platelet-rich plasma (PRP). PRP contains the pool of cytokines and growth factors stored in platelets [20]. Some studies have shown that PRP improves OA symptoms [21, 22]. However, this effect has not been not observed for a prolonged period [22–27]. To improve the effects of PRP, previous studies have investigated the combined injection of PRP with stem cells. Mesenchymal stem cells (MSCs) in conjunction with PRP have been found to mildly improve cartilage healing, and had improved Knee Injury and Osteoarthritis Outcome Score subscores and visual analog pain scores (VAS) compared with PRP-only therapy [28]. Using this approach, it is hypothesized that MSCs differentiate into chondrocytes, which participate directly in cartilage repair and also contribute to immune modulation to inhibit knee joint inflammation.

To date, various stem cell sources have been used to treat OA, such as bone marrow-derived MSCs (BM-MSCs) for autograft [29–32] or allograft [33], adipose-derived stem cells (ADSCs) [34–36], and peripheral blood-derived stem cells [37–39]. Other MSC sources include enriched mononuclear cells (MNCs) from bone marrow or umbilical cord blood, stromal vascular fractions (SVFs) from adipose tissue (AT) and purified MSCs obtained from culture-expanded MNCs.

In their published study, Enea et al. [40] combined autologous bone marrow-derived cells with microfracture to repair cartilage defects. Their results showed that single-stage treatment of focal cartilage defects of the knee with microfracture followed by coverage with a polyglycolic acid (PGA)-hyaluronic acid (HA) matrix augmented with autologous BMCs (PGA-HA-CMBMC) was safe and improved knee function. To date, no clinical studies have compared the efficacy of arthroscopic surgery with and without SVF injection in the treatment of OA. This study, therefore, aimed to evaluate the clinical effects of AM alone and in combination with SVF injection on the function and satisfaction of patients with OA.

## Materials and Methods

All experimental protocols were approved by the National Ethical Committee Ministry of Health, Vietnam. This study was registered at clinicaltrials.gov with identifier NCT02142842.

### Inclusion and Exclusion Criteria

All patients enrolled in this study were required to sign a consent form. Patient inclusion criteria were as follows: patients must be older than 18 years, have OA with grade 2 to 3 cartilage degeneration at the time of presentation, failed drug treatment and autologous cartilage transplantation, a Lysholm score less than 65, committed with an artheroplasty condition, and be HIV negative.

A total of 30 patients were enrolled in the study: 15 patients were treated using traditional AM and 15 patients were treated with AM plus an injected mixture of SVF and PRP. The follow-up time was 18 months for all patients.

### Liposuction

Patients were restricted from taking corticosteroids, aspirin, nonsteroidal anti-inflammatory drugs and oriental herbal medications for a minimum of 1 week before liposuction. For the liposuction, patients were given spinal anesthesia with 2–3 ml (5 g/L) of bupivacaine hydrochloride. The lower abdomen was also anesthetized. Liposuction was performed using a tumescent solution (500 ml of normal saline and 0.5 ml of 1:1,000 epinephrine). We used a TriPort Harvester cannula (Tulip Medical Products, San Diego, CA, http://www.tulipmedical.com) and a 60-ml BD Luer-Lock syringe (BD Biosciences, East Rutherford, NJ, http://www.bd.com) to harvest 100–500 ml of adipose tissue from each patient.

### SVF Isolation

The SVF was isolated from the abdominal adipose tissue of each patient. Approximately 100 ml of lipoaspirate collected from each patient was divided into two 50-ml sterile syringes. The syringes were stored in a sterile box at 2–8°C and immediately transferred to the laboratory. The SVF was isolated using an ADSC Extraction Kit (GeneWorld, Ho Chi Minh City, Vietnam, http://geneworld.vn) according to the manufacturer's instructions. Briefly, 100 ml of lipoaspirate was placed in a sterile, disposable 250-ml conical centrifuge tube (Corning Life Sciences, Tewksbury, MA, https://www.corning.com) and washed twice with sterile phosphate-buffered saline (PBS) by centrifugation at 400*g* for 5 minutes at room temperature. The adipose tissue was then digested using SuperExtract Solution (GeneWorld) containing collagenase at 37°C, for 30 minutes with agitation at 5-minute intervals. The suspension was centrifuged again at 800*g* for 10 minutes, and the SVF was harvested as a pellet. The pellet was washed twice with PBS to remove any residual enzyme, and resuspended in PBS so that the cell quantity and viability could be measured using an automatic cell counter (NucleoCounter; Chemometec, Lillerød, Denmark, https://chemometec.com).

### Activated PRP Preparation

Activated PRP was derived from the peripheral blood of the same patients as the adipose tissue, using a New-PRP Pro Kit (GeneWorld) according to the manufacturer's guidelines. Briefly, 20 ml of peripheral blood was collected in vacuum tubes and centrifuged at 800*g* for 10 minutes. The plasma fraction was collected and centrifuged at 1,000*g* for 5 minutes to produce a platelet pellet. Most of the plasma was then removed, leaving 3 ml of plasma for resuspension of the platelets. The inactivated PRP was then activated using activating tubes containing 100 $\mu$l of 20% $CaCl_2$.

### Preparation of Product for Injection

The final injection product was composed of a mixture of the harvested SVF and activated PRP. Activated PRP was used to dilute the SVF to achieve a suitable dose for injection at $10^7$ SVF cells/ml.

### AM and SVF/PRP Injection

All patients in both groups received AM, which was used to confirm the degree of OA in each patient. Local chondral lesions were removed using medical instruments and an arthroscopic shaver. Microfractures were performed in accordance with the methods described by Steadman et al. [41]. The 30 patients were grouped into a treatment group and a placebo group ($n$ = 15 per group). After arthroscopic marrow stimulation by AM, the water flow was stopped and excess water was aspirated from the joint cavity. In the treatment group, the SVF and activated PRP mixture (5 ml per knee) was injected. Patients in the placebo group were injected with saline.

### Follow-Up and Evaluation

Patients were monitored in the hospital for 1 week postinjection. During this time, all complications, including shock, infection, and inflammation, were noted. After this, patients were followed for 18 months. Western Ontario and McMaster Universities Arthritis Index (WOMAC), Lysholm, and VAS scores were assessed 1, 6, 12,

and 18 months after surgery. Radiographic imaging and magnetic resonance imaging (MRI) were performed 6 and 12 months post-treatment. In this study, we used the modified VAS scores. with 4 indicating no pain; 3, mild pain; 2, moderate pain; 1, severe pain; and 0, worst pain possible.

Patients began continuous passive motion 4–5 days post-treatment. Partial weight bearing was permitted at 2 weeks, progressing to full weight bearing 4 weeks after surgery. Isometric quadriceps and hamstring training with straight-leg raises were advised during the non-weight-bearing period. Light sport activities such as swimming, cycling, or jogging on even, soft ground were permitted at 6 months. Permission to participate in unrestricted sports activity was given after 12 months.

### Statistical Analysis

Results were expressed as the mean ± SD. One-way analysis of variance and two-tailed *t* tests were used for all statistical analyses, which were performed with GraphPad Prism 4.0 (GraphPad Software, La Jolla, CA, https://www.graphpad.com). *p* values <.05 were considered statistically significant.

## Results

### Patient Characteristics

This study was performed from April 2013 to September 2015 at two hospitals (Van Hanh General Hospital and 115 Hospital, both in Ho Chi Minh City, Vietnam). The 30 patients who satisfied the study standard were divided into 2 groups: placebo ($n$ = 15) and treatment ($n$ = 15). Demographic analysis found that these groups had an equivocal age, body mass index, sex, and Kellgren-Lawrence OA grade (Table 1). The Kellgren-Lawrence grade was based on x-rays, and was confirmed during AM (supplemental online Fig. 1).

**Table 1.** Study participant demographic characteristics

| Characteristic | Treatment group | Placebo group | *p* value |
|---|---|---|---|
| Age ± SD, years | 58.60 ± 6.48 | 58.20 ± 5.71 | >.05 |
| Sex, *n* | | | >.05 |
| Male | 3 | 3 | |
| Female | 12 | 12 | |
| BMI, *n* | | | >.05 |
| Normal weight | 4 | 4 | |
| Overweight | 8 | 5 | |
| Obesity I | 3 | 5 | |
| Obesity II | 0 | 1 | |
| Kellgren-Lawrence grading scale, *n* | | | >.05 |
| 2 | 4 | 5 | |
| 3 | 11 | 10 | |

Abbreviation: BMI, body mass index.

### Adverse Effects

No adverse events were observed during the study in either group. We identified four cases with complications not related to the AM or SVF injection; these complications included high blood pressure, chest pain, dyspnea, and urinary retention.

### Changes in WOMAC Scores

Figure 1 shows the WOMAC score results. Pretreatment WOMAC scores were equivocal, with a small nonsignificant difference observed between the placebo and treatment group (47.27 ± 17.13 vs. 42.87 ± 16.29, respectively; $p > .05$). At 6 and 12 months after treatment, the WOMAC scores in both groups significantly decreased compared with the pretreatment scores. In the placebo group, WOMAC scores decreased from 47.27 ± 17.13 to 23.27 ± 15.61 and 25.60 ± 19.69 at 6 and 12 months after surgery, respectively. In the treatment group, WOMAC scores decreased from 42.87 ± 16.19 to 19.27 ± 14.87 and 17.33 ± 14.91 at 6 and 12 months after surgery, respectively. At 6 and 12 months after surgery, the differences in the WOMAC scores between the treatment and placebo groups were nonsignificant ($p > .05$). However, a slight difference was observed between the 2 groups 12 months after surgery. WOMAC scores in the treatment group gradually decreased at 6 and 12 months compared with the pretreatment scores, although the WOMAC score 12 months after surgery was slightly increased compared with the score 6 months after the procedure.

The difference in the WOMAC scores of the placebo and treatment groups became more pronounced after 18 months of monitoring. In the placebo group, the WOMAC score increased from 25.60 ± 19.69 at 12 months to 37.08 ± 21.45 at 18 months. More importantly, WOMAC scores at 18 months in the placebo group were not significantly different compared with pretreatment scores. The WOMAC scores of the treatment group decreased at 6, 12, and 18 months (19.27 ± 14.87, 17.33 ± 14.91, and 12.40 ± 13.44, respectively) after surgery compared with the pretreatment score (42.87 ± 16.29). The 18-month WOMAC scores were also significantly different between the placebo and treatment groups ($p < .05$; Fig. 1).

### Changes in Lysholm Scores

The results presented in Figure 2 show that Lysholm scores changed in both the treatment and placebo groups, but in opposite directions. The Lysholm scores increased significantly in both groups 6 months post-treatment compared with the pretreatment score ($p < .05$). In the placebo group, however, the Lysholm scores were decreased dramatically 18 months after surgery to a level comparable to the pretreatment score (75.80 ± 16.05, 76.47 ± 12.44, and 65.17 ± 14.74 at 6, 12, and 18 months, respectively, compared with 64.13 ± 10.19 pretreatment). In the treatment group, the Lysholm scores gradually increased over 6, 12, and 18 months compared with pretreatment scores (80.53 ± 7.86, 82.13 ± 8.98, 84.73 ± 19.54, and 53.47 ± 14.56, respectively). At 18 months, the mean Lysholm score of the placebo and treatment groups was significantly different ($p < .05$).

### Changes in VAS Scores

Similar to the Lysholm scores, VAS scores in both the treatment and placebo groups changed, but in opposite directions (Fig. 3). In the placebo group, VAS scores significantly increased after 6 months compared with those at pretreatment (2.67 ± 0.62 vs. 1.40 ± 0.51, respectively; $p < .05$). However, the scores then decreased from 12 to 18 months (2.53 ± 0.83 and 2.08 ± 1.08, respectively). In the treatment group, VAS scores continuously increased from 1.60 ± 0.83 at pretreatment to 3.01 ± 0.59, 3.20 ± 0.68, and 3.47 ± 0.74 at 6, 12, and 18 months, respectively ($p < .05$).

### Cartilage Injury Evaluation by MRI

Based on the MRI results and the Outerbridge classification system (OS), changes in cartilage injury were recorded and are presented in Figure 4A. OS scores gradually increased in the placebo group from

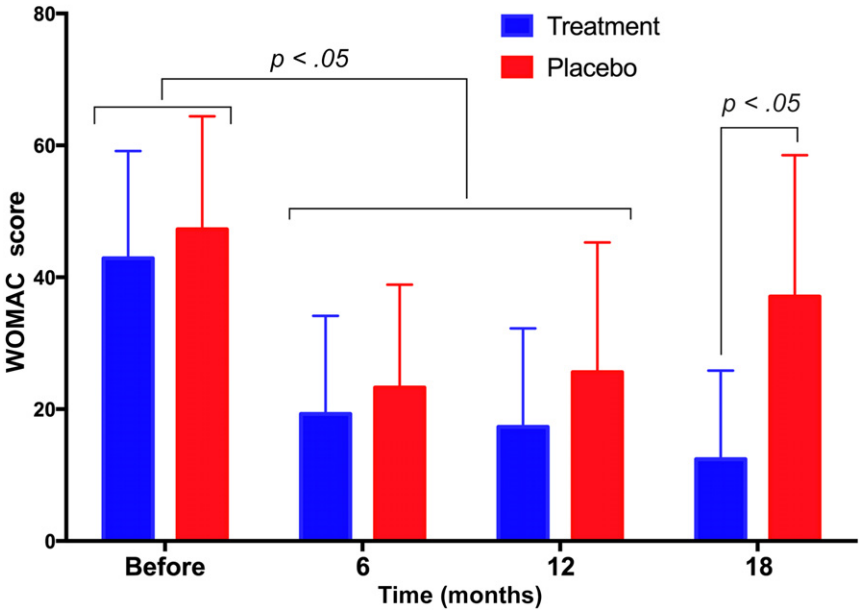


**Figure 1.** WOMAC scores in placebo and treatment groups at 6, 12, and 18 months post-treatment. After 6 months, WOMAC scores significantly decreased in both the treated and placebo groups. At 12 and 18 months, WOMAC scores continued to decrease in the treatment group and increased in the placebo group. Abbreviation: WOMAC, Western Ontario and McMaster Universities Arthritis Index.

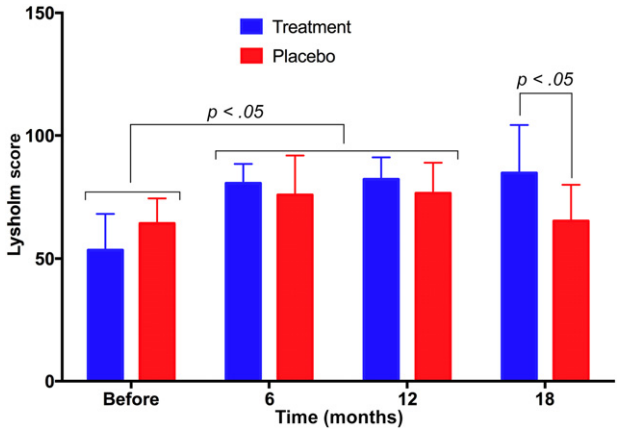


**Figure 2.** Lysholm scores in placebo and treatment groups at 6, 12, and 18 months post-treatment. In both treated and placebo groups, the Lysholm score significantly increased at 6 months post-treatment. At 12 and 18 months post-treatment, the Lysholm scores of the treatment group continued to increase, whereas those of the placebo group gradually decreased.

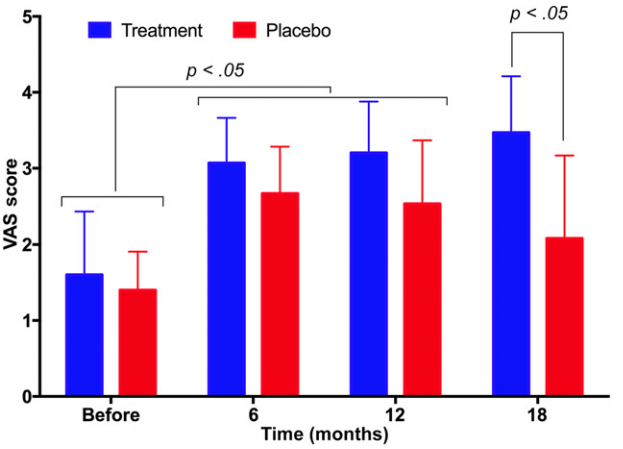


**Figure 3.** VAS scores at pretreatment and 6, 12, and 18 months post-treatment in the placebo and treatment groups. VAS scores in the treatment group gradually increased post-treatment. In the placebo group, scores increased after 6 months and gradually decreased at 12 and 18 months. Abbreviation: VAS, Visual Analog Pain Scale.

pretreatment to 6, 12, and 18 months post-treatment (2.67 ± 1.35, 2.93 ± 1.34, and 3.20 ± 1.08, respectively). However, scores decreased in the treatment group from pretreatment to 12 months post-treatment (3.33 ± 0.97 vs. 2.93 ± 0.88, respectively).

Although differences in OS scores were nonsignificant ($p > .05$), the trend was clearly different between the two groups: OS scores increased in the placebo group over time but decreased in the treatment group. MRI imaging demonstrated that the cartilage layer was thicker in the treatment group 12 months after AM (supplemental online Fig. 3).

## Bone Marrow Edema

Bone marrow edema (BME) was also recorded based on the MRI results. The results presented in Figure 4B and supplemental online Figure 2 show that BME was considerably deceased 12 months after surgery in the treatment group, although it was moderately increased in the placebo group. In the treatment group, BME gradually decreased from pretreatment to 6 and 12 months post-treatment (2.40 ± 0.63, 1.86 ± 0.64, and 1.33 ± 0.62, respectively), with a significant difference at 12 months ($p < .05$).

In the placebo group, BME increased moderately at 6 to 12 months post-treatment compared with pretreatment measurements (1.87 ± 0.74 at pretreatment vs. 2.00 ± 0.53; 2.13 ± 0.64 at 6 to 12 months post-treatment, respectively).

## Correlating OA Stage With Treatment Efficacy

Although the number of patients included in this study was low, we were able to evaluate the relative efficacy of AM plus SVP/PRP

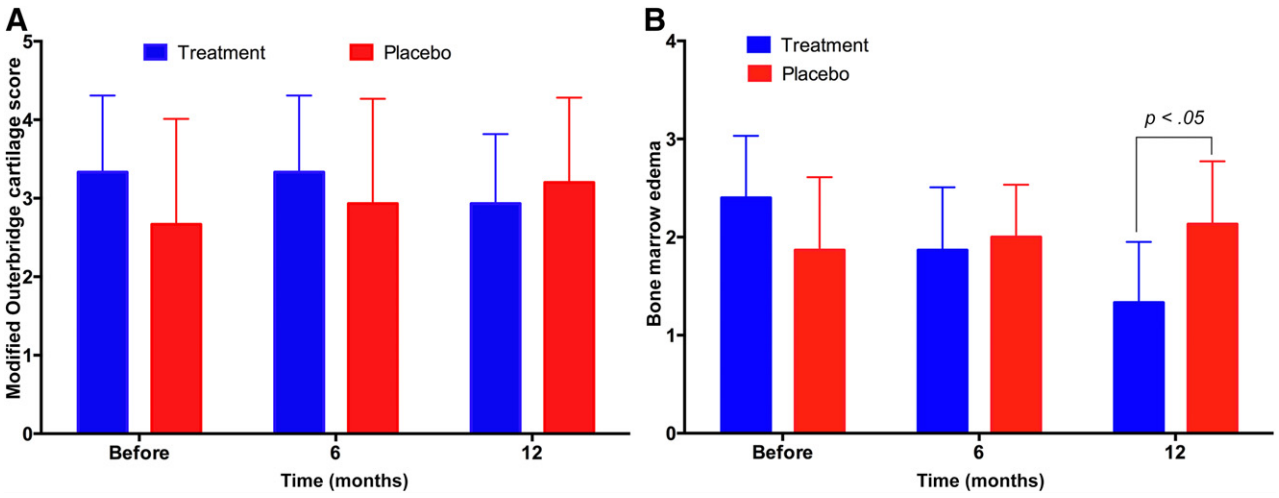


**Figure 4.** OS and BME scores at pretreatment and 6 and 12 months post-treatment. Although the changes were nonsignificant, OS scores increased in the placebo group and decreased in the treatment group **(A);** and BME was significantly decreased in the treatment group 12 months after surgery, and only slightly increased in the placebo group **(B)**. Abbreviations: BME, bone marrow edema; OS, Outerbridge classification system.

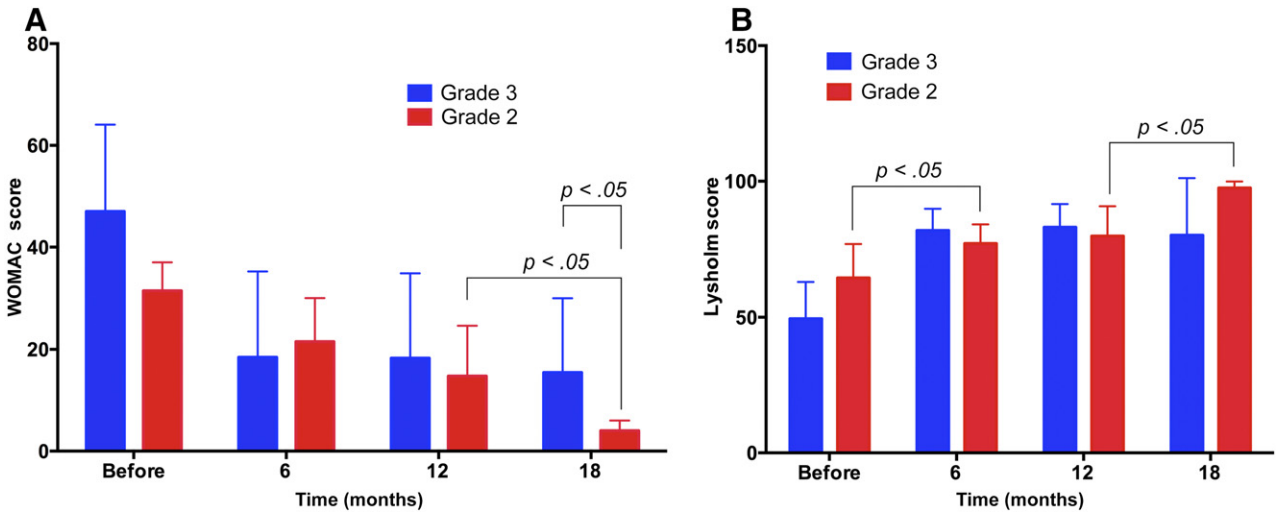


**Figure 5.** WOMAC and Lysholm scores in stage 2 and 3 osteoarthritis (OA; treatment group). Stromal vascular fraction and platelet-rich plasma injection significantly improved WOMAC and Lysholm scores in patients with stage 2 OA compared with those with stage 3 disease. Abbreviations: OA, osteoarthritis; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

treatment between patients with stage 2 ($n$ = 4) and stage 3 ($n$ = 11) OA.

The results presented in Figure 5A and 5B shows that the SVF/PRP injection affected patients with stage 2 and 3 OA differently with respect to both WOMAC and Lysholm scores, with significant differences observed at 18 months post-treatment. Although the WOMAC and Lysholm scores were significantly improved in both stage 2 and 3 OA groups at 18 months post-treatment compared with pretreatment, only in stage 2 OA patients were both WOMAC and Lysholm scores significantly improved at 18 months compared with 12 months post-treatment ($p < .05$).

When we separately compared the stage 2 and stage 3 treatment groups with the placebo group, the differences became clearer (Fig. 6). Compared with the stage 2 OA members of the placebo group, the stage 2 treatment group had significantly improved WOMAC and Lysholm scores. Compared with the stage 3 OA placebo group, the stage 3 treatment group was improved but to a lesser extent. Patients in the stage 2 treatment group continuously improved in both their WOMAC and Lysholm scores at 12 and 18 months post-treatment, whereas the improvement rate was slower in the stage 3 OA group.

## Changes in Knee Joint Function

The knee joint function of treated patients was significantly improved at 18 months post-treatment, and their joint motion amplitude (JMA) increased from 116.2 ± 27.1 at pretreatment to 138.8 ± 12.0 at 18 months post-treatment. JMA also increased in the placebo group from 120.6 ± 24.3 pretreatment to 133.3 ± 17.9 at 18 months post-treatment but to a lesser extent than in the treatment group.

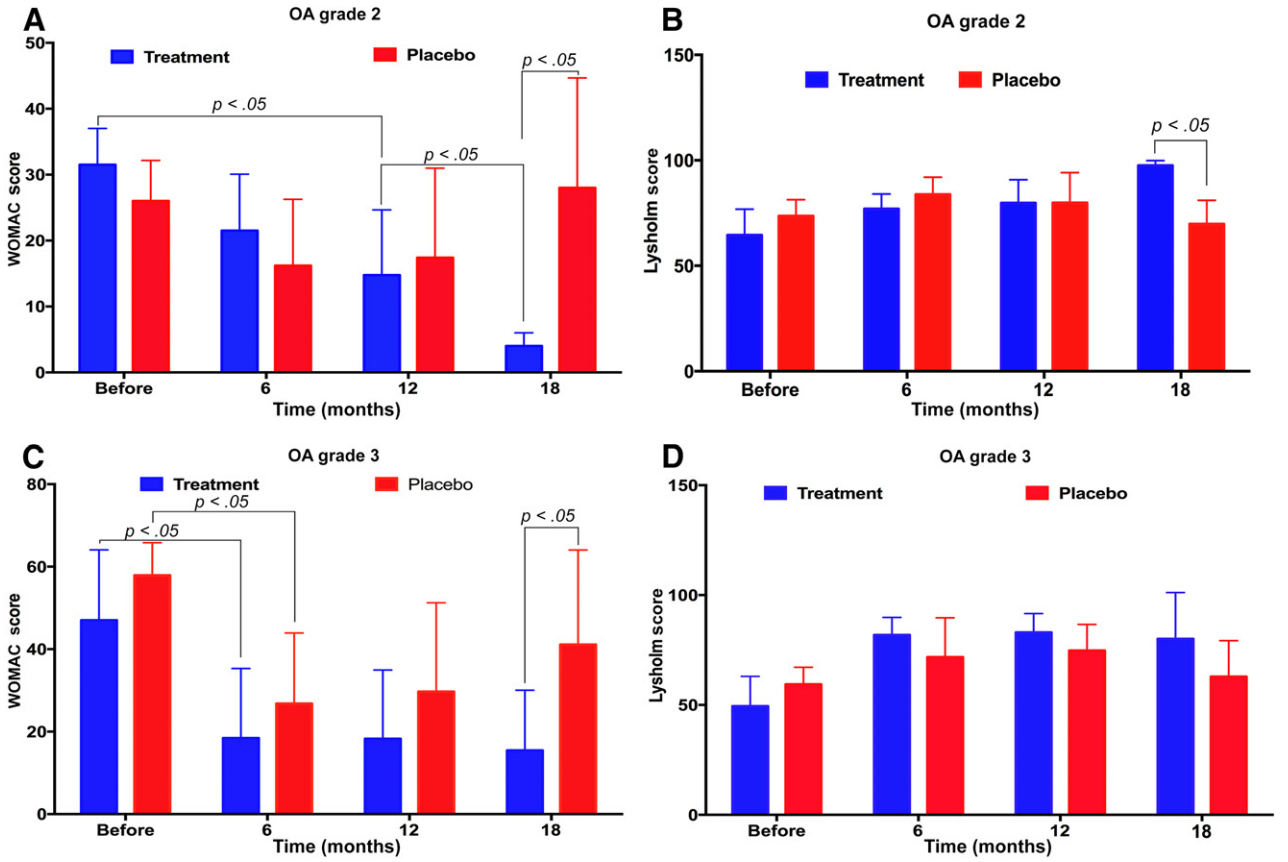


**Figure 6.** WOMAC and Lysholm scores in stage 2 and 3 OA treated and placebo groups. Stage 2 patients improved more rapidly compared with stage 3 patients. Abbreviations: OA, osteoarthritis; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

## Discussion

AM is the conventional method to treat cartilage degeneration, including OA lesions. However, the benefits of AM are gradually lost in the 18 months following treatment. This study aimed to combine the AM approach with an injection of SVF and PRP to improve treatment efficacy. Autologous ADSCs and autologous PRP from the peripheral blood were used in this study. Although previous studies used allogeneic-derived MSCs to effectively improve OA, we used an autologous source to minimize the side effects relating to host factors, specifically inflammation.

Both SVF and ADSCs (the purified form of SVF) have been used clinically in the treatment of conditions such as multiple sclerosis [42], femoral head necrosis [43, 44], chronic myocardial ischemia [45], critical limb ischemia, progressive supranuclear palsy [46], and acute respiratory distress syndrome [47]. Our results indicate that AM with a combined SVF/PRP injection significantly improved and prolonged the treatment efficacy of AM for OA. At 6 months post-treatment, the WOMAC, Lysholm, and VAS scores were significantly improved compared with pretreatment scores. These scores were further and significantly improved at 12 and 18 months post-treatment in the SVF/PRP group. Some of the patients obtained scores similar to that of healthy individuals. The WOMAC is a widely used, proprietary set of standardized questionnaires used by health professionals to evaluate the condition of patients with OA of the knee and hip, including pain, stiffness, and joint function. Higher WOMAC scores correspond with a higher level of pain, stiffness, and functional limitation. In the treatment group, the mean WOMAC score was 12.40 $\pm$ 13.44 at 18 months after surgery. The WOMAC Index is sensitive to change and, therefore, is considered a suitable scale to assess OA.

In addition to the WOMAC Index, the Lysholm scale is one of the most commonly used scoring systems for measuring OA. It was first published in 1982 and comprises 8 questions designed to evaluate joint instability in younger patients. This scale measures disability and focuses on the patient's perception of their ability to perform activities of daily living, as well as various intensities of physical activity [48]. According to this scale, a score of 84–90 is considered a good result. The average Lysholm score of patients in the AM plus SVF/PRP group 18 months after treatment was 84.73 $\pm$ 19.54.

Supporting the change seen in the WOMAC and Lysholm scores, the VAS scale scores also showed clear improvements in the treatment group. The VAS is a psychometric response scale that can be used in questionnaires. It is a measurement approach for subjective characteristics or attitudes that cannot be directly measured. The VAS scale for pain is divided into 4 points: 4 (no pain), 3 (mild pain), 2 (moderate pain), and 0 (severe pain). The WOMAC, Lysholm, and VAS scores demonstrated that at 18 months post-treatment, all patients in the treatment group had significantly improved pain, movement, and capacity for physical activity. Some patients' scores appeared similar to those of healthy individuals.

AM resulted in significantly reduced pain and improved knee function 6 months after the procedure, and these persisted for up to 12 months. However, by 18 months post-AM, the symptoms of

OA in the majority of patients reverted back to pretreatment levels. These results support those of several published studies. Thorlund et al. [16] reviewed 1,789 reports of AM used in degenerative knees. They found that AM had a small, inconsequential benefit in the management of OA, was effective for a limited time, and any benefits were absent 1 to 2 years after surgery. Furthermore, in patients with moderate to severe OA of the knee, Risberg [18] showed that the addition of arthroscopy to a regimen of physiotherapy and medication did not improve the physical function, pain, or health-related quality of life of patients with OA.

Our results showed that SVF in combination with PRP significantly improved the outcomes of AM for OA of the knee. SVF and PRP not only maintained and prolonged the effects of AM, but also increased overall treatment efficacy. All WOMAC, Lysholm, and VAS scores were noticeably improved compared with AM alone at 6 and 12 months post-treatment.

From the MRI results, we showed that OS scores and BME were significantly improved at 12 months post-treatment. Whereas OS scores and BME improved after AM in the placebo group, both of these indicators were decreased in the treatment group. In particular, BME was significantly decreased at 12 months post-treatment. OS classification is a grading system for joint cartilage breakdown: grade 0 represents normal joint cartilage; grade 1 represents cartilage with softening and swelling; grade 2 represents a partial-thickness defect with fissures on the surface that do not reach the subchondral bone or exceed 1.5 cm in diameter; grade 3 represents fissuring to the level of subchondral bone in an area with a diameter more than 1.5 cm; and grade 4 represents exposed subchondral bone. Our results showed that the OS scores decreased from 3.33 $\pm$ 0.97 pretreatment to 2.93 $\pm$ 0.88 at 12 months post-treatment in the treatment group. These results showed that the cartilage layer was thicker 12 months after the knee was injected with SVF and PRP, a finding congruent with our previously published study [36]. Other studies have shown that SVF in combination with PRP stimulates cartilage regeneration, with a thicker cartilage layer observed using post-treatment MRI evaluation [34, 44]. We have shown in a mouse model that SVF and PRP can stimulate knee cartilage regeneration [49]. The impact of a SVF/PRP injection in our study was also similar to effects noted in canine [50–52], rabbit [53, 54], horse [55], rat [56], and goat [57] models. Cartilage regeneration in these models was attributed to neocartilage triggered by SVF and PRP. In a rabbit model, Dragoo et al. [58] showed that autologous ADSCs were able to re-establish the joint surface in rabbits. They found neocartilage was present in 100% of treated rabbits (12 of 12), whereas only 8% of control rabbits (1 of 12) had neocartilage.

The mechanisms of action of SVF and ADSCs have been investigated in previous studies. In 2003, Gimble and Guilak [57] showed that injected ADSCs were able to protect and heal injured cartilage. Other benefits of ADSCs have been reported for cartilage regeneration, including anti-inflammatory properties [59, 60] and immune modulation. ADSCs can produce and secrete cytokines and growth factors that can trigger chondrogenesis, including transforming growth factor-$\beta$ (TGF-$\beta$), bone morphogenic protein 2 (BMP-2), BMP-4, BMP-7, insulin-like growth factor 1, and fibroblast growth factor 2 (FGF-2). ADSCs also produce cytokines that modulate the recipient immune system, including TGF-$\beta$, hepatocyte growth factor, nitric oxide, indolamine-2,3-dioxygenase, TNF-$\alpha$ [61] and interferon-$\gamma$ [62, 63]. In vitro, cultured ADSCs suppress the host's immune response and the T-cell proliferation as effectively as do BM-MSCs [61, 64]. Further studies have demonstrated that ADSCs actually stimulate a lesser proliferative response than do allogeneic PBMCs, but a similar response to BM-MSCs [65–67]. These findings suggest that ADSCs can replace BM-MSCs in the field of regenerative medicine [61].

The anti-inflammatory roles of ADSCs and PRP were also confirmed in our study by the obvious improvement of BME in the treatment group. BME is a condition characterized by the accumulation of excessive fluid in bone marrow-related structures. BME is a predictor for the progression of knee OA in the compartment ipsilateral to the bone marrow lesion [68]. BME was significantly reduced and the cartilage layer thickness was increased in the SVF/PRP-treatment group, indicating that OA was significantly improved. The increased BME observed in the placebo group may have been related to the progression of OA and inflammation after AM.

Cartilage regeneration in OA knees following AM and the combined SVF/PRP injection was likely because of the combination of SVF and PRP. However, SVF is likely to be the main contributor to this healing response. PRP has been used to treat knee OA in previous studies [69–71], but almost all of these studies showed that PRP significantly reduced short-term pain without concurrent cartilage regeneration [21, 69, 71, 72]. In combination with ADSCs, PRP can improve chondrogenesis in vitro and in vivo [73]. The components of PRP play important roles in stimulating grafted and endogenous cell growth and differentiation. PRP contains at least six known growth factors, including: platelet-derived growth factor, which promotes blood vessel growth and cell division; TGF-$\beta$, which promotes cell mitosis and bone metabolism; vascular endothelial growth factor, which promotes blood vessel formation; epidermal growth factor, which promotes cell growth and differentiation, angiogenesis, and collagen formation; FGF-2, which promotes cell differentiation and angiogenesis; and IGF, which is a regulator of all of the body's cell types [74–76].

We also observed that the regeneration response of cartilage to injected SVF/PRP was different between patients with grade 2 and 3 OA. Both WOMAC and Lysholm scores showed that the recovery of patients with grade 2 OA was faster than that of those with grade 3 disease. In particular, the improvement of WOMAC and Lysholm scores in patients with OA grade 2 were significant at 18 months compared with 12 months post-treatment. This demonstrated that OA grade 2 was treated with higher efficacy than OA grade 3 following SVF/PRP injection. Although this study was limited with respect to the sample size of patients with either grade 2 or 3 OA, these results are similar to other treatment options for OA, such as HA and PRP injections [24, 25].

Finally, JMA was compared between treated and placebo group patients. JMA was clearly increased in the treatment group compared with the placebo group, which agrees with both our subjective and radiographic analyses. More importantly, almost all patients in the treatment group exhibited a JMA similar to healthy individuals. The mean JMA was 138.8 $\pm$ 12 at 18 months post-treatment. The mean JMA of healthy individuals has been reported to be 140.0 (range, 113.9–166.4) [77].

We believe that our study is the first to evaluate AM with and without SVF for OA treatment with an 18-month follow-up time. Although Freitag et al. [78] recently performed a similar study to ours, their follow-up time was only 12 months.

## Conclusion

This study showed that AM with SVF/PRP injection was effective for knee OA and had better long-term outcomes than AM alone. Our preliminary analysis also showed that grade 2 knee OA was

improved to a greater extent than grade 3 disease following AM with SVF injection. AM with SVF injection significantly improved WOMAC, Lysholm, and VAS scores over the entire 18-month study period. MRI findings showed that the regenerated cartilage layer of patients treated with AM and SVF was thicker at 12 and 18 months after the procedure. Furthermore, the JMA of SVF/PRP-treatment patients 18 months after surgery was significantly improved and comparable with that of healthy individuals. No adverse effects were recorded in any treated patients. From these findings, we conclude that AM with SVF/PRP injection may be a suitable treatment for grade 2 and 3 OA of the knee.

## Author Contributions

P.D.N.: conception and design, administrative support, provision of study material or patients; T.D.-X.T., H.T.-N.N., and H.T.V.: provision of study material or patients, collection and/or assembly of data; P.T.-B.L.: conception and design, administrative support, provision of study material or patients, collection and/or assembly of data; N.L.-C.P. and N.B.V.: provision of study material or patients; N.K.P.: conception and design, data analysis and interpretation; P.V.P.: conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript.

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## Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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# Comparative Outcomes of Open-Wedge High Tibial Osteotomy With Platelet-Rich Plasma Alone or in Combination With Mesenchymal Stem Cell Treatment: A Prospective Study

Yong-Gon Koh, M.D., Oh-Ryong Kwon, M.D., Yong-Sang Kim, M.D., and Yun-Jin Choi, M.D.

**Purpose:** This study compared the clinical results and second-look arthroscopic findings of patients undergoing open-wedge high tibial osteotomy (HTO) for varus deformity, with or without mesenchymal stem cell (MSC) therapy. **Methods:** This prospective, comparative observational study was designed to evaluate the effectiveness of MSC therapy. The patients were divided into 2 groups: HTO with platelet-rich plasma (PRP) injection only (n = 23) or HTO in conjunction with MSC therapy and PRP injection (n = 21). Prospective evaluations of both groups were performed using the Lysholm score, Knee Injury and Osteoarthritis Outcome Score (KOOS), and a visual analog scale (VAS) score for pain. Second-look arthroscopy was carried out in all patients at the time of metal removal. **Results:** The patients in the MSC-PRP group showed significantly greater improvements in the KOOS subscales for pain (PRP only, 74.0 ± 5.7; MSC-PRP, 81.2 ± 6.9; $P < .001$) and symptoms (PRP only, 75.4 ± 8.5; MSC-PRP, 82.8 ± 7.2; $P = .006$) relative to the PRP-only group. Although the mean Lysholm score was similarly improved in both groups (PRP only, 80.6 ± 13.5; MSC-PRP, 84.7 ± 16.2; $P = .357$), the MSC-PRP group showed a significantly greater improvement in the VAS pain score (PRP only, 16.2 ± 4.6; MSC-PRP, 10.2 ± 5.7; $P < .001$). There were no differences in the preoperative (PRP only, varus 2.8° ± 1.7°; MSC-PRP, varus 3.4° ± 3.0°; $P = .719$) and postoperative (PRP only, valgus 9.8° ± 2.4°; MSC-PRP, valgus 8.7° ± 2.3°; $P = .678$) femorotibial angles or weight-bearing lines between the groups. Arthroscopic evaluation, at plate removal, showed that partial or even fibrocartilage coverage was achieved in 50% of the MSC-PRP group patients but in only 10% of the patients in the PRP-only group ($P < .001$). **Conclusions:** MSC therapy, in conjunction with HTO, mildly improved cartilage healing and showed good clinical results in some KOOS subscores and the VAS pain score compared with PRP only. **Level of Evidence:** Level II, prospective comparative study.

Globally, osteoarthritis (OA) is the most common cause of knee pain. Arthritis of the knee joint commonly affects the medial compartment and is associated with misalignment, thereby placing a greater load on the affected compartment.[1] High tibial osteotomy (HTO) is a treatment option for younger and/or physically active patients who have OA of the medial compartment of the knee. HTO was originally devised to treat varus OA by decreasing pressure on the medial compartment.[2] In this regard, several studies have reported remodeling of the articular cartilage after HTO and attributed improvements to reduced contact stress by altering the weight-bearing axis.[2-5] However, HTO alone induces partial remodeling of the articular cartilage,[3] and therefore additional procedures, such as stem cell transplantation, may further enhance articular cartilage healing in OA patients.

Intra-articular injection of mesenchymal stem cells (MSCs) was reported to be effective for reducing pain in patients with knee OA.[6,7] In a previous study, postoperative magnetic resonance imaging studies also showed notable improvements in medial femoral condyle cartilage defects. On the basis of these findings, stem cell injection was used to achieve greater cartilage remodeling and better clinical results after HTO surgery.

The purpose of this study was to compare the clinical results and second-look arthroscopic findings in patients undergoing open-wedge HTO for varus deformities, with or without MSC therapy. MSC

*From the Center for Stem Cell & Arthritis Research, Department of Orthopaedic Surgery, Yonsei Sarang Hospital, Seoul, South Korea.*

*Yong-Gon Koh and Oh-Ryong Kwon contributed equally to this work and should be considered co-first authors.*

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*Address correspondence to Yun-Jin Choi, M.D., Department of Orthopaedic Surgery, Yonsei Sarang Hospital, 478-3, Bangbae-dong, Seocho-gu, Seoul, South Korea. E-mail: yunjinchoi78@gmail.com*



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therapy with platelet-rich plasma (PRP), in conjunction with HTO, was hypothesized to provide improved cartilage healing and clinical results compared with injection of PRP only.

## Methods

This prospective, comparative observational study was designed to evaluate the effectiveness of MSC therapy. Study protocols were approved by the local ethics committee, and all patients provided written informed consent. From January to October 2011, 44 patients who met the following inclusion criteria were enrolled in this study. The inclusion criteria for surgical treatment reflected those outlined in the literature for this procedure: (1) age younger than 60 years, (2) radiographs showing grade III or lower Kellgren-Lawrence symptomatic isolated medial knee compartment OA, (3) failure of conservative treatment, and (4) absence of additional cartilaginous procedures (autologous chondrocyte transplantation, microfracture). Patients were excluded if they did not consent to undergo a second operation for plate removal and second-look arthroscopy and could not be evaluated at either the 1- or 2-year postoperative visit. In addition, patients were excluded if they had undergone previous cartilage procedures, such as microfracture or chondroplasty, for chondral lesions of the medial femoral condyle because the intention was to examine the effect of MSC therapy on cartilage healing. Patients were also excluded if they met at least 1 of the following criteria: severe cartilage lesions of the lateral compartment or patellofemoral compartment, as observed using preoperative magnetic resonance imaging; inflammatory or postinfectious arthritis; previous arthroscopic treatment for knee OA; previous major knee trauma; intra-articular hyaluronic acid or corticosteroid injection within the preceding 3 months; mechanical pain caused by meniscal tears (including flap tears, bucket-handle tears, and complex tears); chronic anterior cruciate ligament/posterior ligament instability; or inability to provide informed consent.

Patients were randomized into either the PRP-only group or the MSC-PRP group. Simple randomization methods were used in which each patient, when enrolled in the trial, was asked to choose either of 2 identical envelopes with either the PRP-only or MSC-PRP group indicated inside. The randomization process was conducted by a hospital staff member blinded to the patients' data. Patients, however, were not blinded to the interventional method (liposuction) used. A total of 52 patients were enrolled, with 26 knees comprising each group.

The patients were prospectively evaluated by physiotherapists using the Lysholm score,[8] the Knee Injury and Osteoarthritis Outcome Score (KOOS),[9] and a 100-point visual analog scale (VAS) score for pain (0, no pain; 100, worst possible pain). Patients were evaluated preoperatively and postoperatively at 3 months, at 1 year, and at the last follow-up visit (mean, 24.4 months; range, 24 to 25 months). Before surgery, radiographs of the knee joints were obtained, including an anteroposterior (AP) view, a true lateral view at 30° of knee flexion, and an AP long-leg weight-bearing view. To investigate the mechanical effects of HTO, the femorotibial angle (FTA) and percentage of mechanical axis[10] were measured using standing AP radiographs taken immediately before surgery and after surgical removal of the plate. The FTA was determined as the angle between the femoral and tibial shaft axes on the standing AP radiographs.

### Collection of Subcutaneous Adipose Tissue

Subcutaneous adipose tissue was harvested from both buttocks of each patient. One day before HTO, adipose tissue was harvested by tumescent liposuction, with the patient under local anesthesia.[11] Routinely, 140 mL of adipose tissue that had undergone liposuction was collected; 120 mL was used for the injection. The remaining 20 mL was subjected to laboratory analyses to assess the plastic-adherent cells that formed colony-forming unit fibroblasts and to confirm the multilineage differentiation of the adipose-derived stem cells (ADSCs).

### Isolation of Stromal Vascular Fraction and MSCs From Subcutaneous Adipose Tissue

In the operating room, adipose tissue (120 mL) was suspended in phosphate-buffered saline solution, placed in a sterile box, and transported to a laboratory. Mature adipocytes and connective tissue were separated from the stromal vascular fraction (SVF) by centrifugation, as reported by Zuk et al.[12] The volume of the SVF was usually less than 1.0 mL. For injection, SVF cells were prepared with approximately 3.0 mL of PRP. Before injection, bacteriologic tests were performed to ensure the absence of sample contamination, and the cell viability was assessed by methylene blue dye exclusion.

### Assessment of Plastic-Adherent Cells That Form Colony-Forming Unit Fibroblasts and Immunophenotyping of ADSCs

To evaluate the frequency of mesenchymal-like progenitors in patients' SVF, cells were cultured in T-25 flasks at a final concentration of 16 cells/cm$^2$. Colonies consisting of 50-cell aggregates or greater were scored under an optical microscope, and the immunophenotypes of the ADSCs were analyzed by flow cytometry (fluorescence-activated cell sorting). MSC marker phenotyping was performed as previously described.[13]

### Confirmation of Multilineage Differentiation of ADSCs

ADSCs were plated at $2 \times 10^3$ cells/cm$^2$ in Dulbecco's modified Eagle medium containing 10% fetal bovine

serum and allowed to adhere for 24 hours. The culture medium was then replaced with specific media to induce adipogenic, osteogenic, and chondrogenic differentiation, as previously reported.[13]

### PRP Preparation

For PRP preparation, a 60-mL venous blood sample (collected in a tube containing 4 mL of sodium citrate) was collected from each patient. A complete peripheral blood count was determined. The samples were centrifuged twice (at 1,800 rpm for 15 minutes to separate the erythrocytes and then at 3,500 rpm for 10 minutes to concentrate the platelets) to yield 6 mL of PRP. The PRP was divided into 2 units of 3 mL each. One unit was sent to the laboratory for determination of the platelet concentration and for quality testing (bacteriologic tests); the other was used for the first injection, within 2 hours of preparation.

### MSC Implantation and Open-Wedge HTO

The patients were positioned supine on the operating table, and a thigh tourniquet was applied. Before undergoing HTO, each patient underwent arthroscopic surgery. Using arthroscopy, the orthopaedic surgeons (Y-G.K., Y-J.C.) evaluated the medial, lateral, and patellofemoral joint compartments; graded the articular lesions according to the International Cartilage Repair Society Cartilage Injury Evaluation Package[14]; irrigated the compartment with at least 1 L of saline solution; and performed 1 or more treatments, including synovectomy, debridement or excision of the degenerative tears of the menisci, or removal of articular cartilage fragments, chondral flaps, or osteophytes that prevented full extension. After completion of the arthroscopic procedure, the arthroscopic fluid was washed out. In the MSC-PRP group, injection of MSCs plus PRP (isolated 1 day before arthroscopic surgery) was administered under arthroscopic guidance. In the PRP-only group, the injection of PRP alone was performed after the arthroscopic procedure by injection into the medial joint space under arthroscopic guidance.

After injection, HTO was performed according to the technique recommended by the AO International Knee Expert Group.[15] The TomoFix system (Synthes, Solothurn, Switzerland) was used to stabilize the osteotomy, which was performed in a biplanar fashion. Before surgery, the correction angle and open-wedge size were calculated by the operator (Y-G.K. and Y-J.C.), using AP radiographs of the lower extremity (orthoroentgenogram) with the patient in standing (full weight-bearing) position. The aim was to pass the weight-bearing line through a point 62% lateral to the tibial plateau from the medial edge of the medial tibial plateau; the correction angle and size of the open wedge were measured on the orthoroentgenogram before surgery. All measurements were independently calculated by 2 junior surgeons (O-R.K., Y-S.K.), and all osteotomies aimed for mild overcorrection.[16] A β−tricalcium phosphate (Synthes, Bettlach, Switzerland) wedge, corresponding to the open space, was inserted into the osteotomy site. This material is a fully synthetic, resorbable bone graft substitute, consisting of pure β−tricalcium phosphate with a compressive strength similar to that of cancellous bone.

One day after surgery, isometric quadriceps, active ankle, and straight leg−raising exercises began. The patients were allowed to move their knee from 0° to 90° after 2 weeks. Toe-touch weight bearing was allowed for 2 weeks after surgery, followed by partial weight bearing for the next 2 weeks. Full weight bearing was allowed at 4 weeks, after radiographic evaluation of bone consolidation at the osteotomy site.

### Second-Look Arthroscopy

For all patients in this study, second-look arthroscopy was performed during metal removal for fixation. The interval between HTO (first intra-articular observation) and removal of the plate (second intra-articular observation) was 14 to 24 months (mean, 19.8 months). All second-look arthroscopies were video recorded (3 to 5 minutes). The examinations were performed during second-look arthroscopy video review by all members of the surgical team, and the findings were confirmed only when a consensus was reached. Chondral lesions were described, according to the Kanamiya grading system,[4] as follows: grade 1, no regenerative change; grade 2, white scattering with fibrocartilage; grade 3, partial fibrocartilage coverage; and grade 4, even fibrocartilage coverage.

### Power Calculation and Statistical Analysis

A difference of 15 points in the Lysholm score (1 of the main outcome measures) represented a clinically significant difference between treatment groups. Thus, accepting less than 5% probability of a type I error and a power of 80%, we determined that a total sample size of 22 patients was required for each group. Predicting a 10% dropout rate, we enrolled a total of 52 patients, with 26 knees comprising each group.

Statistical analyses were performed by use of SPSS software, version 12.0.1 (SPSS, Chicago, IL), with significance defined as $P < .05$. The principal dependent variables of the clinical outcomes were the KOOS, Lysholm score, and VAS pain score at the final follow-up. The Fisher exact test and a $\chi^2$ test were used to compare categorical data. Differences between groups were analyzed by use of the Mann-Whitney $U$ test. The Wilcoxon rank sum test was used for within-group analyses (preoperative $v$ postoperative in same group). The Spearman rank order correlation test was used to

**Table 1.** Overview of Patient Groups

| | PRP-Only Group | MSC-PRP Group | *P* Value |
|---|---|---|---|
| No. of patients | 23 | 21 | |
| Male/female sex (n) | 6/17 | 5/16 | .53 |
| BMI (kg/m$^2$) | 24.7 ± 3.3 | 25.7 ± 2.9 | .29 |
| Follow-up period (mo) | 24.6 ± 6.4 | 24.2 ± 4.7 | .32 |
| Age (yr) | 52.3 ± 4.9 | 54.2 ± 2.9 | .48 |

NOTE. Values are expressed as mean ± standard deviation unless otherwise indicated.
BMI, body mass index.

analyze the correlation between cartilage healing status and patient demographic factors.

## Results

### Patient Characteristics

The patient demographic data and characteristics are shown in Table 1. Figure 1 shows the trial profile of this study. There were 52 patients recruited into the study, 26 patients in each group. However, 5 patients (2 in the PRP-only group and 3 in the MSC-PRP group) could not be evaluated at either the 1- or 2-year post-operative visit. Second-look arthroscopic data are missing for 1 patient in the PRP-only group and for 2 patients in the MSC-PRP group because they did not consent to undergo a second surgical procedure for plate removal. Finally, for 44 patients (23 in the PRP-only group and 21 in the MSC-PRP group), second-look arthroscopic results and 2-year clinical results were available for the last analysis. There were no significant differences in patient demographic data between the 2 groups.

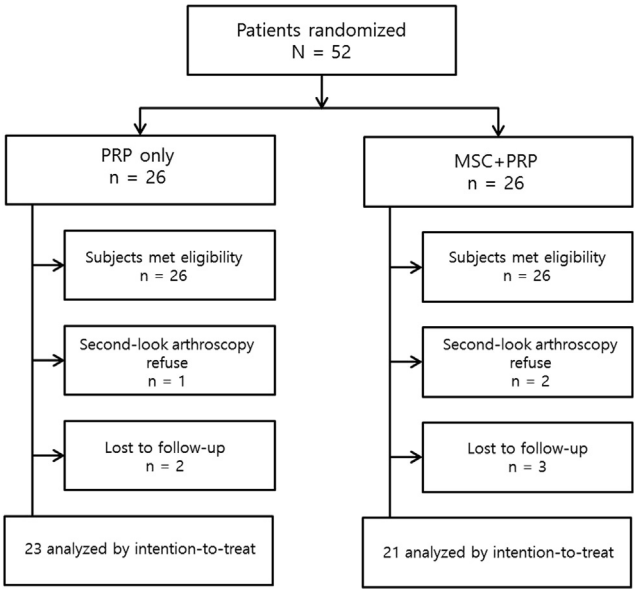


**Fig 1.** Trial profile of patients randomized in study. The patients were randomized into 2 groups of 26 subjects each; 5 patients were lost to follow-up during the 2-year follow-up and 3 patients refused the second-look arthroscopy.

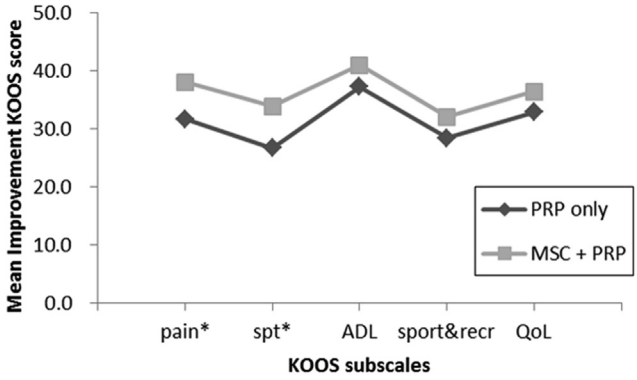


**Fig 2.** Mean improvement from baseline in KOOS subscales at last follow-up. Asterisks indicate statistical significance ($P <$ .05). (ADL, activities of daily living; QOL, quality of life; sports&rec, sports and recreation; spt, symptoms.)

### Cell Isolation and Characterization of ADSCs

The platelet concentrations (mean ± SD) in whole blood and PRP were 208.53 ± 42.9 × 10$^3$/mL and 1,303.27 ± 375.2 × 10$^3$/mL, respectively.

After isolation, ADSCs represented 8.5% of the SVF cells (range, 6.8% to 10.2% of SVF cells), or 4.11 × 10$^6$ stem cells (8.5% of the 4.83 × 10$^7$ SVF cells) were prepared. Flow cytometry characterization showed positive expression of the CD90 (98.34%) and CD105 (91.23%) surface markers and negative expression of CD45 (2.23%), CD34 (6.45%), and CD14 (2.32%). ADSCs treated with conditioned media showed characteristics of adipogenic, osteogenic, and chondrogenic differentiation, as previously reported.[17]

### Clinical and Radiologic Outcomes at Follow-up

The patients in the MSC-PRP group showed a trend toward greater improvements in all of the KOOS subscales, although significant differences were only observed for the pain and symptom subscales at the last follow-up (Fig 2). The MSC-PRP group showed significantly greater improvements in the KOOS pain subscale (PRP only, 74.0 ± 5.7; MSC-PRP, 81.2 ± 6.9; $P <$ .001) and symptom (PRP only, 75.4 ± 8.5; MSC-PRP, 82.8 ± 7.2; $P =$ .006) scores relative to the PRP-only group. The other clinical and radiologic outcomes at the preoperative and final follow-up time points, for both groups, are summarized in Table 2. The mean Lysholm score was also significantly improved in both groups ($P <$ .001), but no differences were seen between the groups ($P =$ .357). Although the mean VAS pain score decreased significantly (i.e., improved) at the final follow-up visit in both groups ($P <$ .001), the MSC-PRP group showed a greater improvement relative to the PRP-only group ($P <$ .001).

The standing AP radiographs taken immediately after implant removal showed improved knee joint mechanics in both groups relative to their preoperative conditions. However, there were no differences in the

**Table 2.** Clinical and Radiologic Results of Patient Groups

| | PRP-Only Group | MSC-PRP Group | *P* Value (95% CI) |
|---|---|---|---|
| Lysholm score | | | |
| Preoperative | 56.7 ± 12.2 | 55.7 ± 11.5 | .747 (−12.12 to 8.83) |
| Last follow-up | 80.6 ± 13.5 | 84.7 ± 16.2 | .357 (−8.4 to 1.2) |
| VAS | | | |
| Preoperative | 45.4 ± 7.1 | 44.3 ± 5.7 | .460 (−0.77 to 0.29) |
| Last follow-up† | 16.2 ± 4.6 | 10.2 ± 5.7 | <.001 (0.23 to 0.98) |
| WBL (%) | | | |
| Preoperative | 16.1 ± 5.7 | 17.7 ± 7.3 | .800 (−2.56 to 3.91) |
| Last follow-up | 60.3 ± 3.0 | 61.1 ± 3.4 | .758 (−3.50 to 4.51) |
| FTA (°) | | | |
| Preoperative | Varus 2.8 ± 1.7 | Varus 3.4 ± 3.0 | .719 (−1.30 to 1.87) |
| Last follow-up | Valgus 9.8 ± 2.4 | Valgus 8.7 ± 2.3 | .678 (−1.32 to 1.90) |
| Initial cartilage status (n)* | | | .876 |
| Grade 2 | 1 | 0 | |
| Grade 3 | 11 | 9 | |
| Grade 4 | 11 | 12 | |

NOTE. Values are expressed as mean ± standard deviation unless otherwise indicated.
CI, confidence interval; WBL, weight-bearing line.
*Initial cartilage status was graded by arthroscopy before HTO; the orthopaedic surgeons (Y-G.K., Y-J.C.) evaluated the medial joint compartments and graded the articular lesions according to the International Cartilage Repair Society Cartilage Injury Evaluation Package.
†Significant difference at last follow-up between groups ($P < .05$).

postoperative FTAs ($P = .678$) or weight-bearing lines ($P = .758$) between the groups.

### Second-Look Arthroscopy

There were no significant differences in the initial cartilage status between the groups ($P = .876$) (Table 2). However, there was a significant difference between the groups with respect to cartilage healing ($P = .023$) (Fig 3). Second-look arthroscopy, during plate removal, showed that 0 of the 23 knees in the PRP-only group had even fibrocartilage coverage (grade 4), determined arthroscopically. One knee (4.3%) had partial fibrocartilage coverage (grade 3), 11 (47.8%) had white scattering with fibrocartilage (grade 2), and 11 (47.8%) did not show any regenerative changes (grade 1). In contrast, in the MSC-PRP group, 3 knees (14.3%) had even fibrocartilage coverage (grade 4), 8 (38.1%) had partial fibrocartilage coverage (grade 3), 9 (42.9%) had white scattering with fibrocartilage (grade 2), and 1 (4.8%) did not show any regenerative changes (grade 1). Figure 4 shows examples of the arthroscopic photographs used in the patient evaluations.

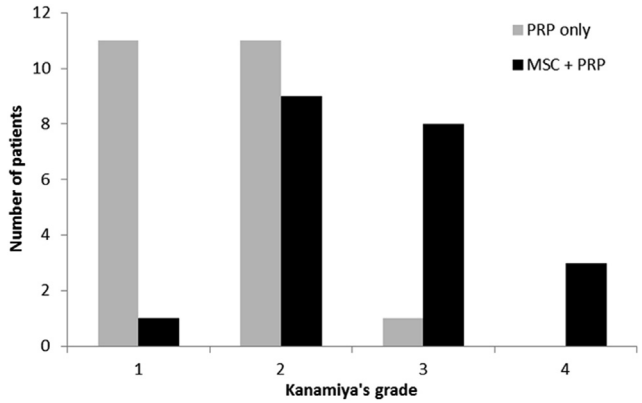


**Fig 3.** Articular cartilage healing status, using the Kanamiya grading system,[4] during second-look arthroscopy in both groups.

### Correlation Between Cartilage Healing Status and Patient Demographic Factors

The correlations between cartilage healing status and other patient demographic factors were analyzed to determine whether there were other reasons for the observed cartilage healing status. However, significant correlations were not found between the cartilage healing status and patient body mass index, age, or radiologic parameters (Table 3).

## Discussion

The principal findings of this study were that HTO in conjunction with the use of MSCs plus PRP resulted in good fibrocartilage repair and improved clinical results compared with HTO and PRP only. Importantly, other patient demographic factors, such as age, were not associated with improvements in cartilage healing, suggesting that the improvements were primarily due to MSC injection. Thus these findings support the hypothesis that MSC therapy with PRP, in conjunction with HTO, provided additional benefits for cartilage healing and clinical results compared with injection of PRP only.

HTO has been recommended for treating varus OA to decrease the pressure on the damaged medial compartment of the joint, provide pain relief, and reduce the progression of medial OA.[18] Although HTO theoretically decreases the stress on the load-bearing cartilage in the medial compartment,[2-5] some studies

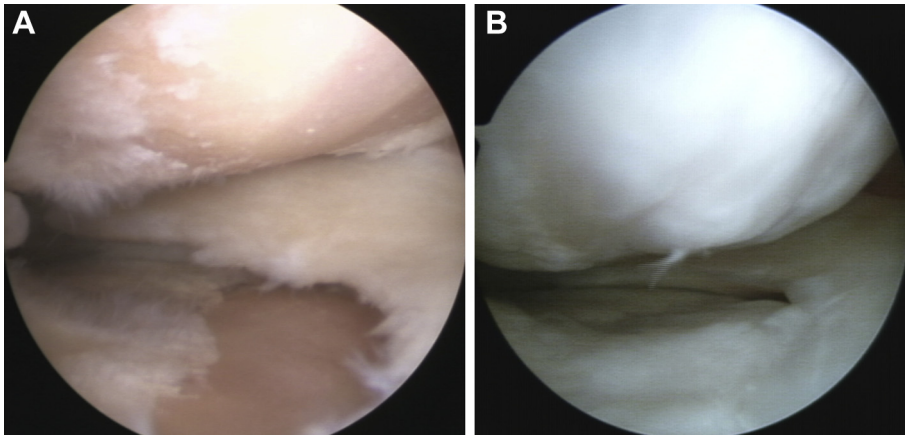


**Fig 4.** Intraoperative arthroscopic images during first- and second-look arthroscopy. (A) Findings in a 53-year-old woman in the MSC-PRP group. During the first arthroscopy, eburnation of the articular surfaces was found. (B) Marked changes in the cartilage defects of the medial femoral condyle are shown. The articular surface shows an even fibrocartilage coverage at 17 months postoperatively.

have reported that partial remodeling of the articular cartilage occurs with cartilage regeneration after HTO.[19,20] For better chondral defect remodeling, HTO combined with chondral resurfacing has been attempted.[3,21] The most popular chondral resurfacing procedures are marrow stimulation techniques. These techniques involve microfractures that promote cartilage repair by stimulating the bone marrow through the subchondral bone and by producing blood clots containing mesenchymal cells on the articular surface. In a 2-year follow-up study of 38 patients, Sterett and Steadman[21] reported that combining a medial open-wedge HTO with a microfracture in the varus knee was an effective method for decreasing pain and increasing function. However, Mithoefer et al.[22] reported that microfractures effectively improved knee function in all patients during the first 24 months after the microfractures, but the durability of the initial functional improvement was inconsistent. Moreover, in patients with degenerative knee arthritis, the cartilage lesion is diffuse and not focal, meaning that microfractures cannot be applied in all HTO cases. Thus, for cartilage defect remodeling, other options are needed.

MSCs are emerging as powerful tools for cartilage repair because of their ability to differentiate into various connective tissues, including cartilage, bone, and fat.[23,24] The intra-articular injection of MSCs was reported to effectively reduce pain while promoting cartilage regeneration in patients with knee OA.[6,7] On the basis of these previous findings, stem cell injection may be used to achieve greater cartilage remodeling and better clinical results after HTO surgery. Thus, in our study, more patients in the MSC-PRP group achieved partial or even fibrocartilage coverage than in the PRP-only group, showing a clear relation between the cartilage healing status and MSC therapy. Furthermore, the patients in the MSC-PRP group showed statistically significantly better clinical outcomes in the VAS pain score and 2 KOOS subscores compared with patients in the PRP-only group. Although better scores were observed in the group receiving MSC therapy than in the group receiving PRP only, there were no differences between the groups with respect to the Lysholm score and the other KOOS subscores.

**Table 3.** Correlation Between Cartilage Healing Status and Patient Demographic Factors

| | Healing Status | |
|---|---|---|
| | Spearman ρ | *P* Value |
| BMI | 0.81 | .60 |
| Age | 0.09 | .56 |
| WBL | 0.10 | .51 |
| FTA | −0.08 | .60 |

NOTE. Data were calculated using the Spearman rank order correlation test.

BMI, body mass index; WBL, weight-bearing line.

In this study, subcutaneous adipose tissue was used as the stem cell source. Adipose tissue is composed of 2 main cell populations, mature adipocytes and the cells in the SVF. The latter comprise a heterogeneous fraction that includes preadipocytes, endothelial cells, smooth muscle cells, pericytes, macrophages, fibroblasts, and ADSCs, which share several characteristics with bone marrow stem cells.[25,26] ADSCs are promising candidates in a broad range of innovative therapies, ranging from regenerative medicine to tissue engineering. Moreover, the use of ADSCs has been proposed for several chronic diseases, such as Crohn disease,[27] autoimmune pathologies (e.g., multiple sclerosis),[28] and allergic pathologies. The effectiveness against these pathologies can be explained by the immunoregulatory and anti-inflammatory activities of ADSCs and non-expanded SVF cells.[28] Unfortunately, because most 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 in addition to MSCs. Recently, ADSCs have been suggested as a new option for the treatment of osteochondral lesions, and the injection of MSCs with marrow stimulation has

been proposed for treating such cases.[29] Moreover, Desando et al.[30] reported that the healing properties of ADSCs, including their promotion of cartilage and meniscus repair and attenuation of inflammatory events in the synovial membrane, may inhibit OA progression. Jurgens et al.[31] evaluated the safety, feasibility, and efficacy of freshly isolated SVF cells and cultured ADSCs in an animal model. They showed the preclinical safety and feasibility of a 1-step surgical procedure for osteochondral defect regeneration using freshly isolated SVF cells and cultured ADSCs. Specifically, they observed similar regeneration induced by either freshly isolated SVF cells or cultured ADSCs.

In OA patients the healing tissue has been shown to be quite different from the surrounding degenerated yellow cartilage. Furthermore, because the cartilage of OA patients has diffuse degenerative lesions, identifying changes in the status of OA patients is difficult. In other words, the grading of severe lesions, used in the Outerbridge classification[32] and the International Cartilage Repair Society grade, does not seem appropriate to describe these changes in the cartilage status of OA patients. Thus the classification of the regenerative progress using the Kanamiya classification,[4] as used in our study, is necessary.

MSC therapy has previously been shown to induce a positive effect in OA treatment through 2 mechanisms, paracrine signaling and end-organ (e.g., cartilage) formation. Paracrine mechanisms likely explain the clinical improvements, whereas cartilage formation explains the differences in cartilage healing status observed between the groups in this study at their final follow-up visit. The MSC therapy method used in this study was a very primitive technique; therefore the method cannot likely be used in isolation. For the application of this technique, several challenges still need to be overcome, including the identification of the optimal sources of stem cells, scaffolds, and growth factors.

### Limitations

This study has several limitations. First, the follow-up period was short, and therefore future studies with longer cartilage formation and survival follow-up periods should be undertaken. Second, the stem cells were delivered with a single injection, whereas optimal results may require providing patients with more than 1 injection over time. Third, pathologic examinations of the cartilage properties in each group were not performed. Fourth, the loss of correction influenced the clinical outcome; because patients were not assessed in the standing position, measurement of correction angles in the immediate postoperative period was not performed. Therefore a measure of the influence of correction loss on clinical outcomes was not possible. Fifth, because several patients were excluded because they did not want to undergo plate removal, there might be the problem of selection bias in this study. Sixth, the Kanamiya grading system[4] was a potential limitation because it was not validated with known interobserver and intraobserver variability. Lastly, an additional limitation is the potential for type II errors because of the small sample sizes. Although an a priori power evaluation was conducted to determine the number of participants required for the trial, the calculations were completed using limited data. Therefore the study may suffer from a type II statistical error, resulting from the effects of stem cells on persons with diffuse cartilage lesions. Thus the lack of significant differences in some of the clinical outcome data, with the exception of the pain scores and symptom subscores, was likely because of a type II error. In addition, although statistically significant improvements in some KOOS subscores and in the VAS pain score were observed, they may not reflect clinically significant improvements. Therefore another study will be needed with a larger number of patients.

## Conclusions

MSC therapy, in conjunction with HTO, induced mild improvements in cartilage healing and good clinical results in some KOOS subscores and the VAS pain score compared with PRP only.

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# Infrapatellar fat pad-derived mesenchymal stem cell therapy for knee osteoarthritis

Yong-Gon Koh, Yun-Jin Choi *

*Department of Orthopedic Surgery, Yonsei Sarang Hospital, Seoul, South Korea*

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ABSTRACT

*Purpose:* The aim of the study was to determine if isolated mesenchymal stem cells (MSCs) derived from the infrapatellar fat pad could effectively improve clinical results when percutaneously injected into arthritic knees.
*Level of evidence:* Therapeutic case–control study; Level III.
*Methods:* Twenty five stem cell injections combined with arthroscopic debridement were administered to patients with knee OA. A mean of $1.89 \times 10^6$ stem cells were prepared with approximately 3.0 mL of platelet-rich plasma (PRP) and injected in the selected knees of patients in the study group.
*Results:* The mean Lysholm, Tegner activity scale, and VAS scores of patients in the study group improved significantly by the last follow-up visit. No major adverse events related to the injections were observed during the treatment and follow-up periods. The results were compared between the study and control groups, in which the patients had undergone arthroscopic debridement and PRP injection without stem cells. Although the preoperative mean Lysholm, Tegner activity scale, and VAS scores of the study group were significantly poorer than those of the control group, the clinical results at the last follow-up visit were similar and not significantly different between the two groups.
*Conclusions:* The short-term results of our study are encouraging and demonstrate that infrapatellar fat pad-derived MSC therapy with intraarticular injections is safe, and provides assistance in reducing pain and improving function in patients with knee OA.


## 1. Introduction

Osteoarthritis (OA) is a cartilage degenerative process involving the immune system, wherein local inflammatory reactions occur with the production of proinflammatory cytokines. Currently, no treatment is available to improve or reverse the process. OA of the knee joint has a particularly significant impact on the affected individual's ability to perform activities of daily living, and combined with the high cost of its management, it poses a major social issue, especially in populations with a long life expectancy [1]. Current treatment options for articular injury and OA itself aim to relieve inflammation and pain, but they do little to delay disease progression [2]. Various surgical methods have been proposed to regenerate articular cartilage, but they all are associated with complications, leaving many patients with inadequately treated cartilage lesions. When left untreated, cartilage lesions can progress to more extensive defects and, ultimately, they may require joint replacement surgery, subject to failure of conservative options. This consequence is the driving force behind numerous ongoing efforts to develop new tissue engineering-based strategies for the treatment of OA [3].

Because of their multilineage potential, immunosuppressive activities, limited immunogenicity, and relative ease of growth in culture, mesenchymal stem cells (MSCs) have attracted attention for clinical use. Although ethical and political issues surround the use of embryonic stem cells, the use of MSCs generally is well accepted by society. Furthermore, MSCs are an autologous source of cells, eliminating concerns regarding rejection and disease transmission, and they are less tumorigenic than their embryonic counterparts [4]. Therefore, MSCs have been suggested for use in the cell-based treatment of cartilage lesions.

In this study, we present the preliminary results (at a minimum of 12 months of follow up) of 25 cases of knee OA treated with intraarticular injections of autologous MSCs. Autologous MSCs were separated from the infrapatellar fat pad of OA patients, isolated in vitro, and then injected into the patients' knee joints. The aim of the study was to determine whether isolated MSCs derived from the infrapatellar fat pad are safe and can effectively improve clinical results when percutaneously injected into arthritic knees.

## 2. Patients and methods

### 2.1. Patients

Between January 2010 and September 2010, 25 stem cell injections combined with arthroscopic debridement were administered

* Corresponding author at: Department of Orthopaedic Surgery, Yonsei Sarang Hospital, 478-3 Bangbae-dong, Seocho-gu, Seoul, South Korea. Tel.: +82 2 2023 5592; fax: +82 2 2023 5598.
*E-mail address:* yunjinchoi78@gmail.com (Y.-J. Choi).

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to patients with knee OA (Table 1). The study group comprised 8 men and 17 women, with an average age of 54.1 (range, 34–69) years. Eligible patients were aged ≥30 years with idiopathic or secondary knee OA. The mean follow-up period was 16.4 months (range, 12–18) months.

The exclusion criteria were inflammatory or postinfectious arthritis, previous arthroscopic treatment for knee OA, varus or valgus deformity of 5° or more, previous major knee trauma, Kellgren–Lawrence grade 4 OA as defined by the modified Kellgren–Lawrence classification [5] in 2 compartments (the medial or lateral compartments of the tibiofemoral joint or the patellofemoral compartment), persons over 70 years of age, intraarticular corticosteroid injection in the preceding 3 months, a major neurologic deficit, serious medical illness (life expectancy of <2 years or a high intraoperative risk), and pregnancy. Patients were also excluded if they had large meniscal tears ("bucket handle" tears), were unable to provide informed consent, or were deemed unlikely to comply with follow up. All the patients provided written informed consent according to regulations, after approval of the ethics committee, and they were operated by the same surgeon (the first author).

### 2.2. Arthroscopic procedure and clinical assessment

The patients received arthroscopic treatment under spinal anesthesia, with the use of a tourniquet. The orthopedic surgeon evaluated the medial, lateral, and patellofemoral joint compartments, graded articular lesions according to the International Cartilage Repair Society (ICRS) Cartilage Injury Evaluation Package, irrigated the compartment with at least 1 L of saline, and performed one or more of the following treatments: synovectomy; debridement; or excision of degenerative tears of the menisci, fragments of articular cartilage, chondral flaps, or osteophytes that prevented full extension. Abrasion or microfracture of chondral defects was not performed.

Clinical assessment was performed retrospectively using the arthroscopic surgery database, medical records, and telephone interviews. The clinical outcome was evaluated using the Lysholm score [6], Tegner activity scale [7], and visual analog scale (VAS) for grading knee pain. On the 10-mm VAS, scores (0–10) for pain (0 = no pain; 10 = worst possible pain) [8] were recorded. Patients were evaluated preoperatively, 3 months postoperatively, and at the last follow-up visit (average, 16.4 months; range, 12–18 months). Radiographic evaluation included the standing weight-bearing anteroposterior view, lateral view, skyline view, and full-length anteroposterior view.

### 2.3. Sample collection and MSC isolation

For 1 week before the infrapatellar fat pad harvesting procedure, the patients were restricted from consuming corticosteroids or nonsteroidal anti-inflammatory drugs. After arthroscopic surgery, we collected the fat pad immediately, followed by arthroscopic surgery. The adipose synovium was harvested from the inner side of the infrapatellar fat pad by extension of the skin incision at the arthroscopic lateral portal site (Fig. 1). Then, the infrapatellar fat pad was collected (average weight, 9.4 g; range, 6.9–11.2 g). The MSCs derived from the infrapatellar fat pad were isolated as described previously [9,10]. Briefly, the pad was minced and washed extensively with phosphate-buffered saline and an equal volume of 0.1% collagenase type 1 (Worthington Biochemical Corporation, Lakewood, NJ). The tissue was placed in a rotary incubator at 37 °C, with continuous agitation for 3 h. After digestion, the lipoaspirates were centrifuged at 1200 × *g* for 10 min to separate the lipoaspirate and the collagenase. The lipoaspirates were then washed 3 times to remove any remaining collagenase. After the last round of centrifugation, cells in the aspirates were counted using a hemocytometer. Before injection, bacteriologic tests were performed on the samples (to ensure the absence of contamination), and the viability of the cells was assessed using the methylene blue dye exclusion test.

**Table 1**
Overview of the different patient groups.

| | Study group | Control group | *P* value[a] |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | (95% CI) |
| Age | 54.2 ± 9.3 | 54.4 ± 11.3 | 0.67 (−7.1–4.65) |
| Follow-up (M) | 16.4 ± 2.3 | 17.2 ± 1.8 | 0.23 (−1.9–0.46) |
| ICRS cartilage (Grade) | 3.7 ± 0.4 | 2.8 ± 0.8 | <0.001 (0.19–1.08) |
| Kellgre–Lawrence (Grade) | 3.3 ± 0.8 | 2.7 ± 0.7 | 0.005 (0.63–1.37) |
| Sex M/F | 8/17 | 8/17 | |

CI = confidence interval.
[a] The independent *t*-test.

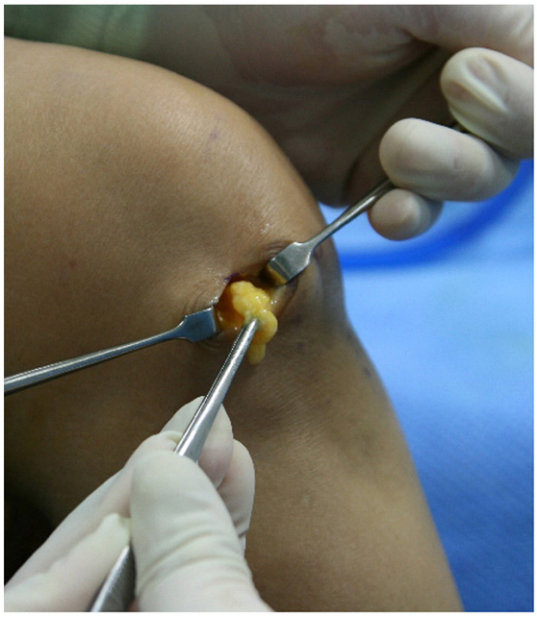

**Fig. 1.** Adipose synovium was harvested from the inner side of the infrapatellar fat pad by skin incision extension of the arthroscopic lateral portal site.

### 2.4. Injection of MSCs

Because the preparation of stem cells takes 3 or 4 h, the first injection time of the stem cells was the same day as the arthroscopic operation. After the stem cells were isolated, a mean of $1.89 \times 10^6$ (range, $1.2–2.3 \times 10^6$) stem cells were prepared with approximately 3.0 mL of platelet-rich plasma (PRP) and injected in the selected knees of patients in the study group. The skin was dressed under aseptic conditions, and the injection was performed through a classic lateral approach of the upper pole of the patella using a 22-g needle. Before injection, the knee first was aspirated for hemarthrosis, and no steroid was injected in the knee joint. All injections were done in an outpatient setting. At the end of the procedure, the patient was invited to bend and extend the knee a few times, in order to allow the stem cells with PRP distribute throughout the joint before becoming a gel. After the injection, the patients were sent home to use cold therapy/ice on the affected area for pain.

During the treatment period, we did not restrict walking, and rest or mild activities (such as exercise biking, mild exercises in a pool) were indicated. Subsequently, the gradual resumption of normal sport or recreational activities was allowed, as tolerated. No analgesics, anti-inflammatory drugs, or immunosuppressive drugs were administered or allowed after the procedure. After the first injection with stem cells and PRP, 3 mL of PRP was administered every 7 days as the second and third rounds of treatment.

### 2.5. PRP preparation

For PRP preparation, a 60-mL venous blood sample (collected in a bag containing 4 mL of sodium citrate) was collected for every lesion treated. The complete peripheral blood count was determined using the first blood sample collected. Then, the samples were centrifuged twice (at 1800 rpm for 15 min to separate the erythrocytes, and then at 3500 rpm for 10 min to concentrate the platelets) to yield 6 mL of PRP. The PRP was divided into 2 units of 3 mL each. One unit was sent to the laboratory for analysis of platelet concentration and quality testing (bacteriologic tests), while the other was used for the first injection within 2 h of preparation.

The total number of platelets per microliter 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. For the second and third rounds of treatment, PRP injections were administered every 7 days. Before all injections, 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.

### 2.6. Control group treatment

For comparison of the clinical results, a control group that matched the study group in terms of patient age and sex and follow-up period was selected from over 500 patients who also had undergone arthroscopic debridement accompanied by postoperative PRP injections between January and September 2010. The selection process was aided by computerized randomization, and the matched-group analysis was performed retrospectively. The group comprised eight men and 17 women, with an average age of 54.4 (range, 36–69) years. On the operative day, PRP was prepared at a mean volume of 3.0 mL and injected without stem cells into the selected knees of the control patients. Then, similar to the study group, the control group was administered PRP without stem cells at 1-week intervals as the second and third rounds of treatment. The other factors (arthroscopic procedure and postoperative rehabilitation) were the same as those for the study patients.

### 2.7. Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Studies (SPSS) software, version 12.0, for Windows (IBM Corporation, Armonk, NY). The clinical scores were given as the mean (SD) at three time points: preoperatively, 3 months postoperatively, and at the last postoperative follow-up visit. We checked the normality of distribution by using the Shapiro–Wilk test. This research followed normal distribution because the probability of the Shapiro–Wilk test was $P>0.05$ and the number of patients was 25 in each group. The paired $t$-test was used for the within group analysis (pre-op. vs post-op in the same group) and the independent $t$-test was used for between group analysis (study group vs control group). The level of significance was $P<0.05$.

## 3. Results

No major adverse events related to the injections were observed during the treatment and follow-up periods, except for 1 case, in which the patient experienced marked pain with swelling after the injection, which resolved spontaneously after 2 weeks. In some cases, slight pain was experienced in the first 2 or 3 days after the injection. A statistically significant improvement from the baseline was noted for all the clinical scores at both the 3-month follow-up visit and the last follow-up visit. No patient was lost to follow up; however, 4 patients were not available for examination in the outpatient clinic, but they were contacted by telephone, and they answered the questionnaire for clinical score.

The mean Lysholm, Tegner activity scale, and VAS scores of patients in the study group improved significantly ($P<0.001$) by the last follow-up visit (Table 2). After the operation, 23 patients (92%) showed an improved Lysholm score, 1 patient's (4%) score did not change, and 1 patient's (4%) score worsened. The Tegner activity score postoperatively improved for 19 patients (76%), remained unchanged for 5 patients (20%), and worsened for 1 patient (4%). The VAS was used to assess patients' pain both pre- and postoperatively. After the operation, 21 patients (84%) reported pain reduction, 1 patient (4%) reported no change, and 3 patients (12%) reported an increase in pain.

**Table 2**
Clinical results of the different patient groups.

| | Study group | Control group | $P$ value[a] |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | (95% CI) |
| Lysholm score | | | |
| Preop | 41.2 ± 12.4 | 50.0 ± 11.1 | 0.01 (−15.50–−2.10) |
| Last F/U | 68.1 ± 18.5 | 69.4 ± 20.4 | 0.81 (−12.40–9.76) |
| Tegner activity scale | | | |
| Preop | 1.5 ± 0.5 | 2.1 ± 0.8 | 0.003 (−0.99–−0.21) |
| Last F/U | 2.8 ± 1.2 | 2.9 ± 1.0 | 0.71 (−0.75–0.51) |
| VAS | | | |
| Preop | 4.9 ± 1.2 | 3.9 ± 1.0 | 0.001 (0.42–1.66) |
| Last F/U | 2.7 ± 1.8 | 2.2 ± 1.7 | 0.34 (−0.52–1.48) |

CI = confidence interval.
[a] The independent $t$-test.

To establish the indications for our treatment, we determined the parameters that influenced the clinical outcome. We found that an increased VAS score and a decreased Tegner activity scale score in older patients (>55 years) at the last follow-up visit (VAS, $P=0.007$; Tegner activity scale, $P=0.049$). This implies that MSC therapy was more effective in younger patients. Furthermore, we found that patients with OA of ICRS grade 3 on the VAS showed greater improvement than those with OA of ICRS grade 4 ($P=0.024$).

To analyze the outcome of our stem cell therapy, the results were compared between the study and control groups, in which the patients had undergone arthroscopic debridement and PRP injection without stem cells. In the control group, the mean Lysholm, Tegner activity scale, and VAS scores improved significantly ($P<0.001$) by the last follow-up visit. Although the preoperative mean Lysholm, Tegner activity scale, and VAS scores of the study group were significantly poorer than those of the control group ($P<0.001$), the clinical results at the last follow-up visit were similar and not significantly different between the 2 groups (Lysholm score, $P=0.812$; Tegner activity scale, $P=0.706$; VAS, $P=0.338$) (Figs. 2–4). However, the degree of improvement was superior in the study group, which had received stem cell injections. Although the scores of the study group tended to improve to a great degree by the last follow-up visit, the difference between the study and control groups was not significant (Lysholm score, $P=0.169$; Tegner activity scale, $P=0.133$; VAS, $P=0.261$), 95% confidence interval (Lysholm score, −3.3–18.3; Tegner activity scale, −0.15–1.11; VAS, −1.55–0.43). The average Lysholm score increased 26.9 points by the last follow-up visit in the study group, whereas it increased only 19.4 points in the control group (Fig. 5). The average Tegner activity scale score increased 1.3 points by the last follow-up visit in the study group, but it increased only 0.8 points in the control group. Finally, the average VAS score decreased 2.2 points by the last follow-up visit in the study group, while it decreased 1.7 points in the control group.

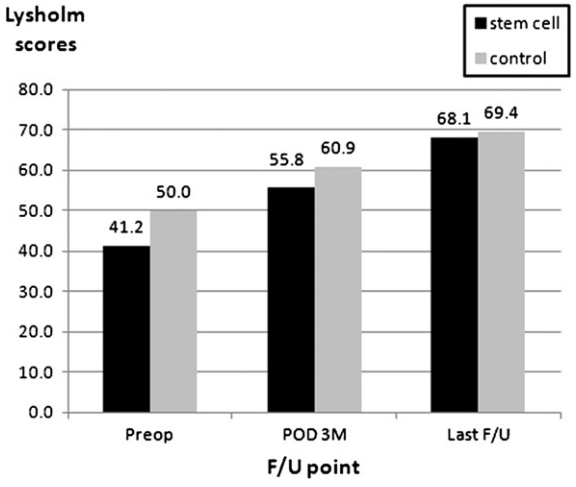


**Fig. 2.** Bar graph showing the Lysholm scores preoperatively, at the 3-month follow-up visit, and at the last follow-up visit. Study group = stem cells + PRP injection; control group = PRP injection.

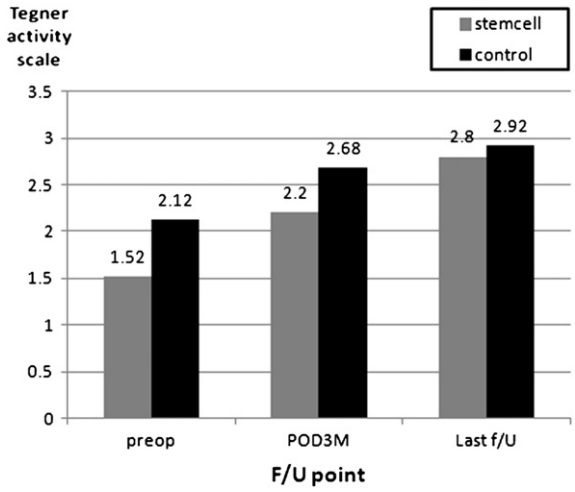


**Fig. 3.** Bar graph showing the Tegner activity scale preoperatively, at the 3-month follow-up visit, and at the last follow-up visit. Study group = stem cells + PRP injection; control group = PRP injection.

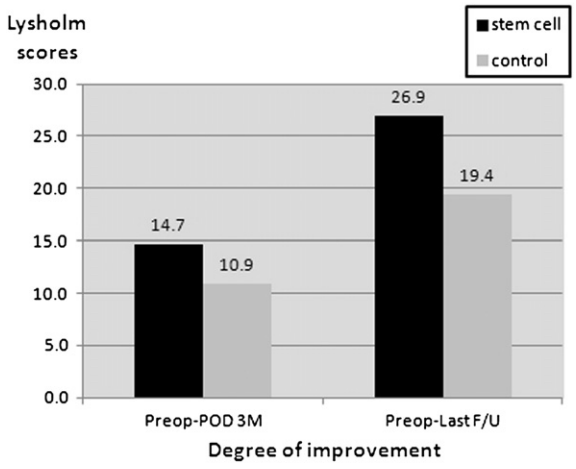


**Fig. 5.** Bar graph showing the degree of improvement, according to Lysholm score, preoperatively to the 3-month follow-up visit, and preoperatively to the last follow-up visit. Study group = stem cells + PRP injection; control group = PRP injection.

## 4. Discussion

The first aim of this study using MSCs was to evaluate the safety of our technique. No complications such as infection, marked muscle atrophy, fever, hematoma, tissue hypertrophy, adhesion formation, or other major adverse events occurred among the study subjects. The secondary aim was to analyze the effectiveness and application modalities for use in further studies: we found that the MSC therapy provides assistance in reducing pain and improving function in patients with knee OA.

Cartilage defects have a very limited intrinsic healing capacity. Small defects can spontaneously undergo repair with the production of hyaline cartilage, but large defects undergo repair only with the production of fibrous tissue or fibrocartilage, which are biochemically and biomechanically different from normal hyaline cartilage. Therefore, degeneration occurs subsequently and can progress to osteoarthritic changes in some cases [11].

Recently, MSCs have been suggested for use in the cell-based treatment of cartilage lesions. Chondrogenesis of MSCs was first reported by Ashton and colleagues [12], and a defined medium for the in vitro chondrogenesis of MSCs was first described by Johnstone and colleagues [13], who used micromass culture with transforming growth factor-beta (TGF-β) and dexamethasone. With regard to in vivo studies, the transplantation of MSCs into full thickness articular cartilage defects has been attempted under various conditions. Although many studies have been successful, several questions still persist that limit the clinical application of these cells for cartilage injury, such as from which tissue are suitable MSCs derived or what conditions are appropriate for cartilage repair.

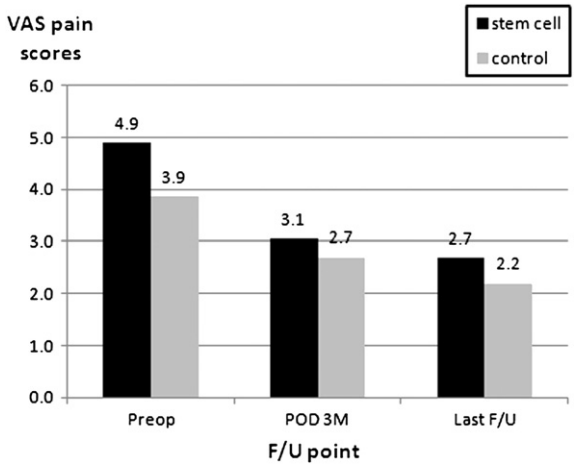


**Fig. 4.** Bar graph showing the visual analog scale pain scores preoperatively, at the 3-month follow-up visit, and at the last follow-up visit. Study group = stem cells + PRP injection; control group = PRP injection.

Currently, very few clinical studies on MSC transplantation for cartilage repair have been reported, though animal experiments on MSC use in the prevention and treatment of experimental OA have showed encouraging results [14,15]. In 2 reports, experiments on humans [16,17] involving the intraarticular injection of autologous MSCs yielded good results after 6 months. In 2008, Centeno and colleagues reported the use of autologous culture-expanded bone marrow-derived stem cells for knee cartilage regeneration in humans [17]. In their study, the patients' pain, as determined by the VAS, and range of motion improved, and MRI showed significant articular cartilage growth and meniscus regeneration. Currently, only one prospective clinical study on MSC transplantation for cartilage repair has been published; in this study, bone marrow-derived MSCs were resuspended in a collagen type I gel and transplanted with an autologous periosteal flap [18]. Patients with knee OA who had undergone high-tibial osteotomy were treated using a cell-containing scaffold with the periosteal flap transplanted into a cartilage defect in the medial femoral condyle, and their outcomes were compared with those of patients in whom a cell-free scaffold with the periosteal flap was transplanted into similar lesions. Although the cell-treated group showed no significant clinical improvements compared with the control group, the arthroscopic and histologic scores were better in the MSC-transplanted group.

As mentioned above, previous reports were almost entire case reports on a few patients, while our study is a study including many patients. Although this is a retrospective study, the results prove that stem cell therapy is safe, and provides assistance in the treatment of knee OA. Although the preoperative status of the study group was poorer than that of the control group, the clinical results at the last follow-up visit were similar. In addition, the degree of improvement from the preoperative status was greater in the study group than in the control group.

Although our technique is primitive, we tried to select a technique that was substantially better than those reported previously on stem cell therapy for knee OA. To obtain good results, the source of the MSCs is very important. The choice of the stem cell source is determined by the ease of harvesting, population density, and differentiation potential of the cells, as their abilities vary among different

tissue sources [19]. Bone marrow- and synovium-derived MSCs have shown good results [19], and we intend to concentrate on these two sources. Bone marrow-derived stem cells have been widely studied, and there is a wealth of information in the literature concerning them [20]. To date, only limited reports have been published on human autologous bone marrow stromal cell implantation for cartilage repair [21,22]. Unfortunately, bone marrow harvesting is painful and is associated with donor site morbidities and risks of wound infection and sepsis [23]. Furthermore, with increasing age, there is a decrease in the MSC numbers [24], lifespan, and proliferation [25] and differentiation potentials [26]. Therefore, an alternative cell source that is easy to obtain, has a low risk of complications, and has a high yield of cells with good proliferation and differentiation potentials that do not decline with age is ideal for enabling optimal cell-based tissue repair therapies in an aging population.

In this respect, MSCs extracted from the infrapatellar fat pad have been induced to exhibit the chondrogenic, adipogenic, and osteogenic phenotypes by using appropriate media [27]. These cells have been shown to maintain their differentiation potential even in the later stages of life [28], and they may have better chondrogenic potential compared to the bone marrow-derived MSCs [19]. In addition, compared with the bone marrow, the infrapatellar fat pad is reported to give a higher yield of adherent colony-forming cells: A 30-mL bone marrow aspirate afforded approximately $1\times10^5$ cells [29], whereas 21 mL of infrapatellar fat pad yielded approximately $5.5\times10^6$ cells [27]. Obtaining a large number of cells at harvest has the advantage of reducing the need for costly and time-consuming tissue culture expansion, which is also associated with the risk of contamination. Moreover, the pain and morbidity associated with the harvesting of infrapatellar fat pad cells are considerably less than that associated with bone marrow cell harvesting [27].

Although we collected an average of just 9.4 g of infrapatellar fat pad in the present study, we could extract an average of $1.89\times10^6$ stem cells. Sekiya compared the MSCs derived from bone marrow, synovium, periosteum, adipose tissue, and muscle and showed that the synovium was the best source of MSCs for use in cartilage regeneration: synovium-derived MSCs had a greater proliferative capacity and chondrogenic potential [19]. An important consideration in tissue engineering is harvesting the greatest number of MSCs with the highest potential. In this regard, the adipose synovium cells have an advantage because of their high chondrogenic potential and accessibility; sufficient amounts of adipose synovium can be harvested with possibly fewer complications. Thus, we chose the infrapatellar fat pad as a source of MSCs for use in cartilage defect treatment. In addition, the infrapatellar fat pad frequently is resected during arthroscopy or total knee arthroplasty for improved surgical visualization and for the treatment of chronic impingement and fiduring of the fat pad (Hoffa's disease) [30]. No long-term adverse effects have been noted following its resection [31]. Even in our study, no adverse effects of infrapatellar fat pad harvesting were noted.

In the present study, we administered injections of patients' stem cells prepared with PRP because PRP is a novel biological scaffold that has been widely used as an MSC carrier for clinical chondrogenesis. PRP is nonimmunogenic, bioabsorbable, and can be easily prepared preoperatively. According to Frechette and colleagues, the platelet augmentation approach is based on the concept that platelets contain key growth factors such as platelet-derived growth factors, TGFs, and various interleukins [32]. They hypothesize that the released growth factors have chemotactic and mitogenic effects on MSCs and osteoblasts when applied to bony tissues [33]. In fact, recent research has reported that treatment with PRP injections is safe and has the potential to reduce pain and improve knee function and quality of life in patients with degenerative osteoarthritic knees [34]. Because the average baseline blood platelet count in an individual is $200{,}000\pm75{,}000/\mu L$, a platelet count of 1,000,000/μL (5-fold greater than the average) commonly is described for therapeutic platelet-rich preparations [35]. In our study, we administered an average of 1,280,000/μL in the patients' knees at every injection.

In this study, we did not culture stem cells but isolated them from the infrapatellar fat pad, and then injected into patients' knees. The number of MSCs that can be isolated from the infrapatellar fat pad is fairly limited. Therefore, most research on cartilage regeneration has focused on the use of culture-expanded cells [36–39]. Various elements of the local microenvironment during culture can affect MSC differentiation [40–44], and culture expansion carries some risk of infection or changes in MSC properties, however; thus, we just isolated stem cells from the infrapatellar fat pad and injected them into the patients' knees. Although the technique of this study was primitive, we obtained good results in the study group at a minimum follow-up period of 1 year, probably because of the paracrine effects of the injected stem cells. It is widely known that stem cell therapy has two main mechanisms of action. The first is that these cells comprise the final tissue in human organs. The second mechanism, the most convincingly proven so far, is the paracrine effects of the cytokines and growth factors released by the grafted cells, which favorably influence the microenvironment by triggering host-associated signaling pathways [45] and lead to increased angiogenesis, decreased apoptosis, and possibly, induction of endogenous generation.

The primary objective of our study was to evaluate the safety of our technique. No complications such as infection, marked muscle atrophy, fever, hematoma, tissue hypertrophy, adhesion formation, or other major adverse events occurred among the study subjects. Only minor adverse events were detected, such as a mild pain reaction and effusion after the injections, which persisted for not more than 2 days. The secondary aim was to analyze the indication criteria and application modalities for use in further studies. In our study, better results were achieved in younger patients, which was expected and easily explained by the high percentage of living and vital cells in the knee joint of younger patients. Therefore, a high response potential to the paracrine effects was expected. At the last follow-up visit, the results were poorer in patients with a higher cartilage grade. Thus, good results are obtained with stem cell therapy of knee OA in young patients and those with early cartilage degeneration.

The present study does have some limitations. The first problem with our stem cell therapy is that the number of cells to be injected to achieve the optimal response is unknown. Second, it is unknown whether a single injection is adequate or $>1$ injection within a time period is necessary to obtain the desired result. Third, we need more experience on a large scale to determine the proper use of costimulators. The other important limitations of our study are that we do not have data on the effects of pure stem cell injections, and it is difficult to distinguish the effects of the stem cells from those of the PRP. Lastly, the number of subjects was small, the follow-up period was short, the data were collected retrospectively, and neither a routine second-look arthroscopy nor an MRI examination was performed.

In the future, tissue-engineering techniques hold promise for repairing damaged cartilage within joints. Several challenges still need to be overcome, however, which include identifying the optimal source of stem cells, scaffolds, and growth factors. Nonetheless, this study proposes a new option for the treatment of knee OA. The positive clinical outcomes obtained support further randomized controlled clinical trials of this treatment modality with a large number of patients and a long follow-up period.

## 5. Conclusions

The short-term results of our study are encouraging and demonstrate that infrapatellar fat pad-derived MSC therapy with intraarticular injections is safe, and provides assistance in reducing pain and improving function in patients with knee OA. However, before MSC

therapy can be widely adopted as a new method for the treatment of knee OA, the techniques involved should be improved.

## Conflict of interest

None.

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STANDARDS, PROTOCOLS, POLICIES, AND REGULATIONS FOR CELL-BASED THERAPIES

# Concise Review: A Safety Assessment of Adipose-Derived Cell Therapy in Clinical Trials: A Systematic Review of Reported Adverse Events

Authored by a member of

IFATS
International Federation for Adipose Therapeutics and Science

NAVID MOHAMADPOUR TOYSERKANI,[a,b,c] MADS GUSTAF JØRGENSEN,[a,b,c] SIAVOSH TABATABAEIFAR,[a,c] CHARLOTTE HARKEN JENSEN,[b,d] SØREN PALUDAN SHEIKH,[b,d,e] JENS AHM SØRENSEN[a,b,c]

**Key Words.** Adipose-derived stromal cells • Stromal vascular fraction • Safety • Adverse events • Complications

[a]Department of Plastic Surgery, Odense University Hospital, Odense, Denmark; [c]Clinical Institute, [e]Institute of Molecular Medicine, University of Southern Denmark, Odense C, Denmark; [d]Laboratory of Molecular and Cellular Cardiology, Department of Clinical Biochemistry and Pharmacology, [b]The Danish Centre for Regenerative Medicine Odense University Hospital, Denmark

Correspondence: Navid Mohamadpour Toyserkani, M.D., Department of Plastic Surgery, Odense University Hospital, Sdr. Boulevard 29, Odense C, Denmark. Telephone: +45 6541 4679;
e-mail: Navid.m.toyserkani@rsyd.dk

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## ABSTRACT

**The popularity of adipose-derived cell therapy has increased over the last decade, and the number of studies published annually is growing. However, concerns regarding safety in the setting of previous malignancy or the use of allogeneic cells have been raised. We therefore aimed to systematically review all clinical studies using adipose-derived cell therapy to identify reported adverse events with a special focus on risk of thromboembolic, immunological, and oncological safety concerns. Our systematic search resulted in 70 included studies involving more than 1,400 patients that were treated with adipose-derived cell therapy. Safety assessment method was not described in 32 of the included studies. For studies involving systemic or cardiac administration, one case of pulmonary thromboembolism and cases of both myocardial and cerebral infarctions were described. In the setting of allogeneic cell therapy studies, where the production of specific antibodies toward donor cells was examined, it was noted that 19%–34% of patients develop antibodies, but the consequence of this is unknown. With regard to oncological safety, only one case of breast cancer recurrence was identified out of 121 patients. Adipose-derived cell therapy has so far shown a favorable safety profile, but safety assessment description has, in general, been of poor quality, and only adverse events that are looked for will be found. We encourage future studies to maintain a strong focus on the safety profile of cell therapy, so its safeness can be confirmed.**

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## SIGNIFICANCE STATEMENT

This study reviewed the safety of adipose-derived cell therapy. Thromboembolic complications were noted following systemic administration of cells. The treatment has so far shown to be safe in the setting of previous cancer. Donor-specific antibodies are produced when using allogeneic cells, documenting that these cells are not immune privileged. The consequences of this needs further research. Future research should focus on higher quality of reporting of adverse events, as the present literature is of low quality.

## INTRODUCTION

The field of regenerative medicine has been rapidly expanding over the last decade and especially cells derived from adipose tissue have received a lot of attention due to the ease of harvest and obtainable number of cells [1, 2]. The cells from adipose tissue can be used for therapeutic purposes, either freshly isolated as the stromal vascular fraction (SVF, also called adipose-derived regenerative cells [ADRC]), or culture-expanded as the adipose-derived stem cells (ASC). Adipose-derived cell therapy has shown potential in almost every preclinical animal model [3–7] and the time is ripe for clinical translation of this potential.

The first results published from a clinical trial using adipose-derived cell therapy, published in 2005, were for the treatment of Crohn's fistula [8]. Since then, a steady increase of publications and in treated conditions has occurred. However, as of yet there is still no clear evidence for the implementation of adipose-derived cell therapy in the daily clinical routine.

The mechanisms of action of adipose-derived cell therapy have been hypothesized to be through different pathways, such as paracrine secretion of growth factors, cytokines and microRNA promoting angiogenesis and modulating the immune response, as well as the ability of cells to differentiate into a variety of different cell types. However, very rarely can a beneficial effect be expected without the risk of adverse events. Consequently, safety concerns have been raised

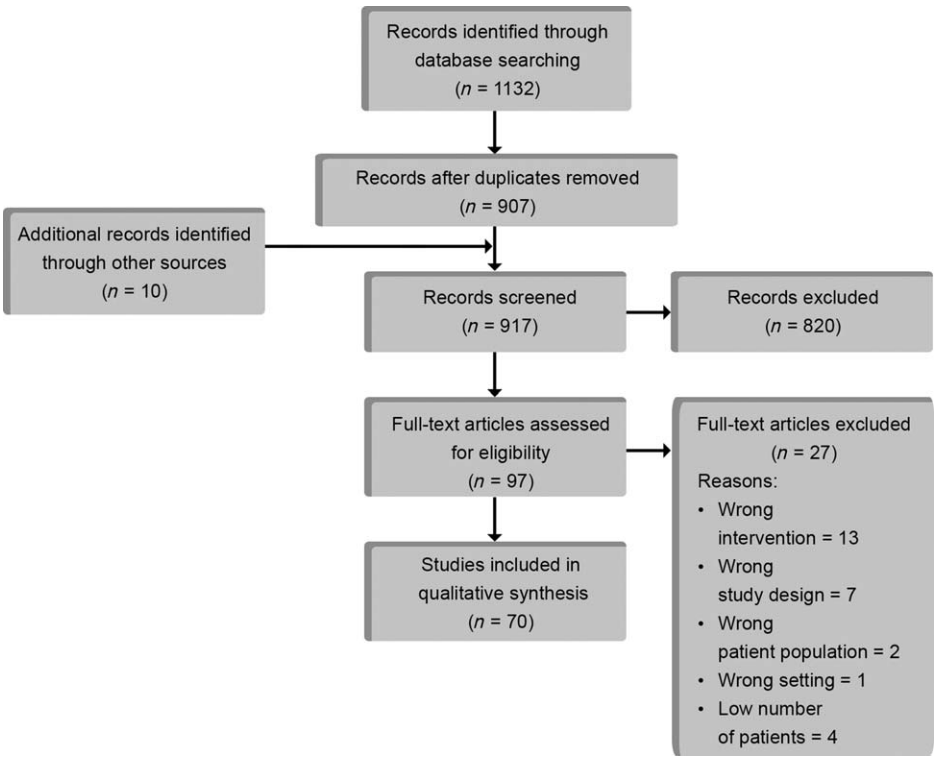


**Figure 1.** Flow chart of article selection process.

regarding the use of systemically administered cell therapy due to risk of thromboembolic complications [9, 10], the use of allogeneic cells and possible rejection [11], and in the setting of previous cancer therapy [12–14].

The aim of this systematic review was therefore to collect and review all reported adverse events related to adipose-derived cell therapy with a focus on thromboembolic, immunological, and oncological safety concerns.

## Materials and Methods

This systematic review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [15]. A systematic search was performed on PubMed using the following search string: "([adipose stem cell] OR [adipose stromal cell] or [adipose regenerative cell] or [stromal vascular fraction] or [processed lipoaspirate]) AND (trial or trials or pilot or 'feasibility study' or 'safety study')." A similar search was performed on EMBASE. All search results were imported to Covidence for further evaluation [16]. Study selection was performed by two independent assessors (NMT and & MGJ). First, all studies were screened based on title and abstract. Secondly, full text versions of included studies were read for further evaluation. A hand search was also performed by skimming the references of included studies.

Inclusion criteria were human studies using adipose-derived cells for treatment of any given disease published no later than 31st of December 2016. Exclusion criteria were non-English language, reviews, case reports, or case series with fewer than five patients and animal or in vitro studies.

Data retrieved from included studies were year of publication, country of origin, disease treated, study design (randomized controlled trial, nonrandomized study, or case series/pilot study), primary aim (safety or efficacy), cell type used (freshly isolated or culture-expanded as well as autologous or allogeneic), cell dosage, cell characterization (cell count/viability, surface marker analysis, and fibroblastoid colony forming units assay [CFU-F]), number of participants, safety reporting described clearly in the Methods section (yes/no), and the reported adverse events including all-cause mortality. The primary aim was set to safety if this was explicitly stated or was mentioned with equal weight as efficacy. The safety reporting was set to "yes" if anything pertaining to the evaluation of adverse events was noted in the Methods section, and it was set to "no" if nothing at all was described.

All studies with a comparison group were also evaluated for risk of bias using the Cochrane Collaboration tool where safety was set as outcome measure [17]. In brief, seven aspects are evaluated for risk of bias and given an evaluation of either low risk, unclear, or high risk of bias. The seven aspects are random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other areas of bias.

## Results

### Description of Included Studies

In total, 907 unique studies were identified using our search string. In addition, 10 studies were found through hand search. After screening title and abstract, 97 studies remained. Full text evaluation excluded a further 27 studies leaving 70 studies to be included in the review with a total of 1,474 patients treated with adipose-derived cell therapy (Fig. 1). Almost all organ system have been implicated in adipose-derived cell therapy. The indication for treatment in the included studies was (in order count) soft tissue

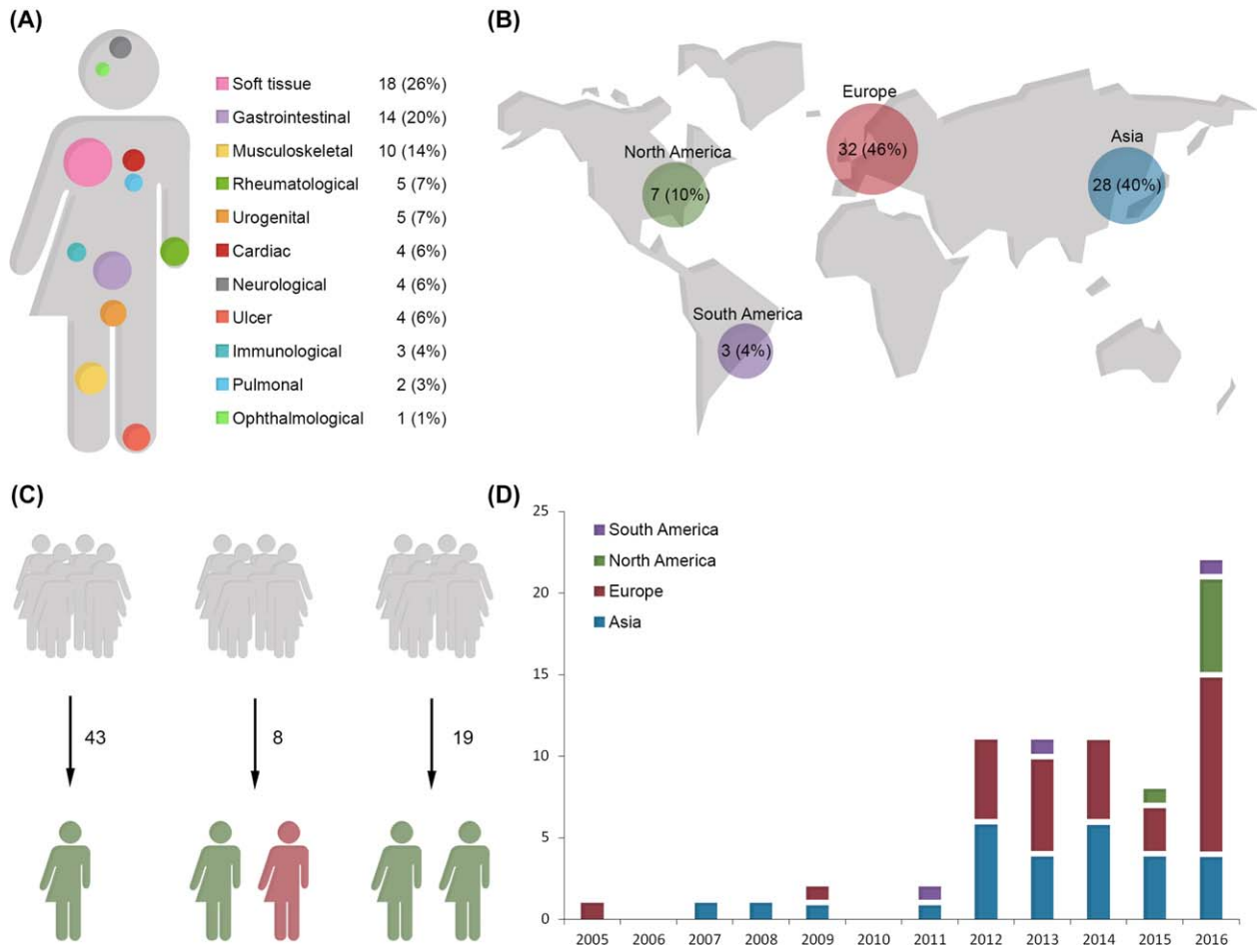


**Figure 2.** An overview of included studies. **(A):** Graphical overview of the range of indications adipose-derived cell therapy has been used for. **(B):** Graphical overview of research activity bias on geographic location showing that the majority of included studies are from either Europe or Asia. **(C):** The majority of studies were of case series quality with no control group for comparison. Eight of studies included a non-random control group and 19 studies were randomized studies. **(D):** Since the first publication in 2005 there has been a steady rise in research output of clinical studies using adipose-derived cell therapy.

[18–35], gastrointestinal [8, 36–48], musculoskeletal [49–58], rheumatological [59–63], ulcer/ischemic limb [64–67], urogenital [68–72], cardiac [73–76], neurological [77–80], immunological [81–83], pulmonary [84, 85], and ophthalmological [86] (Fig. 2A). Studies were performed worldwide but Europe and Asia have so far published the majority of studies using adipose-derived cell therapy (Fig. 2B).

The majority of studies [43] were small in scale and conducted as a series of cases without a comparison group. There were eight studies comparing adipose-derived cell therapy with a nonrandom control group, and 19 studies were conducted as randomized controlled trials (Fig. 2C). The first clinical study using adipose-derived cell therapy was published in 2005. Since then, a steady rise in publications has followed with an explosive growth annually since 2012 (Fig. 2D).

## Overview

In the included studies ADRC treatment was given in 36 studies, whereas ASC was used in the 32 studies (two studies included both ADRC and ASC). The most frequent method to characterize cells was cell count with viability estimation. Cell dosage was not specified in fourteen studies in which the indication was soft tissue reconstruction [20, 22, 23, 25, 26, 28, 29, 31, 32], gastrointestinal [47], musculoskeletal [49], neurological [80], or urogenital disease [71, 72]. In addition, 38 studies performed surface marker analysis of cells or at least had set release criteria for certain surface markers in studies using ASCs. CFU-F was performed in only ten studies.

Almost any cell administration pathway was represented in the included studies. Remarkably, a total of 32 of 70 studies did not clearly describe any form of adverse event evaluation in the Methods section, including four studies in which safety was designated as the primary outcome [8, 50, 52, 73] (Supporting Information Table S1). The severity of adverse events depended on the underlying condition being treated, but no studies identified any adverse event as being related to the adipose-derived cell therapy.

## Thromboembolic Safety and Mortality

For studies administering cells systemically or intramyocardially, possible thromboembolic complications and all-cause mortality were evaluated. Three RCT studies and one case series administered cells for cardiac indications with various methods.

Two double-blinded RCT trials that were published together administered autologous ADRC intramyocardially with the difference between the two studies being cell dosage (40 or 80 $\times$ $10^6$ cells vs. placebo) [76]. Enrollment was terminated prematurely due to adverse events. In total, 31 patients were included and 17 received ADRC. Two patients treated with ADRC experienced cardiac death on day 2 and 291 after administration, and one experienced myocardial infarction at an unknown time point. In addition, a cerebrovascular event occurred in two patients in the ADRC group and one in the placebo group within 24 hours of injection. All neurological symptoms had complete or near-complete recovery. The RCT study by Houtgraaf et al. administered ADRC intracoronarily for myocardial infarction with no mention of specific thromboembolic complications or deaths [74].

In an RCT study by Perin et al., who administered ADRC transendocardially, one out of 21 patients developed myocardial infarction immediately following injection and died [75]. One patient out of six died in the control group. In a case series by Comella et al., which used a similar administration method for ADRC in 28 patients with chronic ischemic cardiomyopathy, three patients died after 1, 7, and 12 months [73].

Three RCT studies and two case series administered cells intravenously for various indications. One dose-escalating RCT study by Álvaro-Gracia et al., administered allogeneic ASC intravenously for the treatment of rheumatoid arthritis and observed a case of lacunar infarction in the low-dose treatment group, eight days after cell administration [61]. No further thromboembolic events occurred.

Vanikar et al. conducted a three-armed randomized trial administering ASC together with hematopoietic stem cells versus hematopoietic stem cells alone versus no cell treatment to minimize rejection following renal transplantation [82]. Herein the distribution of cardiovascular deaths was not significantly different across the groups of which 6/95 treated with ASCs died of either cardiovascular or cerebrovascular events compared with 9/190 in the two other groups combined. The all-cause mortality in patients treated with ASCs was 7/95 compared with 20/190, which was not statistically significant. The remaining RCT and case series administering cells systemically or using near-systemic administration did not describe cases of thromboembolic events [81, 83, 84]. See Table 1 for overview of reported complications.

The evaluation of thromboembolic complications and mortality included two subgroups of studies that used either autologous ADRC for cardiac indications, administered within the heart, or allogeneic ASC, administered intravenously for a variety of indications. It can be difficult to assess the mortality rate in studies without a control group, especially considering that the patient categories included were of poor prognosis to begin with. For the studies including controls there was no indication of an increased mortality for patients receiving cell therapy.

Thromboembolic complications were few, and the comparison across studies was difficult due to heterogeneous cell administration, as three different administration routes were applied in the four cardiac studies. It must also be taken into account that the complications noted were not necessarily due to the cell treatment, as the time frame between cell treatment and complications was not always clearly described.

## Immunological Safety

Possible immunologic complications were noted for studies administering allogeneic cells (Table 1). This included four RCTs and seven case series.

Two RCTs evaluated the production of donor-specific antibodies as a measure of immune reaction. In the study of Panés et al., 34% of patients without prior IgG HLA class I antibodies generated anti-HLA class I antibodies during the study period [44]. There were, however, no noted immune reactions or adverse events associated with the donor-specific antibodies, and the presence of the antibodies was not associated with the therapeutic response. In another study using similar allogeneic ASC for intravenous administration, it was noted that 19% of patients developed donor-specific antibodies [61]. The most frequent adverse event was transient fever following cell administration (9/46 treated patients).

The remaining two RCTs did not evaluate the immune response to allogeneic cells biochemically. Vanikar et al. conducted a study in which allogeneic ASC were coinfused with allogeneic hematopoietic stem cell transplantation; they found no evidence of graft versus host disease [82]. Zheng et al. described no side effects during allogeneic ASC infusion in the six treated patients [84]. Two adverse events were noted the first day (1 diarrhea and 1 skin rash) , both of which resolved by the next day.

Two case series evaluated the possibility of immune reaction by measuring the ratio of CD4/CD8. In the study by Park et al., it was shown that the ratio of CD4/CD8 did not change [45]. In the study by Lee et al., they found no immunological rejection responses in any of the subjects, based on the ratio of CD4-positive to CD8-positive T cells [55].

The remaining case series did not use any biochemical assays to evaluate the possible immune reaction. Fang et al. was the first to use allogeneic ASC for the treatment of steroid resistant graft versus host disease, and they presented a case series of six patients [81]. They noted no adverse events related to the ASC infusion. Fang et al. also conducted a study using allogeneic ASC obtained from haploidentical donors, and cells were infused intravenously for the treatment of chronic refractory immune thrombocytopenic purpura, also without any mention of adverse effects [83]. De la Portilla described the use of an immunological assessment, but it was not mentioned in the Results section [38]. It was described that two patients were withdrawn from the study due to adverse events possibly due to treatment (pyrexia and perianal abscess); however, in the setting of treating perianal fistulas these events cannot necessarily be attributed to the allogeneic cells used. Oner et al. presented their results of a phase I trial administering subretinal allogeneic ASC for treatment of advanced stage retinitis pigmentosa [86]. They included 11 patients and did not describe any form of immunological reaction.

In the present studies, only ASCs were administered in allogeneic fashion. Several different biochemical tests were applied in the studies of which the CD4/CD8, cytokine levels and unspecific IgM and IgG did not reveal any sign of activated immune response toward the foreign cells. Only the two studies testing for donor-specific antibodies revealed that 19%–34% of patients developed these which suggest that the cells are not as immune privileged as once thought. The consequences of these reactions are unknown.

## Oncological Safety

The oncological safety was evaluated for studies administering cells in the setting of previous malignancy (Table 1). This included five studies (all case series) with a follow-up in the range of 3–12 months. Perez-Cano et al. published a study including 67 patients where ADRC were injected into patients with previous breast cancer, where treatment was given as a cell-assisted lipotransfer [27].

**Table 1.** Overview of safety analysis regarding thromboembolic safety and mortality, immunological as well as oncological safety

| Thromboembolic safety and mortality | | | | | | |
|---|---|---|---|---|---|---|
| **Author** | **Study type** | **Administration route** | **Cell type** | **TE complications** | **Mortality** | **Follow-up** |
| **Cardiac** | | | | | | |
| Comella et al. [73] | Case series | Transendocardial | ADRC | 1/28 | 3/28 | 6 |
| Henry et al. [76] | RCT | Intramyocardial | ADRC | 3/17 (1/14) | 2/17 (0/14) | 12 |
| Houtgraaf et al. [74] | RCT | Intracoronary | ADRC | – | – | 6 |
| Perin et al. [75] | RCT | Transendocardial | ADRC | 1/21 (1/6) | 3/21 (2/6) | 36 |
| **Immunological** | | | | | | |
| Vanikar et al. [82] | RCT | Intravenous | alloASC | 6/95 (9/190) | 7/95 (20/190) | 6 |
| Fang et al. [81] | Case series | Intravenous | alloASC | 0/6 | 2/6 | 40 |
| Fang et al. [83] | Case series | Intravenous | alloASC | 0/7 | 0/7 | 8 |
| **Pulmonary** | | | | | | |
| Zheng et al. [84] | RCT | Intravenous | alloASC | 0/6 (0/6) | 1/6 (2/6) | 1 |
| **Rheumatological** | | | | | | |
| Álvares Garcia et al. [61] | RCT | Intravenous | alloASC | 1/46 (0/7) | 0/46 (0/7) | 6 |

| Immunological safety | | | | | | |
|---|---|---|---|---|---|---|
| **Author** | **Study type** | **Administration route** | **Cell type** | **Complications** | **Biochemical reaction** | **Follow-up** |
| **Gastrointestinal** | | | | | | |
| Park et al. [45] | Case series | Wall of fistula | alloASC | 0/6 | CD4/CD8: N.s.i. | 6 |
| Panés et al. [44] | RCT | Wall of fistula | alloASC | N.d. | ASC/HLA-I: 34% | 6 |
| Garcia-Arranz et al. [39] | Case series | Wall of fistula | alloASC | 0/10 | Cytokine/US: N.s.i. | 12 |
| De la Portilla et al. [38] | Case series | Wall of fistula | alloASC | Fever: 1/24 | – | 4 |
| **Immunological** | | | | | | |
| Fang et al. [83] | Case series | Intravenous | alloASC | 0/7 | – | 8 |
| Fang et al. [81] | Case series | Intravenous | alloASC | 0/6 | – | 40 |
| Vanikar et al. [82] | RCT | Intravenous | alloASC | N.d. | – | 6 |
| **Muskuloskeletal** | | | | | | |
| Lee et al. [55] | Case series | Intratendinous | alloASC | 0/12 | CD4/CD8: N.s.i. | 12 |
| **Ophthalmological** | | | | | | |
| Oner et al. [86] | Case series | Subretinal | alloASC | 0/11 | – | 6 |
| **Pulmonary** | | | | | | |
| Zheng et al. [84] | RCT | Intravenous | alloASC | 0/6 (0/6) | – | 1 |
| **Rheumatological** | | | | | | |
| Álvaro-Gracia et al. [61] | RCT | Intravenous | alloASC | Fever: 9/46 (0/7)<br>Infections: 20/46 (0/7)<br>Rash: 2/46 (0/7) | ASC/HLA-I: 19%. | 6 |

| Oncological Safety | | | | | | |
|---|---|---|---|---|---|---|
| **Author** | **Study type** | **Administration route** | **Cell type** | **Local recurrence** | **Metastasis** | **Follow-up** |
| **Soft tissue, breast** | | | | | | |
| Aronowitz et al. [19] | Case series | Subcutaneous | ADRC | 1/54 | 0/54 | 12 |
| Pérez-Cano et al. [27] | Case series | Subcutaneous | ADRC | 0/67 | 1/67 | 12 |
| **Urogenital** | | | | | | |
| Choi et al. [72] | Case series | Transurethral | ADRC | 0/6 | 0/6 | 3 |
| Gotoh et al. [68] | Case series | Periurethral | ADRC | 0/9 | 0/9 | 6 |
| Haahr et al. [70] | Case series | Corpus cavernosum | ADRC | 0/17 | 0/17 | 6 |

Abbreviations: –, not described/not performed; CD4/CD8, CD4 to CD8 ratio; N.d., no difference in adverse events between groups. no immunological adverse events; N.s.i., no sign of immune rejection; alloASC, allogeneic ASC; ASC/HLA-I, ASC-specific anti-HLA-I antibodies; Cell type, ADRC were autologous in all cases; cytokine/US, cytokine and unspecific antibodies. Data shown as treatment group count/total (control count/total); mortality, all-cause mortality; TE, thromboembolic.

**Table 2.** Risk of bias analysis


All studies with a control group were included for the analysis. Green: Low risk of bias, Yellow: Unclear risk of bias, Red: High risk of bias.

Herein no cases of local recurrence were described, but one case of pelvic metastasis was observed without exact note of timing. Four other serious adverse events were noted; however, only one of these was described, which was donor site bleeding following liposuction. In another study, Aronowitz et al. found that of the 54 patients with previous breast cancer, one patient developed a recurrence after cell-assisted lipotransfer with ADRC 11 months after treatment [19].

A study by Gotoh et al. gave a similar treatment with ADRC as a cell-assisted lipotransfer for urinary incontinence, in which 9/11 patients were previously treated for prostate cancer [87]. Here they found no evidence of recurrence during the 1-year follow-up. In a similar population group Haahr et al. injected ADRC intracavernosal to patients with erectile dysfunction due to previous prostate cancer surgery [70]. The authors did not describe any case of recurrence in their 6-month follow-up study. Similarly, to treat urinary incontinence following prostatectomy, Choi et al. administered ADRC as a cell-assisted lipotransfer to six patients [72]. They described no cases of recurrence or any other side effects in their 3-month follow-up study.

All studies included in the evaluation of oncological safety used autologous ADRC for treatment. There was only one case of local breast cancer recurrence out of a total of 121 patients across two studies within the 12-month follow-up periods. The remaining three studies applied ADRC in 32 previous prostate cancer patients and observed no recurrences within the follow-up period, ranging from 3 to 6 months. For determining long-term oncological safety, follow-up periods of several years are necessary to ensure that cell therapy is safe.

## Bias

All studies with a comparison group were included in the analysis for bias looking specifically at the safety outcome (Table 2); 27 studies were eligible for inclusion. Only seven studies had proper randomization and allocation concealment. For the outcome safety, only five studies described proper blinding of patient, personnel and outcome assessor. Three of these studies were with ADRC and described proper blinding with the introduction of liposuction and subsequent sham ADRC injection [74–76].

## Discussion

The clinical translation process of adipose-derived cell products has begun, and it is crucial that implementation of cell therapy is based on the standard principles of evidence-based medicine. Presently, cell therapy is widely considered as being equivalent to pharmaceutical drugs, which implies that cell therapies should adhere to the same standards for implementation as any newly developed drug, including the assessment of safety and adverse events.

This review includes more than 1,400 patients treated with adipose-derived cell therapy with follow-up ranging from less than a month to 3 years [75, 84]. Very few adverse events have been reported that can be related directly to the cell therapy, Events were rather related to the harvesting of adipose tissue, trauma associated with injection, or the nature of the underlying condition being treated. Of all studies administering ASCs systemically, a case of pulmonary thromboembolism [73], as well as cases of myocardial and cerebral infarctions were described [75, 76]. These are serious adverse events that can be fatal and since there is no clear clinical evidence for the efficacy of adipose-derived cell therapy as of yet, future studies administering cells systemically should be cautious and monitor for these possible serious adverse events. The studies did not describe whether the cells were filtered before administration to ensure that the injected cells were single cell suspensions. Thromboembolic complication risk can be assumed to be higher when injecting clumped cells compared with single cell suspensions. In addition, the underlying condition must also be taken into account as the included patients had a poor prognosis.

Several studies used allogeneic ASC treatment and there was no clear evidence of a clinical immune response. However, for studies examining the presence and later production of donor-specific antibodies, 19%–34% of patients developed these antibodies suggesting that indeed there is a cellular response occurring toward the allogeneic cells [44, 61]. The consequence of this, if any, still remains unknown. In many instances it can be questioned whether the use of allogeneic ASCs has any value over autologous cells, as many of the treated conditions are not acute and life threatening, leaving room and time for the easy, simple isolation and culture-expansion of ASCs. The use of ADRCs has the advantages of being completely autologous and requires much less advanced facilities as treatment can be offered as a same day procedure with everything needed being available in the operating theatre [88]. On the other hand, the advantage of ASCs is the fact that an almost unlimited number of cells can be obtained and is also a more realistic option if one was to consider cell banking either as autologous or allogeneic treatment modalities, and already some studies have been published with funding from companies seeking to offer off-the-shelf allogeneic ASC therapy [44, 61].

Another concern is the use of cell therapy in areas with previous malignancy, as preclinical data have suggested that cell therapy can aggravate any remaining cancer cells [12–14]. However, this has so far not been shown in the clinical setting, as we only could identify one case of recurrence following cell-assisted lipotransfer among 121 breast cancer patients, which is well within what could be expected [89]. It is vital in the setting of previous cancer treatment that safety evaluations are conducted thoroughly with sufficiently long follow-up times, so these initial uplifting results can be confirmed.

During the last 5 years, there has been a marked increase in the number of adipose-derived cell therapy clinical trials that have been published; however, at present most of them are at the case series level (Level IV evidence). In general, it is recognized that low quality studies increase risk of bias, which leads to an increasing chance of findings that do not represent reality [90]. Therefore, it is important to transition toward well-conducted randomized controlled trials with adequate blinding, which also includes the safety assessment.

A systematic review can only be as good as the available literature allows it to be, and this review was limited by the fact that so many studies did not clearly describe their method of assessing safety, and in the end, you will only find the adverse events that you are looking for. Another limitation of the review is the possibility of small overlap in some of the included studies, as case series were published over time from the same research groups with an increasing number of patients; this was deemed to be of such a small magnitude that it was insignificant.

While adipose-derived cell therapy has shown great potential so far, there is very sparse clinical evidence to promote routine clinical implementation. There is a need for higher quality studies before rational conclusions can be made regarding the efficacy of adipose-derived cell therapies. Future studies should place a larger emphasis on including a placebo/sham treatment for proper blinding of both patients and assessors. This is especially crucial when the primary outcome is subjective due to the placebo response [91].

## Author Contributions

N.T., M.J., S.T., and J.S.: conception and design; N.T., S.P., and J.S.: financial support; S.P. and J.S.: administrative support; N.T., M.J., and S.T.: provision of study material or patients; N.T., M.J., S.T., and C.J.: collection and/or assembly of data, data analysis and interpretation; N.T., M.J., and S.T.: manuscript writing; N.T., M.J., S.T., C.J., and S.P.: final approval of manuscript.

## Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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*Review Article*

# Cartilage Regeneration in Human with Adipose Tissue-Derived Stem Cells: Current Status in Clinical Implications

**Jaewoo Pak,[1] Jung Hun Lee,[1,2] Wiwi Andralia Kartolo,[3] and Sang Hee Lee[2]**

[1]*Stems Medical Clinic, 32-3 Chungdam-dong, Gangnam-gu, Seoul 06068, Republic of Korea*
[2]*National Leading Research Laboratory, Department of Biological Sciences, Myongji University, 116 Myongjiro, Yongin, Gyeonggido 17058, Republic of Korea*
[3]*FMN Wellness & Antiaging Centre, Jalan Sangihe 15A, Jakarta Pusat 10150, Indonesia*

Correspondence should be addressed to Sang Hee Lee; sangheelee@mju.ac.kr

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Osteoarthritis (OA) is one of the most common debilitating disorders among the elderly population. At present, there is no definite cure for the underlying causes of OA. However, adipose tissue-derived stem cells (ADSCs) in the form of stromal vascular fraction (SVF) may offer an alternative at this time. ADSCs are one type of mesenchymal stem cells that have been utilized and have demonstrated an ability to regenerate cartilage. ADSCs have been shown to regenerate cartilage in a variety of animal models also. Non-culture-expanded ADSCs, in the form of SVF along with platelet rich plasma (PRP), have recently been used in humans to treat OA and other cartilage abnormalities. These ADSCs have demonstrated effectiveness without any serious side effects. However, due to regulatory issues, only ADSCs in the form of SVF are currently allowed for clinical uses in humans. Culture-expanded ADSCs, although more convenient, require clinical trials for a regulatory approval prior to uses in clinical settings. Here we present a systematic review of currently available clinical studies involving ADSCs in the form of SVF and in the culture-expanded form, with or without PRP, highlighting the clinical effectiveness and safety in treating OA.

## 1. Introduction

Osteoarthritis (OA) is a common painful and debilitating disorder in the elderly [1, 2]. All current medical treatments for OA, such as nonsteroidal anti-inflammatory drugs (NSAIDs), steroids, and hyaluronic acids (HAs), physical therapy, aim to remedy the symptoms, as opposed to treating the underlying causes. When failed with symptomatic medical treatments, patients usually resort to receiving total knee replacement (TKR) or total hip replacement (THR) surgery. Both TKR and THR surgeries carry relatively high morbidity and mortality rates [1, 2]. Even with improved surgical technique, anesthesia, and rehabilitation, the thirty-day mortality rate after total knee arthroplasty is reported to be 0.18%, and 5.6% of the patients experienced complications [3]. Also, the overall 30- and 90-day mortality rates for total hip arthroplasty are reported to be 0.24% and 0.55%, respectively [4]. These approaches do not address the morbidity associated with early disease or the limitations of arthroplasty surgery, which include the possibility of adverse outcomes and the finite lifespan of prostheses [5].

Mesenchymal stem cells (MSCs) are found in numerous human tissues including bone marrow and adipose tissue [6, 7]. These MSCs have been shown to differentiate into bones, cartilage, muscle, and adipose tissue [6–8]. Because of their potential capabilities in regenerating cartilage, MSCs have been successfully used in animals [9, 10]. In 2008, Centeno et al. have showed successful cartilage regeneration in humans with MSCs [11]. Subsequently, in 2010, the same group also reported safety data of using MSCs in humans for cartilage regeneration [12].

Adipose tissue-derived stem cells (ADSCs) are one type of MSCs. In 2001 and 2002, Zuk et al. showed that adipose tissue in the form of stromal vascular fraction (SVF) contains stem cells that have the capacity to differentiate into cartilage, bone, muscle, and adipose tissue, similar to MSCs [13, 14]. Likewise,

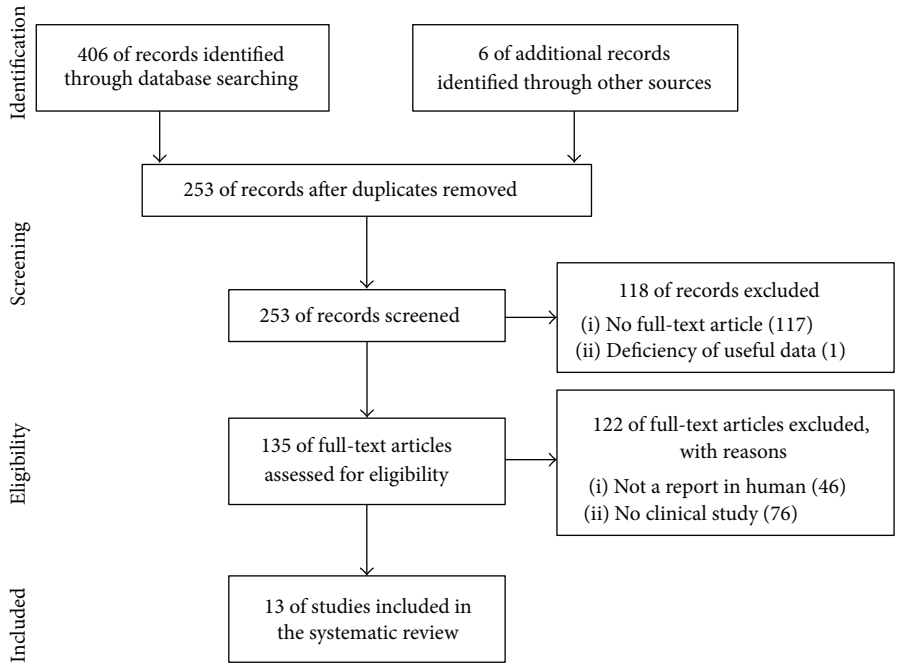


Figure 1: Literature selection process (PRISMA flow diagram).

ADSCs also have been investigated in treatment of cartilage injuries and osteoarthritis in animals. The results from these studies showed evidence of cartilage regeneration by using ADSCs [15–19].

Consequently, in 2011, Pak successfully treated 2 human patients with OA of the knees by using autologous ADSCs in the form of SVF along with platelet rich plasma (PRP) and hyaluronic acid (HA). He documented the regeneration of cartilage-like tissue in these patients through magnetic resonance imaging (MRI) studies [20].

More studies have recently become available, providing more evidence of cartilage regeneration in human patients with OA of the knees [21–23]. Such continued research and interests hold great promises in the field of regenerative medicine.

Although the successful regeneration of cartilage with ADSCs in humans may represent a promising, minimally invasive, nonsurgical alternative, many issues need to be resolved and clarified before the general application of this procedure. The mechanism of regeneration remains unclear: (i) it could be due to the secretory effects of the stem cells injected [24, 25]; (ii) it could be due to direct engraftment and differentiation of the stem cells that were introduced into the diseased joints [26, 27]; or (iii) it could be due to the combination of secretory effects and direct engraftment of the stem cells.

Adipose stem cells excrete a variety of cytokines, chemokines, growth factors, and exosomes [28, 29]. These factors have positive effects on the surrounding progenitor cells. However, there is some evidence that these stem cells injected may actually become engrafted into the tissue and differentiate into tissue-specific stem cells [30]. It is also very possible that these two mechanisms play a role in cartilage regeneration.

Furthermore, the method of the cell transplant needs to be studied in detail: the most optimal dosage of the stem cells to be injected, the best mode of injection, the best method of promoting stem cell adherence to the lesions, and the most potential growth factors (e.g., PRP) to be added, as well as the best scaffolding materials (e.g., HA and extracellular matrix (ECM)).

Here we will present a comprehensive and systematic review of cartilage regeneration in human joints by using ADSCs in the form of adipose SVF and assess the possibility of the clinical application of these stem cells.

## 2. Method

We used the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) in our review (Figure 1) [31]. We conducted a systematic literature search in PubMed, Medline, and Embase. We used the keywords as our search terms. We combined terms for selected indications (stem cell, osteoarthritis, and adipose). The literature search included all studies published in English between 2000 and 2015. We identified 253 references after removing duplicates. We independently assessed full-text articles for inclusion in our review. The criteria for the inclusion of studies in our review encompassed clinical studies on ADSC injection conducted on humans for cartilage regeneration. Finally, we found 13 articles showing clinical studies on ADSC treatments for cartilage defects (Figure 1).

## 3. ADSCs in the Form of SVF along with PRP and/or HA/ECM

At present, most of the ADSCs being used in clinical settings are in the form of SVF. To obtain adipose SVF, liposuction is performed on easily accessible areas of the body, such as the abdomen, buttocks, or thighs. These lipoaspirates are then digested with collagenase to extract stem cells that exist within the matrix of the adipose tissue [13, 14]. The collagenase is then washed off using a centrifuge and dilution method. The pellet, including the bottom portion of the centrifuge, is considered to be SVF [13, 14]. SVF contains a variety of cells in different proportions: ADSCs, a type of mesenchymal stem cells, pericytes, vascular adventitia cells, fibroblasts, preadipocytes, monocytes, macrophages, red blood cells, fibrous tissue, ECM, and so forth [13, 14].

The process of preparing adipose SVF is considered to be a medical procedure in Korea and a few other countries when performed by a physician as a single surgical procedure within the same day and with minimal manipulations [32]. Unlike adipose SVF, culture-expanded stem cells are usually considered to be pharmaceutical products, requiring clinical trials and governmental approval.

*3.1. Number of Stem Cells in Human Adipose Tissue.* The number of stem cells that can be extracted from each individual varies greatly.

Currently, it is well accepted that ADSCs exist within the matrix of adipose tissue. More specifically, it has been shown that ADSCs exist around blood vessels of adipose matrix [33]. These stem cells can be released from the matrix by processing the lipoaspirate with collagenase. Such stem cells are shown to regenerated cartilage as shown by Zuk et al. [13, 14]. However, the number of stem cells that can be extracted from one gram of adipose tissue can be very variable in different individual patients [13, 34–39].

The number of stem cells that can be obtained from one gram of adipose tissue can range from 5,000 to 200,000 cells [40], which have been measured by flow cytometry and indirect immunofluorescence [41, 42]. Such large individual variability may result in inconsistency of results in treating patients. Patients with high number of stem cells will have great cartilage regeneration. However, patients with low number of stem cells will not a great response, as shown by Jo et al. [21].

*3.2. Autologous Platelet Rich Plasma (PRP).* Autologous PRP was used in most of 13 articles showing clinical studies on ADSC treatments for cartilage defects.

PRP contains a variety of growth factors: transforming growth factor-$\beta$ (TGF-$\beta$), epidermal growth factor (EGF), and fibroblast growth factor (FGF), along others [43]. These growth factors are known to proliferate stem cells. Centeno et al. used autologous platelet lysate to grow bone marrow-derived stem cells, which were injected in human patients for cartilage regeneration [11]. Likewise, PRP has been used to increase the number of stem cells injected into a joint.

Also, activated PRP may act like a scaffold for stem cells. Autologous PRP has been prepared by centrifuging autologous blood with anticoagulant citrate dextrose solution [11, 44]. When autologous PRP has been activated by adding calcium chloride, thrombin, or collagen [11, 44–46], PRP may become a "curd-like" substance [11], which may function like a scaffold.

*3.3. Hyaluronic Acid (HA) and Extracellular Matrix (ECM).* Scaffolding materials were used in some [20, 47–49] of the 13 articles showing clinical studies on ADSC treatments for cartilage defects.

HA and ECM are two naturally occurring scaffolding materials. HA has a high affinity for cartilage defects and provides an environment for stem cells to adhere to the lesion and differentiate [50]. ECM also provides an environment for stem cells to adhere and differentiate [51]. When autologous ECM is provided, immune reactions are not likely to occur. In addition, ECM contains a variety of growth factors, which further enhance the growth and differentiation of the injected stem cells [51].

*3.4. ADSCs with PRP and/or HA/ECM.* The combination of ADSCS with PRP and/or scaffolding materials was used in 13 articles showing clinical studies on ADSC treatments for cartilage defects.

PRP or platelet lysate provides a variety of growth factors for stem cells [11, 43]. HA/ECM scaffolding materials provide the environment for stem cells to adhere and differentiate into cartilage [50, 51]. Together, this combination may provide the best optimal strategy for stem cells to adhere, grow, and differentiate into cartilage [20, 22, 44, 47–49, 52–55].

## 4. Clinical Applications of ADSCs

The main features of clinical studies on ADSC treatments for cartilage defects were summarized in Table 1.

*4.1. Case Report by Pak [20].* This is the very first study that showed the possibility of ADSCs in the form of SVF regenerating cartilage in human patients. Pak used approximately 100 g of adipose tissue obtained from the abdomen. This adipose tissue was digested with collagenase. The collagenase was washed off. The resulting adipose SVF, containing ADSCs, was injected percutaneously with calcium chloride-activated PRP, HA, and dexamethasone into joints of 2 patients with OA. Three months after the injections, the visual analog score (VAS) for pain, functional rating index, and range of motion (ROM) improved along with the MRI evidence of cartilage-like tissue regeneration in these patients.

This study used 100 g of adipose tissue. Thus, the total estimated number of ADSCs injected can range from 500,000 to 20,000,000 [40]. Also, it should be noted that this study used PRP and HA, along with ADSCs.

*4.2. Nonrandomized, Retrospective, and Comparative Study by Koh and Choi [52].* This study involved 25 patients with OA of the knees. The patients were injected with adipose SVF derived from approximately 19 g of adipose tissue obtained from the knee fat pad while performing arthroscopic lavage

Table 1: Clinical studies on ADSC treatments for cartilage defects.

| Study (yr) | Intervention treatment | Study type | Number of subjects | Subject characteristic [age (yr); gender] | Previous therapy | Concurrent treatment | Follow-up (mo) | Outcome measures | Results | Authors' conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| Pak (2011) [20] | Adipose SVF (ADSC) + PRP via percutaneous injections | Case report | 2 | 70 and 79; 2 F with chronic knee pain | Various treatments without any success | None | 3 | VAS; functions (FRI, ROM); MRI | VAS/function improvements and MRI evidence of cartilage regeneration | ADSC + PRP: potentially effective in regenerating cartilage in humans |
| Koh and Choi (2012) [52] | Adipose SVF (ADSC) + PRP via percutaneous injections | Nonrandomized, retrospective, comparative study: ADSC + PRP versus PRP alone | 25 Study group (ADSC + PRP): 25; control group (PRP alone): 25 | Study group: mean 54.1 (range, 34–69); 8 M and 17 F | Various treatments without any success | None | 16.4 | VAS; functions (Lysholm, Tegner) | ADSC + PRP: more effective than PRP-control group | ADSC + PRP: potentially effective in patients with cartilage defects |
| Pak et al. (2013) [44] | Adipose SVF (ADSC) + PRP via percutaneous injections | Retrospective cohort study | 91 | Mean 51.23 ± 1.50 (range, 18–78); 45 M and 46 F | Various treatments without any success | None | 26.62 ± 0.32 | VAS; functions | Statistically significant improvement in both VAS and functions | ADSC + PRP: safe and potentially effective |
| Pak et al. (2013) [47] | Adipose SVF (ADSC) + PRP via percutaneous injections | Case series | 3 | 43 and 63; 2 F 54; 1 M All with chronic knee pain | Various treatments without any success | None | 3 | VAS; functions (FRI, ROM); MRI | VAS/function improvements and MRI evidence of cartilage regeneration | ADSC + PRP: effective in treating chondromalacia patellae patients |
| Koh et al. (2013) [53] | Adipose SVF (ADSC) + PRP via percutaneous injection | Case series | 18 | Mean 54.6; 6 M and 12 F | Various treatments without any success | Arthroscopic lavage before knee-fat-pad-derived adipose SVF + PRP injection | 24.3 | VAS; functions (WOMAC, Lysholm); MRI | VAS/function/MRI improvements | ADSC + PRP: effective in treating OA of knees |
| Koh et al. (2015) [22] | Adipose SVF (ADSC) + PRP under arthroscopic guidance | Case series | 30 for adipose SVF + PRP injection; 16 for second-look arthroscopy | Mean 70.3 (range, 65–80); 5 M and 25 F | Various treatments without any success | Arthroscopic lavage before ADSCs + PRP injection | 24 | VAS; functions; 2nd-look arthroscopy | VAS/function improvements; improved and maintained cartilage status | ADSCs + PRP: effective in treating elderly patients with OA |

Table 1: Continued.

| Study (yr) | Intervention treatment | Study type | Number of subjects | Subject characteristic [age (yr); gender] | Previous therapy | Concurrent treatment | Follow-up (mo) | Outcome measures | Results | Authors' conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| Pak et al. (2014) [48] | Adipose SVF (ADSC) + PRP via percutaneous injections | Case report | 1 | 32; 1 F with chronic knee pain due to meniscus tear | Various treatments without any success | None | 3 | VAS; functions (FRI, ROM); MRI | VAS/function improvements and MRI evidence of cartilage regeneration | ADSC + PRP: effective in treating cartilage defect lesions, including meniscus tear |
| Bui et al. (2014) [54] | Adipose SVF (ADSC) + PRP via percutaneous injections | Case series | 21 | >18; ND | Various treatments without any success | None | 8.5 | VAS; functions; MRI | VAS/function/MRI improvements | ADSC + PRP: effective in treating OA of knees |
| Jo et al. (2014) [21] | Culture-expanded ADSC via arthroscopic injections | Randomized double-blind dose escalation study (a proof-of-concept clinical trial) | 18 | 61–65; 3 M and 15 F | Various treatments without any success | None | 6 | VAS; functions; MRI; arthroscopy; histology | VAS/function/MRI/ arthroscopic/histological improvements | $1.0 \times 10^8$ ADSCs into the osteoarthritic knee improved function and pain of the knee joint. Radiological, arthroscopic, and histological measures demonstrated regeneration of hyaline-like articular cartilage |
| Koh et al. (2014) [23] | Adipose SVF (ADSC) + PRP under arthroscopic guidance | Case series | 35 with 37 knee joints | Mean 57.4 (range, 48–69); 14 M and 21 F | Various treatments without any success | Arthroscopic lavage before adipose SVF + PRP injection | 12.7 | VAS; functions; arthroscopy | 94% patients had excellent clinical improvement; 76% had abnormal repair tissue | Scaffolds may be needed to treat patients with large cartilage lesions |

Table 1: Continued.

| Study (yr) | Intervention treatment | Study type | Number of subjects | Subject characteristic [age (yr); gender] | Previous therapy | Concurrent treatment | Follow-up (mo) | Outcome measures | Results | Authors' conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| Koh et al. (2014) [55] | Adipose SVF (ADSC) + PRP under arthroscopic guidance | Comparative study: adipose SVF + PRP versus PRP only | 44 | ND | Various treatments without any success | Open-wedge high tibial osteotomy | 24 | VAS; functions; arthroscopy | Adipose SVF + PRP is more effective than PRP alone | ADSC therapy, in conjunction with HTO, mildly improved cartilage healing and showed good clinical results compared with PRP only |
| Kim et al. (2015) [49] | Adipose SVF (ADSC) under arthroscopic guidance | Comparative study: adipose SVF versus adipose SVF + fibrin glue (as a scaffold) | 54 | Mean 57.5 ± 5.8; 22 M and 32 F | Various treatments without any success | None | 28.6 | VAS; functions; arthroscopy | No significant difference | Clinical and arthroscopic outcomes of ADSC implantation were encouraging for OA knees in both groups, although there were no significant differences in outcome scores between groups |
| Michalek et al. (2015) [56] | Adipose SVF (ADSC) via percutaneous injection | Multicenter case control study | 1,114 | Median 62 (range, 19–94); 589 M and 525 F | Various treatments without any success | None | Median 17.2 | VAS; functions | VAS/function improvements | Adipose SVF is a novel and promising treatment approach for patients with degenerative OA. ADSC is safe and cost-effective |

SVF: stromal vascular fraction; ADSC: adipose tissue-derived stem cells; PRP: platelet rich plasma; OA: osteoarthritis; yr: year; mo: month; M: male; F: female; ND: not described; HTO: high tibial osteotomy; VAS: visual analogue scale; FRI: functional rate index; ROM: range of motion; WOMAC: Western Ontario and McMaster Universities osteoarthritis index; Lysholm: Lysholm scores; Tegner: Tegner activity scale.

and debridement. Thereafter, the adipose SVF was percutaneously injected with calcium chloride-activated PRP. A mean of $1.89 \times 10^6$ ADSCs was presented in 19 g of adipose SVF. The results showed that the mean Lysholm knee scoring scales, Tegner activity scales, and VAS scores in the study group had improved significantly compared to the control group. No major adverse events were observed.

In this study, the approximate number of ADSCs obtained was little less than 2,000,000, and this was calculated to be little less than 100,000 stem cells per gram of adipose tissue. The study concludes that little less than 2 million of ADSCs with PRP were effective.

*4.3. Retrospective Cohort Study by Pak et al. [44].* This is the very first safety report involving human ADSCs in the form of SVF. Between the period of 2009 and 2010, Pak et al. injected joints percutaneously with the autologous, non-culture-expanded ADSCs in 91 patients. In 2013, Pak et al. reported that all 91 patients had no serious side effects and no cancer was reported. However, the study reported that a few minor side effects occurred, mainly swelling and tendonitis, both of which were ameliorated with NSAIDs. The average efficacy reported was 65% at 3 months after the treatment.

All these patients were injected with approximately 100 g of adipose tissue. Thus, the total estimated number of ADSCs injected can range from 500,000 to 20,000,000 [40].

*4.4. Case Series by Pak et al. [47].* This study involved 3 patients with chondromalacia patellae of the knees. The patients were treated with ADSCs in the form of SVF, calcium chloride-activated PRP, and HA. The mixture was injected into the knees percutaneously. After 3 months of the treatment, the patients' VAS pain scale, functional rating index (FRI), and ROM had improved. The study also showed positive regeneration of hyaline cartilage-like tissue at the patellofemoral joints of all 3 patients.

This is the very first study showing the possibility of treating chondromalacia patellae with ADSCs with PRP and HA.

*4.5. Case Series by Koh et al. [53].* This study involved 18 patients with OA of the knees. The patients received non-culture-expanded ADSCs in the form of SVF obtained from the knee fat pad. The ADSCs were percutaneously injected into the knees with calcium chloride-activated PRP after arthroscopic debridement of the knees. A mean of $1.18 \times 10^6$ ADSCs was prepared from approximately 9.1 g of adipose tissue from the knee fat pad. Thereafter, Western Ontario and McMaster Universities osteoarthritis index (WOMAC), Lysholm, and VAS scores were measured and improved. The whole-organ MRI score, particularly the cartilage whole-organ MRI score, also improved. The authors concluded that improvements in the clinical and MRI results were positively related to the number of ADSCs injected.

This study used little over one million ADSCs obtained from mean of 9.1 g of adipose tissue obtained from the knee fat pad along with PRP. The number of ADSCs extracted from 1 g of adipose tissue was approximately 129,700 ADSCs per gram of adipose tissue.

*4.6. Case Series by Koh et al. [22].* This study involved 30 patients with OA of the knees. The patients were injected with adipose SVF containing ADSCs extracted from 120 g of adipose tissue from the buttocks. The adipose SVF were injected with calcium chloride-activated PRP under arthroscopic guidance after arthroscopic lavage. Of these patients, 16 patients went through the second-look arthroscopies in a median of 25 months after the initial treatment. At a minimum of 2 years after the operation, almost all patients showed significant improvement in the knee injury, OA outcome scores (KOOS), VAS pain scale, and Lysholm score. In the second-look arthroscopy, 10 patients (63%) had improved cartilage, 4 patients (25%) had maintained the cartilage, and 2 patients (12%) failed in healing cartilage defects.

This study used 120 g of adipose tissue from buttock. Unlike other previous reports, the study reported extracting only little over 4 million ADSCs from 120 g of adipose tissue. However, this study is the very first study showing direct evidence of cartilage regeneration via arthroscope.

*4.7. Case Report by Pak et al. [48].* This study involved 1 patient with a meniscus tear of the knee. The patient was treated with autologous adipose SVF containing ADSCs derived from approximately 40 g of packed adipose tissue obtained from the abdomen. The adipose SVF was injected with calcium chloride-activated PRP and HA. After 3 months, the patient's VAS for pain, FRI, and ROM had improved. Furthermore, the meniscus tear had improved, if not entirely disappeared, in the subsequent follow-up MRIs after 3 months.

This is another first case report showing the possibility of treating meniscus tear with ADSCs with PRP and HA.

*4.8. Case Series by Bui et al. [54].* This study involved 21 patients with OA of the knees with grades 2 and 3. The patients were treated with autologous ADSCs in the form of SVF obtained from the abdomen. The ADSCs were injected percutaneously into the joints with calcium chloride-activated PRP. All 21 patients showed improved joint function after 8.5 months, measured by VAS pain score and the Lysholm score. In addition, significant improvements were noted in the MRI findings with increased thickness of the cartilage layer.

This study used 50–100 g of lipoaspirates. Thus, the number of ADSCs injected may range from 250,000 to 20,000,000. All these ADSCs were injected with PRP with good response.

*4.9. Double-Blind, Randomized Dose Escalation Study by Jo et al. [21].* This is the very first double-blind, randomized clinical trial involving ADSCs in 18 patients. The patients received autologous culture-expanded ADSCs via arthroscopy. No arthroscopic lavage was performed and no PRP was injected. The ADSCs suspended in 3 mL of normal saline were injected. Initially, there were 3 groups: low-dose ($1.0 \times 10^7$ ADSCs), mid-dose ($5.0 \times 10^7$ ADSCs), and high-dose ($1.0 \times 10^8$ ADSCs) groups with 3 patients each. In the high-dose group, there was a significantly increased volume of cartilage regeneration compared to mid-dose and low-dose group. The regeneration of the cartilage was confirmed by MRI and

arthroscopy. Furthermore, the histology of the regenerated tissue was consistent with hyaline cartilage in characteristics. After such results in the first 9 patients, the remaining 9 of the 18 patients received high-dose ($1.0 \times 10^8$) ADSCs. There were no treatment-related adverse events and the WOMAC score improved.

This is the very first double-blind, randomized study with 3 different dosages of ADSCs. Unlike other studies, Jo et al. used only autologous culture-expanded ADSCs without PRP and without HA. This study clearly shows that ADSCs are effective in regenerating cartilage. This study also showed that higher dosage of ADSCs (100 million) is more efficacious than lower number of ADSCs (10 million).

*4.10. Case Series by Koh et al. [23].* This is a second-look arthroscopic study involving 35 patients with a total of 37 knee joints with OA. The patients were treated with ADSCs contained in SVF obtained from a mean of 22.6 g of fat originating from the buttocks. The mean ADSCs obtained from SVF were $3.83 \times 10^6$. The ADSCs were injected with calcium chloride-activated PRP under arthroscopic guidance after arthroscopic lavage.

After the mean follow-up period of 12.7 months, second-look arthroscopy was performed. The mean International Knee Documentation Committee (IKDC) and Tegner activity scale scores significantly improved in 94% of the patients. However, 76% of the patients had abnormal repair tissue at second-look arthroscopies. The authors concluded that a scaffolding material may be needed for large lesions.

This study used little less than 4 million ADSCs obtained from 22.6 g of adipose tissue from buttocks. Although PRP was injected with ADSCs, some of the patients did not respond well, necessitating a scaffolding material for better results.

*4.11. Comparative Study by Koh et al. [55].* This study involved 44 patients and compared the clinical results and second-look arthroscopic findings of a PRP-only treatment group and ADSCs in the form of SVF with a PRP treatment group. Both groups underwent open-wedge high tibial osteotomies (HTO). ADSCs were obtained from 120 g of adipose tissue and injected with PRP in 23 patients. The other 21 patients who went through HTO were injected with PRP only. After following the patients for 24 months, the ADSC with PRP group showed significantly greater improvement in the VAS for pain and KOOS subscales for pain and symptoms, compared to the PRP-only group. However, the Lysholm score was similarly improved in both groups. Arthroscopic evaluation showed that fibrocartilage was regenerated in 50% of the ADSCs with PRP group. Only 10% in the PRP-only group had their fibrous cartilage regenerated. The authors concluded that ADSCs with PRP are more effective than PRP alone.

This study used 120 g of adipose tissue. Thus, the number of ADSCs injected may range from 600,000 to 24,000,000 cells. This study also showed that ADSCs with PRP are more effective than PRP alone.

*4.12. Comparative Study by Kim et al. [49].* This study involved 54 patients with a total of 56 affected knees in comparing the efficacy of ADSCs in the form of SVF-only group to that of ADSCs-with-fibrin-glue group. The fibrin glue was used as a scaffold. Adipose SVF were obtained from 120 g of adipose tissue. A total of 37 patients (39 knees) were treated with ADSCs only, and the other 17 patients were injected with ADSCs with fibrin glue. After a mean follow-up period of 28.6 months, the mean IKDC score and Tegner activity scale in both the groups significantly improved. However, better International Cartilage Repair Society (ICRS) scores were achieved in the ADSCs-with-fibrin-glue group in the second-look arthroscopies.

This study used 120 g of adipose tissue in comparing ADSCs versus ADSCs with fibrin glue as a scaffold. As expected, ADSCs-with-fibrin-glue scaffold were more effective.

*4.13. Multicenter Case Control Study by Michalek et al. [56].* This study involved 1,114 patients with OA of the knee and hip from the USA, Czech Republic, Slovakia, and Lithuania. The patients were percutaneously injected with ADSCs in the form of SVF obtained from 20–90 g of adipose tissue. These patients were then followed up for a median of 17.2 months. The clinical effects were measured on the basis of pain, nonsteroid analgesic usage, limping, extent of joint movement, and stiffness. There were no serious side effects reported, including cancer. At the 12 months of follow-up period, approximately 75% of symptom improvement was noticed in 63% of patients and approximately 50% of symptom improvement was documented in 91% of patients.

This is the first study that involves a large number of human patients. The amount of adipose tissue varies: 20–90 g. Thus, the estimated number of ADSCs injected may range from 100,000 to 18,000,000 cells. Further, no PRP nor HA was used. However, the results are encouraging.

## 5. Discussions

Adipose tissue is considered to be a preferable source of MSCs due to its ease of accessibility and the availability of a large number of stem cells per gram of adipose tissue. In adipose tissue, 1% to 10% of nucleated cells are considered to be ADSCs whereas only 0.0001–0.01% of nucleated cells in the bone marrow are stem cells [14]. In addition, the number of nucleated cells in adipose SVF can range from 500,000 to 2,000,000 cells per gram of adipose tissue [40]. The range of MSCs in 1 g of adipose tissue may be 5,000–200,000 stem cells [40]. Thus, theoretically, 0.5–20 million ADSCs can be extracted from 100 g of adipose tissue. If the number of MSCs in adipose SVF is 5%, approximately 10 million ADSCs can be obtained from 100 g of adipose tissue.

ADSCs, as one specific form of MSCs, have been shown to regenerate cartilage in animals [15, 57, 58]. However, some authors claim adipose SVF alone may not be sufficient to regenerate cartilage in animals [18]. Interestingly, in this review, 11 of thirteen human studies had used autologous PRP in addition to ADSCs in the form of SVF.

Autologous PRP may play an important role in cartilage regeneration. PRP releases a variety of growth factors when activated. Centeno et al. used platelet lysate to grow MSCs that were injected into a human knee for cartilage regeneration [11]. The TGF-$\beta$ contained in PRP may be necessary for differentiation of MSCs into cartilage cells [43].

Autologous PRP may also play a role as a scaffold, influencing stem cell adherence to lesions, as well as stem cell growth and differentiation. When properly activated, autologous PRP can become a "curd-like" substance and can thus operate as scaffold, as shown by Kim et al. [49]. Although autologous PRP alone may not regenerate cartilage as shown by Koh et al. [55], PRP may enhance ADSCs in SVF to adhere to the cartilage lesion and proliferate.

The randomized, double-blind dose escalation clinical study reported by Jo et al. clearly showed the likelihood of cartilage regeneration with ADSCs alone without any additives such as PRP or HA [21]. In the study, Jo et al. showed a direct relationship between the number of stem cells injected and the amount of cartilage regenerated. The amount of cartilage regenerated was much greater with 100 million ADSCs than 50 million ADSCs injected. This was documented by arthroscopies and MRIs [21].

On the other hand, the study by Michalek et al. did not use any other additives although the numbers of ADSCs injected are estimated to be less than the number of ADSCs used in the study by Jo et al. Also, the study by Michalek et al. did not use PRP or HA. Among all the studies reviewed in this paper, Michalek et al. study is the only one that did not have any visible objective data, such as MRI or arthroscopic photos, although significant clinical improvement has been documented.

Although most of the studies in this review used a relatively large volume (approximately 100 g) of adipose tissue, three studies used a relatively small volume (approximately 20 g) of adipose tissue. However, these three studies used PRP with low amount of adipose tissue and showed clinical improvement in patients. Therefore, it can only be estimated that adipose tissue from different regions of patients' abdomens may contain different number of stem cells.

It has been shown that different individuals have different density in the adipose tissue, indicating different amount of matrix [59]. ADSCs exist within matrix of adipose tissue around the blood vessels. Consequently, it can be concluded that higher density of adipose tissue may contain higher density of matrix and thus yields higher number of stem cells. Furthermore, the method of liposuction may affect the results of ADSCs yield in the SVF. Compared to surgical resection of adipose tissue, liposuction has been shown to produce higher percentage of viable cells in lipoaspirates [60].

In addition to differences in adipose tissue and its extraction, the concentration and incubation time of collagenase are other important factors affecting the yield of ADSCs and their viability in SVF. Since high dosage or exposure to collagenase may be toxic to ADSCs, excess amount of collagenase can decrease the ADSC viability while insufficient amount of collagenase may result in inefficient and inadequate amount of ADSC yield [61].

Based on the study by Jo et al., it is logical to expect higher rates of improvement with a higher amount of ADSCs obtained and used for cartilage regeneration. However, the direct dose relationship was not clearly observed when comparing the 12 studies that involved ADSCs in the form of SVF. This may be due to variability in adipose SVF obtained from different individuals, stem cell viability when processing adipose tissue and injecting SVF, stem cell adherence, and stem cell growth. Also addition of growth factors, such as PRP, and scaffold material, such as HA, may be important as shown by Koh et al. [49, 55]. Dregalla et al. showed that local anesthetics can also have very significant negative effects on stem cell survival and adherence [62].

Another factor can be the scaffolds themselves. HA works as a scaffold [50], and the studies [20, 44, 47, 48] reported by Pak et al. used HA for such purposes. Adipose SVF contains a variety of cell types including ADSCs and extracellular matrix (ECM) [13, 14]. Such ECM contained in the adipose SVF may also work as scaffold and assist ADSCs to adhere to the lesion, proliferate, and differentiate [51]. ECM also may excrete a variety of cytokines and growth factors, affecting the cartilage regeneration by MSCs [51, 63–65].

The mode of injection does not seem be a major determining factor in cartilage regeneration. Most studies reported by Koh et al. used intra-articular injections of adipose SVF under arthroscopic guidance. However, it is unclear whether such an injection is better than a percutaneous injection. Arthroscopic examination of knees requires spinal or general anesthesia; thus, it is not considered to be a minimally invasive procedure. In addition, arthroscopic lavage and debridement for OA of the knee are ineffective [66]. A head-to-head study may be necessary to determine if such an invasive procedure outweighs the efficacy of percutaneous injections.

## 6. Conclusions

At present, there is no cure for painful OA in stages 2 and 3. For these patients, the intra-articular injection of ADSCs in the form of SVF can be an alternative treatment for now. As described in this review, the joint injection of ADSCs in the form of SVF with PRP can be safe and efficacious. Moreover, obtaining approximately 100 g of adipose tissue and percutaneous joint injections is considered to be a minimally invasive procedure and can be readily accepted by patients. These procedures carry relatively low rates of morbidity and side effects.

Although a large amount of injecting ADSCs is more efficacious in regenerating cartilage, the studies reviewed in this paper have shown that ADSCs in the form of SVF with PRP can be efficacious in symptom improvement.

However, lack of well-designed studies with control on using different methods and components of the injections still leaves many questions unanswered. In addition, the lack of understanding of the mechanism of action of ADSCs dictates the need for more clinical trials.

## Conflict of Interests

The authors have no conflict of interests.

## Authors' Contribution

Jaewoo Pak and Jung Hun Lee contributed equally to this work.

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REVIEW Open Access

# Current use of autologous adipose tissue-derived stromal vascular fraction cells for orthopedic applications

Jaewoo Pak[1,2,3†], Jung Hun Lee[1,4†], Kwang Seung Park[4], Moonhee Park[4,5], Lin-Woo Kang[6*] and Sang Hee Lee[4*]

## Abstract

Autologous adipose stromal vascular fractions (SVFs) containing adipose tissue-derived stem cells (ASCs) are currently being used in clinical settings for various orthopedic applications for human patients. Due to its potential capability of regenerating cartilage, bone, and tendons, autologous adipose SVFs are being tried in treating patients with osteoarthritis (OA), chondromalacia, meniscus tear, osteonecrosis of the femoral head, and tendon injuries. Here, we have reviewed available human clinical studies with regard to patient applications of autologous adipose SVF containing ASCs, specifically assessing effectiveness and safety in the field of orthopedic disorders. All studies reviewed in this article presents potential benefits of autologous adipose SVF in various orthopedic applications without any serious side effects.

Keywords: Mesenchymal stem cell, Stromal vascular fraction, Autologous adipose tissue-derived stem cells, Effectiveness and safety, Orthopedic applications

## Background

Musculoskeletal injuries and damage are common health problems in both young and old patients [1]. Various treatment modalities are available for such musculoskeletal injuries. However, most of these modalities provide only symptomatic relief [2]. The regenerative potential of injured and damaged tissue with stem cells is a promising new treatment strategy in the field of orthopedics. Stem cells can be categorized into two major forms: embryonic stem cells and adult stem cells [3]. Adult stem cells, which include mesenchymal stem cells (MSCs), can be further divided into non-culture expanded forms, also known as stromal vascular fractions (SVF), and culture expanded forms [3]. Often, the SVFs are autologous in nature and the process of obtaining SVFs may require a procedure with a physician. On the contrary, culture expanded stem cells involve cell growth and cell expansion using various nutrients in a laboratory setting. Thus, culture expanded stem cells are usually considered to be a pharmaceutical product requiring government regulatory clearance and approval in Korea [4]. Due to such government regulatory issues, adipose SVF has been more commonly used for various orthopedic applications in clinical settings. Currently two common forms of SVFs are readily available: bone marrow and adipose tissue [5].

Although MSCs can be found in numerous human tissues, a clinically applicable quantity of autologous non-culture expanded MSCs can be obtained only from bone marrow and adipose tissue [5, 6]. MSCs contained in adipose tissue are called adipose tissue- derived stem cells (ASCs) and are considered to be one specific type of MSCs, and they have been shown to differentiate into bones and cartilage [5–9]. In 2001 and 2002, Zuk et al. showed that adipose tissue contains MSCs in SVF and that these MSCs have the capacity to differentiate into cartilage and bone [8, 9]. The earliest clinical application of autologous adipose SVF with one surgical procedure to treat widespread traumatic calvarial defects was reported in 2004 by Lendeckel et al. [10].

* Correspondence: lkang@konkuk.ac.kr; sangheelee@mju.ac.kr
†Equal contributors
[6]Department of Biological Sciences, Konkuk University, 1 Hwayangdong, Gwangjingu, Seoul 05029, Republic of Korea
[4]National Leading Research Laboratory, Department of Biological Sciences, Myongji University, 116 Myongjiro, Yongin, Gyeonggido 17058, Republic of Korea
Full list of author information is available at the end of the article

In 2011, Pak had successfully used autologous adipose SVF for cartilage and bone regeneration in human patients without a surgical procedure [11]. Afterward, numerous clinical studies have been published about OA treatment with autologous adipose SVF. We conducted a literature search in the PubMed, Medline, and Embase. We used the keywords as our search terms. We combined terms for selected indications (stromal vascular fraction, stem cell, orthopedic, and adipose). The literature search included all studies published in English between 2010 and 2016. The criteria for the inclusion of studies in our review encompassed clinical studies on autologous adipose SVF injection conducted on humans for orthopedic applications. These studies will be reviewed in this article and summarized in Table 1.

## Autologous adipose SVF

### Preparation of autologous adipose SVF

In order to obtain autologous adipose SVF, liposuction is first performed. The resulting adipose tissue is called lipoaspirate. The lipoaspirates are then digested with collagenase to break down the matrix. Consequently, MSCs are released from the matrix of the adipose tissue [8, 9]. These MSCs are termed adipose tissue-derived stem cells (ASCs). Afterward, by using the centrifugation-and-dilution method, the ASCs are isolated and collagenase is washed off. After 3 to 4 rounds of centrifugation and dilution, the bottom few milliliters of the end-product are obtained. The end-product is considered to be SVF [8, 9]. Autologous adipose SVF contains a variety of cells: MSCs, pericytes, vascular adventitial cells, fibroblasts, pre-adipocytes, monocytes, macrophages, red blood cells, fibrous tissue, and extracellular matrix (ECM) [8, 9].

### Stem cells in autologous adipose SVF

The number of stem cells contained in the adipose SVF can fluctuate widely. In adipose tissue, the numbers of nucleated cells can range from 500,000 to 2,000,000 cells per gram (g) of adipose tissue, and 1 to 10% of these nucleated cells are considered to be ASCs [12]. The number of ASCs in 1 g of adipose tissue may vary from 5000 to 200,000 stem cells [12]. Theoretically, in 100 g of adipose tissue, 0.5–20 million ASCs can be extracted in the SVF form. One of the reasons for such variation can be attributed to individual differences. Different patients have different adipose tissue texture and density [13]. Some of the adipose tissue is denser than the other, probably due to different amount of ECM.

In addition to differences in individual adipose tissue, collagenase may also play an important role, affecting the yield and viability of stem cells in SVF. High dosage or prolonged exposure to collagenase may be toxic to stem cells. Thus, an excess amount of collagenase can decrease stem cell viability. However, insufficient amount of collagenase may result in an inefficient and inadequate amount of stem cell yield [14]. Thus, using the correct amount of collagenase is very important. In addition, the correct type of collagenase is just as important. There are numerous types of collagenase available commercially. Collagenase is produced by two separate and distinct genes in the bacterium *Clostridium histolyticum*. The *col*G gene codes for type I collagenase and the *col*H gene codes for type II collagenase. Various enzymes such as elastase, trypsin, and/or papain can be added to these two types of collagenase to increase the specificity for certain tissues [15].

## Current clinical applications of autologous adipose SVF in cartilage regeneration

### Cartilage regeneration in OA

OA is a debilitating health problem common in elderly patient populations worldwide. Painful OA lowers quality of life by limiting the normal daily activities of patients [16, 17]. Current existing medical treatments aim to remedy symptoms only. Commonly prescribed treatments include non-steroidal anti-inflammatory drugs (NSAIDs), steroids, hyaluronic acids (HAs), and physical therapy. However, MSCs, in the form of autologous adipose SVF or culture expanded form, are an alternative therapy that can potentially treat the underlying cause of OA by regenerating cartilage.

One of the major drawbacks of applying autologous adipose SVF in orthopedic conditions is the lack of availability of randomized controlled studies. Most, if not all, literature available with regard to the human application of autologous adipose SVF are either in the format of case reports or cohort studies. Due to such constraints, despite the successful results reported by these articles, it is not yet readily accepted as a mainstream medical treatment.

In 2011, for the first time, Pak reported a case series of treating patients with OA of the knees with autologous adipose SVF and regenerating cartilage-like tissue [11]. Pak obtained autologous adipose SVF from digesting about 100 g of adipose tissue with collagenase and going through the centrifugation-dilution washing cycle as described by Zuk et al. [8, 9]. This autologous adipose SVF, with platelet rich plasma (PRP) and HA, was then injected percutaneously into the knee joints of two patients. After 3 months, the visual analog score (VAS) for pain, functional rating index (FRI), and range of motion (ROM) of the patients were assessed and shown to be improved along with MRI evidence of cartilage regeneration [11]. The inclusion criteria and exclusion criteria were listed as follows: Inclusion criteria: (i) chronic or degenerative joint disease causing significant functional disability and/or pain; (ii) the failure of

Table 1 Current use of adipose SVF containing ASCs for orthopedic applications

| Study (yr) | Intervention treatment | Study type | Number of subjects and diseases | Subject characteristic [age (yr); gender] | Previous therapy | Concurrent treatment | Follow-up (mo) | Outcome measures | Results | Authors' conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| Pak (2011) [11] | Adipose SVF (ASC) + PRP + HA via percutaneous injections | Case report | 2 OA | 70 and 79; 2 F with chronic knee pain | Various conservative treatments without any success | None | 3 | VAS; functions (FRI, ROM); MRI | VAS/function improvements and MRI evidence of cartilage regeneration | ASC + PRP + HA: potentially effective in regenerating cartilage in humans |
| Pak et al. (2013) [18] | Adipose SVF (ASC) + PRP + HA via percutaneous injections | Retrospective cohort study | 91 various orthopedic applications including OA | Mean 51.23 ± 1.50 (range, 18–78); 45 M and 46 F | Various conservative treatments without any success | None | 26.62 ± 0.32 | VAS; functions | Statistically significant improvement in both VAS and functions | ASC + PRP + HA: safe and potentially effective |
| Pak et al. (2016) [19] | Adipose SVF (ASC) + PRP + HA + ECM via percutaneous injections | Case report | 3 OA | 68; 1 M.<br>60 and 87; 2 F. | Various conservative treatments without any success | None | 3.5 | VAS; functions (FRI, ROM); MRI | VAS/function improvements and MRI evidence of cartilage regeneration | ASC + PRP + HA + ECM: potentially effective in regenerating cartilage in humans |
| Koh and Choi (2012) [20] | Adipose SVF (ASC)+PRP via percutaneous injections | Non-randomized, retrospective, comparative study: ASC + PRP vs PRP alone | 25 OA Study group (ASC + PRP): 25; control group (PRP alone): 25 | Study group: mean 54.1 (range, 34–69); 8 M and 17 F | Various treatments without any success | None | 16.4 | VAS; functions (Lysholm, Tegner) | ASC + PRP: more effective than PRP-control group | ASC+PRP: potentially effective in patients with cartilage defects |
| Koh et al. (2013) [21] | Adipose SVF (ASC)+PRP via percutaneous injections | Case series | 18 OA | Mean 54.6; 6 M and 12 F | Various treatments without any success | Arthroscopic lavage before knee-fat-pad-derived adipose SVF + PRP injection | 24.3 | VAS; functions (WOMAC, Lysholm); MRI | VAS/function/MRI improvements | ASC+PRP: effective in treating OA of knees |
| Koh et al. (2014) [22] | Adipose SVF (ASC) + PRP under arthroscopic guidance | Case series | 35 with 37 knee joints of OA | Mean 57.4 (range, 48–69); 14 M and 21 F | Various treatments without any success | Arthroscopic lavage before adipose SVF + PRP injection | 12.7 | VAS; functions; arthroscopy | 94% patients had excellent clinical improvement; 76% had abnormal repair tissue. | Scaffolds may be needed to treat patients with large cartilage lesions. |
| Koh et al. (2014) [23] | Adipose SVF (ASC) + PRP under arthroscopic guidance | Comparative study: adipose SVF + PRP vs PRP only | 44 OA | ND | Various treatments without any success | Open-wedge high tibial osteotomy | 24 | VAS; functions; arthroscopy | Adipose SVF + PRP is more effective than PRP alone. | ASC therapy, in conjunction with HTO, mildly improved cartilage healing and showed good clinical results compared with PRP only. |
| Koh et al. (2015) [24] | Adipose SVF (ASC) + PRP under arthroscopic guidance | Case series | 30 for adipose SVF + PRP injection; 16 for second look arthroscopy for OA | Mean 70.3 (range, 65–80); 5 M and 25 F | Various treatments without any success | Arthroscopic lavage before ASC + PRP injection | 24 | VAS; functions; 2nd look arthroscopy | VAS/function improvements; improved and maintained cartilage status | ASC+PRP: effective in treating elderly patients with OA |

Table 1 Current use of adipose SVF containing ASCs for orthopedic applications *(Continued)*

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|---|---|---|---|---|---|---|---|---|---|---|
| Kim et al. (2015) [25] | Adipose SVF (ASC) under arthroscopic guidance | Comparative study: adipose SVF vs adipose SVF + fibrin glue (as a scaffold) | 54 OA | Mean 57.5±5.8; 22 M and 32 F | Various treatments without any success | None | 28.6 | VAS; functions; arthroscopy | No significant difference | Clinical and arthroscopic outcomes of ASC implantation were encouraging for OA knees in both groups, although there were no significant differences in outcome scores between groups. |
| Bui et al. (2014) [26] | Adipose SVF (ASC) + PRP via percutaneous injections | Case series | 21 OA | >18; ND | Various treatments without any success | None | 8.5 | VAS; functions; MRI | VAS/function/MRI improvements | ASC + PRP: effective in treating OA of knees |
| Michalek et al. (2015) [27] | Adipose SVF (ASC) via percutaneous injections | Multi-center case control study | 1114 OA | Median 62 (range, 19–94); 589 M and 525 F | Various treatments without any success | None | Median 17.2 | VAS; functions | VAS/function improvements | Adipose SVF is a novel and promising treatment approach for patients with degenerative OA. ASC is safe and cost-effective. |
| Fodor et al. (2016) [28] | Adipose SVF (ASC) percutaneous injections | Case report | 6 OA | Mean 59 (range, 51–69); 1 M and 5 F | Various conservative treatments without any success | None | 12 | VAS; functions (WOMAC, ROM, TUG); MRI | VAS/function improvements but no MRI evidence of cartilage regeneration | Autologous adipose SVF is a safe and potential new therapy for pain reduction in knee OA. |
| Pak et al. (2013) [32] | Adipose SVF (ASC) + PRP via percutaneous injections | Case series | 3 chondromalacia patellae | 54; 1 M.<br>43 and 63; 2 F. All with chronic knee pain. | Various treatments without any success | None | 3 | VAS; functions (FRI, ROM); MRI | VAS/function improvements and MRI evidence of cartilage regeneration | ASC + PRP: effective in treating chondromalacia patellae patients |
| Pak et al. (2014) [37] | Adipose SVF (ASC) + PRP via percutaneous injections | Case report | 1 patient with meniscus tear | 32;1 F with chronic knee pain due to meniscus tear | Various treatments without any success | None | 3 | VAS; functions (FRI, ROM); MRI | VAS/function improvements and MRI evidence of cartilage regeneration | ASC + PRP: effective in treating cartilage defect lesions, including meniscus tear |
| Pak (2011) [11] | Adipose SVF (ASC) + PRP + HA via percutaneous injections | Case report | 2 osteonecrosis of femoral head | 47; 1 M.<br>29; 1 F. | Various conservative treatments without any success | None | 3 | VAS; functions (FRI, ROM); MRI | VAS/function improvements and MRI evidence of bone regeneration | ASC + PRP + HA: potentially effective in regenerating bone in humans |
| Pak (2012) [40] | Adipose SVF (ASC) + PRP + HA via | Case report | 2 osteonecrosis of femoral head | 34 and 39; 2 M | Various conservative treatments | None | 7 and 16 | VAS; functions (FRI, ROM); MRI | VAS/function improvements and MRI evidence | Regenerated bone by using ASC + PRP + HA may persist, representing |

Table 1 Current use of adipose SVF containing ASCs for orthopedic applications *(Continued)*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | percutaneous injections | | | | without any success | | | | of bone regeneration | potential future therapy for osteonecrosis. |
| Pak et al. (2014) [41] | Adipose SVF (ASC) + PRP + HA via percutaneous injections | Case report | 1 osteonecrosis of femoral head | 43; 1 M | Various conservative treatments without any success | None | 3 | VAS; functions (FRI, ROM); MRI | VAS/function improvements and MRI evidence of bone regeneration | ASC + PRP + HA: potentially effective in regenerating bone in humans |
| Saxer et al. (2016) [42] | Adipose SVF (ASC) + ceramic granules + fibrin gel | Case report | 8 proximal humeral fractures | 68; IM. Mean 70.4 (range, 62–84); 7 F. | None | Open reduction and internal fixation | 12 | VAS; biopsies; mCT | VAS improvements; biopsies and mCT evidence of bone (tissue) regeneration | Spontaneous bone tissue and vessel formation within a fracture-microenvironment with autologous adipose SVF |
| de Girolamo et al. (2016) [49] | Adipose SVF (ASC) vs PRP | Randomized prospective clinical trial | 56 patients with Achilles tendinopathy PRP group: 28; SVF group: 28 | PRP group: 46.6 6.2; ND. SVF group: 47.3 3.8; ND | None | None | 6 | VAS; functions (VISA-A, AOFAS, SF-36); US and MRI | VAS, VISA-A, AOFAS, SF-36 improved and structural changes on US and MRI | Both PRP and adipose SVF are safe and effective for Achilles tendinopathy but adipose SVF yields faster results. |
| Lee et al. (2015) [50] | Allogeneic adipose ASC+ fibrin glue | Open-label pilot study | 12 patients with lateral epicondylosis | Mean 51.8±9.5; 5 M and 7 F | None | None | 13 | VAS; functions (MEPI); US | VAS, MEPI improved; tendon defects improved | Allogeneic ASC is safe and effective in treating lateral epicondylosis |

*SVF* stromal vascular fraction, *ASC* adipose tissue-derived stem cells, *PRP* platelet-rich plasma, *HA* hyaluronic acid, *ECM* extracellular matrix, *OA* osteoarthritis, *yr* year, *mo* month, *M* male, *F* female, *ND* not described, *MRI* magnetic resonance imaging, *mCT* microcomputed tomography, *US* ultrasonography, *HTO* high tibial osteotomy, *VAS* visual analogue scale, *FRI* functional rate index, *ROM* range of motion, *WOMAC* western Ontario and McMaster Universities osteoarthritis index, *Lysholm* Lysholm scores, *Tegner* Tegner activity scale, *TUG* Timed up-and-go, *MEPI* mayo elbow performance index, *AOFAS* American Orthopaedic Foot & Ankle Society, *SF-36* Short Form-36, *VISA-A* Victorian Institute of Sport Assessment for Achilles tendinopathy

conservative treatments; and (iii) an unwillingness to proceed with surgical intervention. Exclusion criteria: (i) active inflammatory or connective tissue disease thought to impact pain condition (i.e., lupus, rheumatoid arthritis, and fibromyalgia); (ii) active non-corrected endocrine disorder that might impact pain condition (i.e., hypothyroidism and diabetes); (iii) active neurologic disorder that might impact pain condition (i.e., peripheral neuropathy and multiple sclerosis); (iv) pulmonary and cardiac disease uncontrolled with medication usage; (v) history of active neoplasm within the past 5 years; (vi) blood disorders documented by abnormal complete blood count (CBC) within 3 months including severe anemia, thrombocytopenia, leukocytosis and/or leukopenia; and (vii) medical conditions precluding the injection procedures.

Subsequently in 2013, Pak et al. reported a retrospective cohort study involving 91 patients with various orthopedic conditions [18]. Between the period of 2009 and 2010, Pak et al. treated 91 patients with OA of the knees, OA of the hips, and osteonecrosis of the femoral heads with percutaneous injections of autologous adipose SVFs along with autologous PRPs and HAs. The study reported the average efficacy of the regenerative treatment to be 65% at 3 months without any serious side effects and without any development of tumors. Some of the side effects reported were swelling and tendonitis [18].

In 2016, Pak et al. published a case series reporting that addition of autologous adipose ECM along with the SVF may also be effective when used together with autologous PRP and HA [19]. As in other reports, Pak et al. obtained autologous adipose SVF from digesting 100 g of adipose tissue with a collagenase. However, this time, unlike other reports, they added autologous adipose tissue-derived ECM, extracted by using an adipose tissue homogenizer, into the mixture of autologous adipose SVF, along with autologous PRP and HA. The mixture was injected into the knees of three patients with OA of the knees. Three months after treatment, all three patients' symptoms, measured using FRI, ROM, and VAS pain score, improved. In addition, comparison of pre-treatment and post-treatment MRI data of all three patients demonstrated cartilage-like tissue regeneration [19].

In 2012, Koh and Choi also reported a retrospective cohort study treating 25 OA patients with autologous adipose SVF with autologous PRP [20]. This group obtained autologous adipose SVF from digesting only 19 g of adipose tissue extracted from the knee fat pad. Koh et al. also used the centrifugation-dilution method described by Zuk et al. [8, 9]. As performed by Pak et al., these adipose SVFs with autologous PRP was percutaneously injected into the knees of 25 patients with OA after performing arthroscopic debridement and lavage. The article states that the mean Lysholm knee scoring scales, Tegner activity level scales, and VAS scores improved significantly in the treated group compared to the control group. No imaging studies were carried out. No major side effects were reported [20].

In 2013, Koh et al. reported a case series involving 18 patients with OA of the knees receiving autologous adipose SVF obtained from digesting only 9 g of adipose tissue from the knee fat pad [21]. The autologous adipose SVF with autologous PRP were percutaneously injected into knees of 18 patients after arthroscopic debridement and lavage. After a few months, the patients were evaluated with Western Ontario and McMaster Universities osteoarthritis index (WOMAC) scores, Lysholm knee scoring scales, and VAS scores and MRI studies. The patients improved on all criteria, including the cartilage whole-organ MRI scores. No serious complications were reported [21].

In 2014, Koh et al. reported a case series involving second-look arthroscopy results in 35 patients with knee OA treated with autologous adipose SVF [22]. In this report, Koh et al. incorporated arthroscopic guidance when injecting the knees with adipose SVF. Initially, the patients underwent arthroscopic examinations with debridement and lavage. Afterward, autologous adipose SVF with autologous PRP were injected under arthroscopic guidance. Only about 23 g of adipose tissue was used. About 12.7 months after treatment, second-look arthroscopy was performed. The results showed that the mean International Knee Documentation Committee (IKDC) and Tegner activity level scales significantly improved, but 76% of the patients had abnormal repair tissue observed during arthroscopy [22].

In another study reported by Koh et al. in 2014, the clinical results and second-look arthroscopy findings were compared between an autologous adipose SVF/PRP injection group and a PRP-only group [23]. This study involved 44 patients undergoing open-wedge high tibial osteotomies (HTO). This time, autologous adipose SVF were obtained from 120 g of adipose tissue from the patients' buttocks. Afterward, the autologous adipose SVFs were injected with autologous PRP in 23 patients under arthroscopic guidance and the other 21 patients were injected with autologous PRP alone under arthroscopic guidance. After 24 months of the treatment, the results showed that the autologous adipose SVF/PRP group showed significantly greater improvement than the PRP-only group, as measured by VAS for pain, Knee injury Osteoarthritis Outcome Score (KOOS) subscales for pain and symptoms, and second-look arthroscopic evaluation. Arthroscopic exams showed fibrocartilage regeneration in 50% of the adipose SVF/PRP group

versus 10% in the PRP-only group. However, the Lysholm score was similarly improved in both groups [23].

Later in 2015, Koh et al. reported another case series involving second-look arthroscopy results of 30 patients with OA of the knees treated with autologous adipose SVF obtained from 120 g of adipose tissue from the patients' buttocks [24]. The autologous adipose SVF was injected with PRP under arthroscopic guidance. Of the 30 patients, 16 patients underwent second look arthroscopies about 25 months after the initial treatment. Of the 16 patients, 10 patients (63%) had improved cartilage, 4 patients (25%) had maintained the cartilage, but 2 patients (12%) failed in healing cartilage defects. The study reported that all patients showed significant improvement in OA outcome scores (KOOS), VAS pain scale, and Lysholm score [24].

In another study, Kim et al. compared the efficacy of autologous adipose SVF alone to that of autologous adipose SVF with fibrin glue [25]. The fibrin glue was used as a scaffold for stem cells to attach. This study involved 54 patients with knee OA. Autologous adipose SVF was obtained from digesting 120 g of adipose tissue with collagenase. Of the 54 patients, 37 patients were treated with autologous adipose SVF only and the other 17 patients were injected with autologous adipose SVF with fibrin glue. After about 28 months, the mean IKDC score and Tegner activity level scale in both groups were compared and had improved significantly; the improvement was comparable in both groups. However, in second-look arthroscopies, International Cartilage Repair Society (ICRS) scores were better in the adipose SV with fibrin glue group [25].

In 2014, Bui et al. reported a case series involving 21 patients with OA of the knees [26]. The patients were treated with autologous adipose SVF with PRP. The adipose SVF was obtained from digesting 50–100 ml of lipoaspirates originating from the abdomen. Then, the autologous adipose SVF with autologous PRP was injected percutaneously into the diseased knees. After 8.5 months of treatment, all 21 patients showed improved VAS pain score and the Lysholm score. There was also a significant increase in the thickness of the cartilage, as depicted on the MRIs [26].

In early 2015, Michalek et al. reported a multi-center case-control study involving 1114 patients with OA of the knees and hips from four different countries (USA, Czech Republic, Slovakia, and Lithuania) [27]. These patients were percutaneously injected with autologous adipose SVF and followed for average 17 months. No serious side effects were reported and no incidents of cancer were reported. The clinical effects, measured on the basis of pain, non-steroid analgesic usage, limping, extent of joint movement, and stiffness, all improved. At 12 months after treatment, 63% of all patients reported approximately 75% symptom improvement and 91% of all patients reported approximately 50% of symptom improvement [27].

In 2016, Fodor et al., another group in the USA, reported clinical improvement of 8 knee OA patients treated with autologous adipose SVF obtained by digesting 150–250 ml of lipoaspirates [28]. All patients attained full activity with decreased knee pain. WOMAC scores, VAS pain scale score, ROM, and timed up-and- go (TUG) results all improved. The improvement in WOMAC scores and VAS scores were maintained at 1 year. Comparing preoperative MRI to 3-months postoperative MRI showed no detectable structural differences. No major side effects were observed [28].

### Chondromalacia patellae (CMP)

CMP is a knee joint disorder defined by cartilaginous softening of patellar bone cartilage and may cause patellofemoral pain syndrome (PFPS), which is characterized by anterior knee pain (AKP) along with malalignment of the tibio-patello-femoral joint [29, 30]. CMP can be diagnosed with MRI along with clinical history and physical examination [29, 30]. Currently, only symptomatic treatment is available. As in OA, commonly prescribed treatments include NSAIDs and physical therapy. Thus, CMP poses a major therapeutic challenge. However, as a few recent studies have shown the possibility of cartilage recovery using MSCs [31], the combination of autologous adipose SVF with correction of alignment may be a novel approach to treating CMP.

In 2013, Pak et al. reported a case series involving three patients with CMP of the knees [32]. Pak et al. treated these patients with autologous adipose SVF using 100 g of adipose tissue obtained from the abdomen of the patients. The adipose SVF was injected percutaneously with PRP and HA. After 3 months of treatment, the patients' symptoms improved in terms of VAS pain scale, FRI, and ROM. The study also showed positive regeneration of hyaline cartilage at the patellofemoral joints of all three patients between pre- and post-treatment MRIs [32].

### Meniscus tear

The meniscus is a fibrocartilaginous disk that functions to transfer weight, absolve shock to the knee, and to protect the hyaline cartilage at the knee joint [33]. With knee injuries, the meniscus may be damaged causing it to be torn. Such meniscus tears are initially treated conservatively with NSAIDs and physical therapy [34, 35]. If conservative treatment fails, an arthroscopic meniscectomy is traditionally performed. However, arthroscopic meniscectomy, either full or partial, is associated with early onset of OA of the knees [36]. Thus, potential

cartilage regeneration with MSCs, or autologous adipose SVF, may offer a major therapeutic breakthrough.

In 2014, Pak et al. reported that autologous adipose SVF may be effective in treating meniscus tears [37]. This case report involved one patient treated with autologous adipose SVF obtained from digesting approximately 40 g of packed adipose tissue with collagenase. Afterward, the autologous adipose SVF was injected with PRP and HA. After 3 months of treatment, the patient's symptoms, measured with VAS scores for pain, FRI, and physical therapy ROM, had improved. In addition, probable regeneration of the meniscus cartilage was documented by pre- and post-treatment MRIs [37].

## Current clinical applications of autologous adipose SVF in bone regeneration

Bone has an innate capability to regenerate. Upon fracture, resident progenitor stem cells work to form scarless healing [38]. However, a few clinical instances require therapeutic interventions to facilitate bone repair and regeneration.

### Osteonecrosis of the femoral head

Osteonecrosis of the femoral head is a debilitating skeletal disorder of unknown etiology that usually occurs in young males, can lead to collapse of the hip joint and may necessitate a total hip replacement [39].

In 2011, Pak reported that autologous adipose SVF may have the capability to regenerate bone in the lesion of osteonecrosis of the femoral head [11]. Pak obtained autologous adipose SVF from digesting 100 g of adipose tissue with collagenase. This autologous adipose SVF was then injected percutaneously with PRP and HA into hip joints of two patients. After 3 months, VAS for pain, FRI, and ROM of the hips were improved, and there was MRI evidence of bone regeneration [11].

Subsequently in 2012, Pak reported the long-term effect of autologous adipose SVF on bone regeneration in patients with osteonecrosis of the femoral head [40]. Of the two patients involved, one patient was followed for 7 months and the other patient for 16 months. The patients' symptom improved and the MRI showed positive bone regeneration in both patients. Both patients clearly showed maintenance of the regenerated bone for a relatively long time period [40].

In another case report, Pak et al. treated a patient with stage 1 osteonecrosis of femoral head with autologous adipose SVF [41]. Pak et al. obtained adipose SVF from digesting 100 g of adipose tissue with collagenase. The autologous adipose SVF with PRP and HA was injected into the femoral head under ultrasound guidance. Three months after the injection, patient's symptom completely resolved and the MRI findings of necrosis resolved completely as well. A subsequent MRI taken a few months later showed maintenance of the regenerated bone [41].

### Bone fracture

In a case report by Saxer et al. in 2016, autologous adipose SVF was used with ceramic granules within fibrin gel to treat proximal humeral fractures in conjunction with standard open reduction and internal fixation in eight patients [42]. Up to 12 months after the procedure, biopsies of the repair tissue were performed and demonstrated formation of bone ossicles that were structurally disconnected and morphologically distinct from osteoconducted bone, which suggests the osteogenic nature of implanted SVF cells. This study demonstrated spontaneous bone tissue and vessel formation within a fracture microenvironment with autologous adipose SVF [42].

### Non-union fracture

Although autologous adipose SVF may be indicated for treatment of a non-union fracture, there have not been any reports so far.

## Current clinical applications of autologous (or allogeneic) adipose SVF in tendon/ligament regeneration

For patients with chronic tendinopathy, conservative medical management, including anti-inflammatory drugs, physiotherapy, braces, and therapeutic exercises, has produced unsatisfactory outcomes [43, 44]. Although corticosteroid injection has been widely used for short-term pain relief, the effectiveness of the treatment is transient [45, 46]. In addition, by suppressing the cellular activity of human tenocytes and collagen synthesis, corticosteroid injections weaken the tendon, thereby increasing the risk of rupture [46, 47]. Injection approaches with dextrose solutions, whole blood, and platelet-rich plasma have been tried with limited evidences of success [48]. Potential regenerative MSC therapy, on the other hand, is emerging as a novel treatment for chronic tendinopathy.

### Achilles tendinopathy

In 2016, de Girolamo et al. reported a result of randomized prospective clinical trial involving 56 patients with Achilles tendinopathy [49]. Of the 56 patients, 28 patients were randomly assigned to a single autologous PRP injection and the other 28 patients were assigned to a single autologous adipose SVF injection. All patients were assessed clinically using VAS, Victorian Institute of Sport Assessment for Achilles tendinopathy (VISA-A), the American Orthopaedic Foot & Ankle Society (AOFAS) and Short Form-36 (SF-36) forms. Before the

treatments, all patients also underwent ultrasound imaging studies and MRIs; these were then repeated at 4 and 6 month follow-ups. At the final follow-up, both patients group showed significant improvements in all scores compared to baseline ($p < 0.05$). In the adipose SVF injection patients, these improvements were faster and more pronounced. After 6 months, the MRI and ultrasound studies showed no significant difference. No side effects were observed in either group. The study concluded that both PRP and SVF are safe and effective treatments for Achilles tendinopathy, although adipose SVF may allow faster clinical results than PRP [49].

### Lateral epicondylosis

Lee et al. published an article in 2015 involving 12 patients with lateral epicondylosis treated with allogeneic adipose-derived MSCs [50]. Although the scope of this article is limited to autologous adipose SVF, the study by Lee et al. is significant in light of the fact that an insufficient number of human studies are available with regards to tendon and ligament repair. The study is a pilot study assessing the safety and efficacy of culture expanded ASCs in treating human patients with lateral epicondylosis. The ASCs were injected with fibrin glue under ultrasound guidance into the hypoechoic tendon lesions of chronic lateral epicondylosis. Then, patients' VAS score, modified Mayo clinic performance index, and longitudinal and transverse ultrasound images of the tendon defect areas were evaluated at 6, 12, 26, and 52 weeks. Through 52 weeks of follow-up, VAS scores progressively decreased and elbow performance scores improved. Tendon defects, assessed by ultrasound images, also significantly decreased throughout the follow-up period. No significant adverse effects were observed [50].

## Discussion

Due to the current regulatory environment, culture expanded MSCs are considered to be a pharmaceutical product and require governmental clearance and approval. Autologous adipose SVF injection, on the other hand, is considered to be a medical procedure, and thus allowed in many parts of the world. Consequently, autologous adipose SVF is slowly being tried as an alternative treatment in the field of orthopedics, treating disorders involving cartilage, bone, and tendons/ligaments. Compared to bone marrow SVF, adipose tissue is considered to be a preferred source of MSCs in the form of SVF due to its ease of accessibility and the availability of a large number of stem cells per gram of adipose tissue [12].

Although numerous studies available that show the effectiveness of autologous adipose SVF treatment in OA patients, the comparison of these studies show lack of standardization. Lacking standardization may lead to differences in results of the treatment. Most of the standardization may be improved with availability of culture expanded stem cells [50, 51]. With differences of procedure in processing adipose tissue, the yield of viable stem cells may differ from one group to the other. However, with the availability of culture expanded stem cells, all variables that exist in the manual process may be eliminated, providing consistent quantity and quality of stem cells. With the standardized availability of culture expanded stem cells, the effectiveness would become more improved.

In addition, it should be well known that OA, CMP, and meniscus tear are all diseases of the joint, not just cartilage. In these joint problems, cartilage, ligaments, tendons, muscles, and bone are all involved. For example, CMP involves alignment of the knee. In patients with CMP, correction of only cartilage may not dramatically improve the symptoms unless the misalignment is also corrected. As for meniscus tear is involved, improving muscles, tendon, and ligament may also be important in addition to cartilage regeneration.

It seems the amount of autologous adipose tissue used in producing adipose SVF has no direct relationship with the efficacy and safety observed. Some of the studies used only 20 g of adipose tissue while others have used more than 100 g of adipose tissue. However, Jo el al. showed in a double-blind randomized clinical trial that higher number of stem cells may result in improved cartilage regeneration [51].

Although number of ASCs contained in autologous adipose SVF should play an important role in regenerative medicine, other components in the adipose SVF may also play important roles. Autologous adipose SVF contains various cells including ASCs and ECM [11, 12]. It is well known that ECM excretes a variety of cytokines and growth factors [52–54]. In addition, ECM may work as a scaffold, assisting ASCs to adhere to the lesion [55].

As Zuk et al. showed in 2001 and 2002, ASCs in the adipose SVF have the capacity to regenerate bone, cartilage, muscle, and adipose tissue. Likewise, human data is accumulating in the field of orthopedics that ASCs contained in adipose SVF can be applied to treat various disorders by regenerating cartilage and bone. Recently, a study clearly showed that regeneration of a tendon in a human patient is possible with autologous adipose SVF.

As shown by de Girolamo et al. [49], and to a certain extent by Lee et al. [50] since this group used culture expanded ASCs, adipose SVF can be used to treat tendon injuries. These results may be used to further extrapolate that adipose SVF and MSCs may be used in the treatment of ligament injuries.

Although the successful applications of autologous adipose SVF in humans may represent a promising,

minimally invasive, non-surgical alternative, many issues (challenges and limitations) need to be resolved and clarified before the general application of this procedure in clinics. Firstly, how ASCs in the form of SVF may help joint diseases remains unclear: (i) it could be due to the secretory effects of the stem cells injected [56, 57]; (ii) it could be due to direct engraftment and differentiation of the stem cells that were introduced into the diseased joints [58, 59]; or (iii) it could be due to the combination of secretory effects and direct engraftment of the stem cells. Adipose stem cells excrete a variety of cytokines, chemokines, growth factors, and exosomes [60, 61]. These factors have positive effects on the surrounding progenitor cells. However, there are some evidence that these stem cells injected may actually become engrafted into the tissue and differentiate into tissue-specific stem cells [62]. It is also very possible that these two mechanisms play a role in cartilage regeneration.

Secondly, how long can ASCs or SVF (after injection) stay in the joint before they are cleared out? To the best of our knowledge, most of fluid is reabsorbed within few days after the injection of SVF. However, the fate of ASCs injected into a joint is not yet clear. It can be assumed that ASCs may stay in the joint and be attached to the lesion via scaffold. ASCs that are attached and integrated may be able to survive prolonged period of time. However, it can be assumed that ASCs that are not integrated into the tissue may die slowly while excreting various trophic factors.

Thirdly, is proper control (e.g., control group only receiving saline washes but not stem cells) needed in order to make a fair conclusion that the effect of SVF seen here is due to SVF injection but not the washout of inflammatory cytokines by saline? Since it is possible that PRP may have a regenerative potential, three are studies comparing PRP to autologous SVF to confirm the regenerative effects of SVF [20, 23, 49]. However, it is necessary to have a controlled study comparing saline control group to autologous SVF group to confirm the real effect of SVF.

Fourthly, whether would the quality of ASCs affect the therapeutic effect? For example, will ASCs from obese vs non-obese patients have similar results of healing? It is very well known that people have different texture of adipose tissue as well as differences in adipose cell size [63]. Thus, the lipoaspirate tissue must be different in different individuals. Since the lipoaspirate processing step, including the dosage of the collagenase, is usually constant within the treating group, the end result of the different tissue must yield difference adipose SVF. Therefore, it is very probable that there may be some differences in quantity and quality of ASCs in obese and non-obese patients. Compared with ASCs from non-obese individuals, ASCs from obese individuals have showed increased proliferation and migration capacity but decreased differentiation capacity [64]. Multiple studies have documented the reduction in the osteogenic differentiation capacity of ASCs in obese individuals [65–67]. Therefore, there is a need for investigating whether ASCs from obese vs non-obese patients have similar results of healing of human orthopedic disorders.

Lastly, are all the cell types contained in SVF beneficial for disease healing? The adipose SVF contains numerous cells types, including red blood cells (RBCs), white blood cells (WBCs), adipocytes, along with MSCs [8, 9]. In addition, the adipose SVF may contain left-over collagenase, which can cause connective tissue damage, as it is being used to breakdown the connective tissue in the adipose tissue. These extra cells (RBCs and WBCs), either intact or fragmented, may elicit other responses. It is probable that the joint swelling after injecting the autologous adipose SVF may be due to these extra cells and/or collagenase contained in the SVF [18].

## Conclusions

Autologous adipose SVF, containing MSCs that are termed ASCs, has a great clinical potential to treat various orthopedic disorders as seen in human studies. Along with autologous adipose SVF, double-blind, randomized human clinical trials are being conducted using culture expanded MSCs with promising results. Until culture expanded stem cells are available for various orthopedic applications, autologous adipose SVF may be worthwhile to try in individuals for whom medical treatment has failed and for whom surgical options are not available.

### Abbreviations

AKP: Anterior knee pain; AOFAS: American Orthopaedic Foot & Ankle Society; ASC: Adipose tissue-derived stem cell; CMP: Chondromalacia patellae; ECM: Extracellular matrix; FRI: Functional rating index; HA: Hyaluronic acid; HTO: High tibial osteotomies; ICRS: International Cartilage Repair Society; IKDC: International Knee Documentation Committee; KOOS: Knee injury Osteoarthritis Outcome Score; MEPI: Mayo elbow performance index; MRI: Magnetic resonance imaging; MSC: Mesenchymal stem cell; NSAID: Non-steroidal anti-inflammatory drug; OA: Osteoarthritis; PFPS: Patellofemoral pain syndrome; PRP: Platelet rich plasma; ROM: Range of motion; SF-36: Short Form-36; SVF: Stromal vascular fraction; TUG: Timed up-and-go; VAS: Visual analog score; VISA-A: Victorian Institute of Sport Assessment for Achilles tendinopathy; WOMAC: Western Ontario and McMaster Universities osteoarthritis index

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Authors' contributions
JP and JHL conceived the idea and wrote the manuscript. KSP provided inputs for the design and final edition of the article. JP, JHL, KSP and MP participated in literature survey. LK and SHL critically revised the manuscript. All authors read and approved the final manuscript.

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Author details
[1]Stems Medical Clinic, 32-3 Chungdamdong, Gangnamgu, Seoul 06068, Republic of Korea. [2]TEDA-Puhua International Hospital, Tianjin 300457, People's Republic of China. [3]Life Science Institute, Komplek Permata Senayan, Jalan Tentara Pelajar, Jakarta Selatan 12210, Indonesia. [4]National Leading Research Laboratory, Department of Biological Sciences, Myongji University, 116 Myongjiro, Yongin, Gyeonggido 17058, Republic of Korea. [5]DNA Analysis Division, Seoul institute, National Forensic Service, 139 Jiyangro, Yangcheongu, Seoul 08036, Republic of Korea. [6]Department of Biological Sciences, Konkuk University, 1 Hwayangdong, Gwangjingu, Seoul 05029, Republic of Korea.

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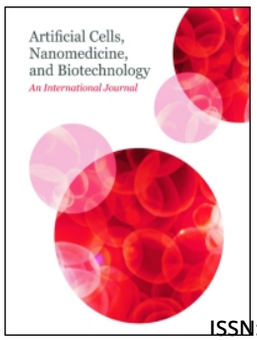

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# The use of stromal vascular fraction (SVF), platelet-rich plasma (PRP) and stem cells in the treatment of osteoarthritis: an overview of clinical trials

Sahar Mehranfar[a,b], Isa Abdi Rad[a,b], Ebrahim Mostafavi[c] and Abolfazl Akbarzadeh[d,e]

[a]Department of Genetics and Immunology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran; [b]Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran; [c]Department of Chemical Engineering, Northeastern University, Boston, MA, USA; [d]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; [e]Department of Medical Nanotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran;

**ABSTRACT**

Osteoarthritis (OA) is a major cause of disability across the world, which its prevalence is relatively high in elder population. Current accepted therapies such as exercise, anti-inflammatory drugs and intra-articular inoculation of corticosteroids are aimed at controlling symptoms in the affected patients. Surgical options including arthroplasty, osteotomy and joint replacement are other choices of treatment, which are invasive and can be applied in case of failure of conventional therapies. In the last few decades, efforts to treat musculoskeletal diseases are being increasingly focused on regenerative cellular therapies. Stromal vascular fraction (SVF), which obtained from adipose tissue, contains a variety of cells include mesenchymal stem cells (MSCs) and has shown to be effective in cartilage repair. Autologous blood products such as platelet-rich plasma (PRP) act as an adjuvant of surgical treatment and its intra-articular delivery has shown beneficial effects for OA treatment. Given the efficacy of such treatment approaches in OA, this paper discusses both preclinical and clinical evidence with major focus on clinical trials.

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Clinical trial; osteoarthritis; stem cell; platelet-rich plasma; stromal vascular fraction

## Introduction

Osteoarthritis (OA) is the most prevalent degenerative joint disease, which mostly impairs mobility and subsequent quality of life in elder individuals. Patients experience signs of pain, morning stiffness and a grating sound during joint motion known as crepitus. Although the pathogenesis of OA has been poorly understood, it has often defined with changes in articular cartilage. Tissue fluid, proteoglycans and type 2 collagen form the main structure of cartilage. Furthermore, chondrocytes, as the main cell type found in this area, can generate and maintain the extracellular environment. It has been reported that chondrocytes have no mitotic and regenerating capacities under physiologic condition. These cells can maintain the minimal turnover of collagens to make permanent structures in front of mechanical forces exerted on the joints. However, any mechanical stress or injury can stimulate chondrocytes to proliferate and increase their ability to synthesize the extracellular matrix as part of the repair process. The subsequent changes in matrix composition can induce chondrocytes to release catabolic factors leading to cartilage degradation. This can cause friction between bones and make pain and immobility in the affected patients [1].

Several risk factors include genetic, ageing, obesity and low-grade systemic inflammation have been described and are being the subject of ongoing research in OA [2]. Data from twin and familial aggregation studies have estimated 40–65% genetic risk for OA. The strongest genetic association has been reported with growth differentiation factor 5 (*GDF5*) gene, which originally identified with candidate gene-based approach. Moreover, during the last 10 years, genome-wide association studies (GWAS) have established the remaining association with 21 genetic loci. These associated loci include genes that are involved in pathways related to cell signalling, apoptosis, mitochondrial damage and extracellular matrix remodelling. Although each individual allele exerts moderate to small risk in OA pathogenesis, their identification helps to discover the whole mechanism of the disease. In addition, it helps to find biomarkers to detect high-risk individuals or improve disease outcomes in the affected patients [3].

Among several aforementioned risk factors of OA, the most prevalent one is ageing. Evidence has shown that OA and ageing are two linked but independent processes. To date, several mechanisms have been proposed to declare how the ageing-associated changes promote OA development [4]. The low-grade systemic inflammation, as one of the OA risk factors, is created when the mass of muscle decreased and the fat mass increased in the body. This metabolic condition, as seen in obesity, can change mechanical loading, which further increases adipokines and cytokines in the joint space [5]. Other mechanisms include mitochondrial

CONTACT Abolfazl Akbarzadeh dr.akbarzadeh2010@gmail.com Department of Chemical Engineering, Northeastern University, Boston, MA 02115, USA

dysfunction [6], oxidative stress [7], and reduced autophagy in chondrocytes [8], which increase the production of catabolic over anabolic factors. A kind of senescence has also been observed in chondrocytes that results in reduced sensitivity to different growth factors [9]. This phenomenon can increase inflammatory mediators as well as matrix-degrading enzymes in the joints. Moreover, senescence can cause telomere shortening as the much probable mechanism in cartilage damage [10]. Strategies for killing or modulating immune response in these senescence cells can be used as treatment options in OA.

Although OA is a very common illness, the existence of similar signs with other arthritis conditions makes it a difficult disease to diagnose. To date, no precise blood test has been recognized for OA diagnosis. However, imaging aids rheumatologists and radiologists for both diagnosis and longitudinal evaluation of the disease. In this regard, basic radiographs have been considered as the gold standard diagnostic tool in OA. In case of diagnostic uncertainty, other imaging techniques such as magnetic resonance imaging (MRI) and multidetector computed tomography (MDCT) can be used [11]. It has been reported that the structural changes of the joints can be seen with MRI, especially in early stages of the disease [12]. Computed tomography (CT)-based methods include four dimensional (4D)- and cone beam (CB)-CT can evaluate kinematic and weight-bearing characteristics of the joints. All the aforementioned techniques are cross-sectional and any changes in metabolic activities during synovial inflammation can be revealed through positron emission tomography (PET)-based analyses [13]. Moreover, recent research has reported that non-invasive detection of infiltrated innate immune cells can help to identify high-risk individuals [14].

## Treatment approaches

Many experiments in pathogenesis have revealed that OA is a generalized disease that affects different cells and tissues in the body. Therefore, a wider treatment approach is needed to target all the affected regions in the disease [15]. The goal of treatment in patients with OA is to reduce pain and stiffness, maintain the functional capacities as well as improving quality of life [16]. After diagnosis, physicians advise the patients to have low-impact aerobic exercise [17], lose their weight [18] and use nutraceuticals such as glucosamine and chondroitin sulfate [19]. Exercise helps to strengthen the muscle around the affected joints, which, in turn, reduces pain and instability in patients. There is also some evidence that patients can benefit from knee braces and shoe orthotics [20]. Aside from these non-pharmacological suggestions, patients may benefit from nonsteroidal anti-inflammatory drugs (NSAIDS) as well as intra-articular inoculations of corticosteroids and hyaluronic acid (HA) [21,22]. HA is a natural glycosaminoglycan, which provides lubricating and shock-absorbing capacities through acting as an osmotic buffer in the joints [23]. Some patients are advised to use fluoroscopic- and ultrasound-guided neural blockade [24,25]. These kinds of treatments can help to relieve symptoms and pain as well as preventing cartilage destruction in OA patients.

Surgical strategies in symptomatic patients are also beneficial for the management of OA in different types of tissues such as knee, hip and hands. These strategies can be conservative, in which the damaged cartilage is left in place, or radical such as arthroplasty, in which the total joint is replaced with an artificial prosthesis [26]. Arthroplasty has shown promising results in patients who have unsuccessful or contraindicated treatment modalities. However, like other treatment approaches, it has little but serious complications [27]. It should be noted that physicians should never prescribe arthroplasty for individuals younger than 60 years. These approaches are limited to focal lesions and may not be suitable in the field of OA, where the volume of cartilage loss is more generalized [28].

Limitations of previous treatment strategies include poor cell viability, limited supply and adverse effects on joint congruency have encouraged researchers to focus on regeneration rather than replacement of the affected tissues [29]. Progenitor cells are able to regenerate any damaged region in articular cartilage. However, the lack of vascularity in cartilage space prevents the infiltration of these cells [30]. Therefore, regenerative medicine helps to achieve this aim through the application of stromal vascular fraction (SVF), platelet-rich plasma (PRP) and stem cells in orthopaedics. Here, in this review, we aimed to discuss recent attempts about the use of SVF, PRP and stem cells in OA with a specific focus on clinical trial studies.

### *Stromal vascular fraction*

The ability of MSCs to differentiate into several cell lines such as chondrocytes makes them alternative treatment options in OA. Moreover, the anti-inflammatory effect of soluble factors released from these cells can halt cartilage destruction, a process that is created as the result of inflammation [31]. Those MSCs derived from adipose tissue (known as ADSC) gain several advantages over other tissue sources include abundance, the ease of harvest and stable phenotype after many culture passages [32]. Animal studies have proved the efficacy of ADSCs as a treatment option in OA and other related diseases.

The stromal vascular fraction (SVF), which is obtained after enzymatic digestion of adipose tissue, acts as a treatment choice and contains heterogeneous population of stem, progenitor and adult cells [33]. Those ADSCs available in SVF secrete several soluble factors with anti-inflammatory, immunomodulatory and analgesic effects. There are two approaches for delivery of SVF; intra-articular injection of cells suspended in platelet-rich plasma (PRP) and surgical implantation. In this regard, PRP has two advantages as an adjuvant. On the one hand, it provides growth factors to help better proliferation of stem cells, however, on the other hand, acts as scaffold for attaching cells to the site of cartilage damage. It has been reported that injection has several advantages over implantation including less invasiveness, better patient compliance and lower costs [34]. SVF has also its own advantages including the abundant stem cells it has, ease of extraction, availability of tissue supply and minimal invasiveness of the harvest procedure [15]. Nowadays, only one

randomized clinical trial is available for the application of SVF in orthopaedic situations [35]. Therefore, unlike successful results were achieved in many studies, no one can recognize SVF as a conventional treatment.

Almost all the studies evaluate the effect of SVF treatment on knee OA. A 3–36 months follow-up has shown improvements in pain and functional scores in all investigations. In 2011, Pak et al. conducted the first case-series that reported the efficacy of autologous SVF in OA patients. The visual analogue score (VAS), functional rating index (FRI) and range of motion (ROM) improved after three months, which is in line with cartilage regeneration [36]. In a retrospective cohort study, Pak and his colleagues injected SVF with PRP and HA into the knee, hip and femoral joints of patients with OA and observed the 50–60% improvement in ROM and VAS scores. Moreover, MRI has confirmed significant regeneration of cartilage defects. Similar efficacy of this combination has been reported at 3-month follow-up in this study. Some serious side effects such as swelling and tendonitis may somehow limit the SVF use in patients [37]. Kim et al. conducted another retrospective cohort study and found that SVF injection enhanced the efficacy of osteotomy regarding clinical outcomes. In some studies, the cartilage thickness increased as observed in MRI analysis [36,38,39]. Aside from injection approach, SVF implantation has been evaluated through second-look arthroscopy in different studies [40]. Again, all the studies showed improved condition, except one, which showed hyaline-like regenerative tissue in histological analysis of the joints. This observation was only seen in high-dose administration of SVF [38]. Koh et al. have conducted related studies for consecutive years. The study by Koh and Choi compared the effect of SVF with PRP alone, as treatment approach in control group. The authors have shown that SVF injection is safe; however, no significant difference in disease outcome has been reported between the groups [34]. In 2013, Koh et al. used autologous SVF and PRP after arthroscopic debridement in 18 patients with knee OA. The study has reported that this combination is safe and able to improve all related clinical criteria including VAS and Western Ontario and McMaster Universities Osteoarthritis (WOMAC) scores. No serious complications have also been reported upon treatment [39]. In another study by the same authors, outcomes from second-look arthroscopy and other clinical observations have shown that SVF plus PRP therapy mildly improved indices for pain and symptoms compared with another group who received PRP alone. Moreover, findings from arthroscopic examinations have shown more fibrocartilage regeneration in patients receiving SVF/PRP than PRP alone [40]. In a retrospective study in 2014, patients with knee OA received implanted ADSCs, which at first seemed to have great potential for treatment. However, second-look arthroscopy was shown that it had 76% success in repair [41]. Bui et al. conducted a case-series in 21 patients with knee OA who received SVF and PRP. The improved VAS and Lysholm scores, as well as increased cartilage thickness, have been reported after 8.5 months of treatment [38]. Another study in 2015 has reported improved cartilage defect in 63% of the patients who received autologous SVF. The treatment group have also shown better VAS, Lysholm and outcome scores [40]. A multi-center case-control study by Michalek et al. in 2015 has been conducted in patients with knee and hip OA. Upon receiving autologous adipose SVF, no serious side effects were reported in these patients. Clinical symptoms such as pain, stiffness, analgesic usage and extent of joint movement were improved which was estimated 75% in 63% of the patients [42]. In 2016, Pak et al. have found that autologous adipose extra-cellular matrix, when used in combination with SVF and PRP, can increase the effectiveness of treatment. All FRI, ROM and VAS scores were improved after three months of treatment in patients [43]. Fodor et al. have reported full activity and decreased pain in eight OA patients after autologous SVF therapy. Improvements in WOMAC and VAS scores were maintained after 1 year; however, no detectable structural differences were observed in MRI [44]. According to study by Bansal et al., pain levels of those patients who received SVF plus PRP have been reduced, especially after 3 months. Moreover, combinations of these treatment approaches with traditional exercise can make better improvements in the quality of lives of the OA patients [35]. Despite the observed benefits in above studies, all of them classified as case series with some limitations. Since SVF is always suspended in a volume of PRP, there is no information regarding the regenerative effects of pure SVF in OA patients. Moreover, the optimum times and modality of administration remain unknown. This underscores the need for randomized, double blind and placebo-controlled clinical trials of SVF therapy in OA (Table 1).

### *Platelet-rich plasma*

Platelet-rich plasma (PRP) is an autologous plasma product, which has four to five times more platelets than unprocessed blood plasma. Those many growth factors and inflammatory mediators, which released upon activation from pooled platelets, make PRP be potentially effective in orthopaedics. Moreover, the acceptability, non-invasiveness, and safety profile increase demands of PRP use in patients with OA [45]. Evidence suggests that direct injection of PRP can control the inflammatory environment of the joint [46]. One of the molecular mechanisms by which PRP exerts this controlling effect is preventing the activation of nuclear factor (NF)-kB target genes [47]. The inflammatory environment in chondrocytes from patients with OA contains interleukin (IL)-1β, which stimulates NF-kB to inhibit synthesis of anabolic related genes such as type 2 collagen [48]. Moreover, IL-1 receptor antagonist has been concentrated in PRP to exert the anti-inflammatory effect. Other anti-inflammatory effects of PRP are related to growth factor components in it. Some of these growth factors have the ability to control the NF-kB, however, others can suppress the expression of special chemokine receptor on the surface of cells at the site of inflammation [49]. Moreover, PRP increases the synthesis of proteoglycans and collagen as the same levels as in normal chondrocytes [50].

Many studies have reported that PRP administration has positive effects on patients with knee OA. In 2012, Gobbi et al. had shown that intra-articular injection of autologous

**Table 1.** Chronological list of studies regarding the application of SVF, PRP and MSCs in patients with knee OA.

| Type of therapy | Publication Year | Study type | Patient population | Study design | Follow-up | Outcome | References |
|---|---|---|---|---|---|---|---|
| SVF therapy | 2011 | Case-series | 3 women 1 man | ADSC HA PRP Calcium chloride | 3 months | Positive changes in MRI; Improvements in pain, physical therapy outcomes and functional status | [36] |
| | 2012 | Therapeutic case-control level III | 25 | SVF + PRP | 12 months | Improved Lysholm, Tegner and VAS scores; no adverse side effects | [34] |
| | 2013 | Case-series | 18 | SVF + PRP | 24.3 months | Improved WOMAC, Lysholm, VAS and whole-organ MRI scores | [39] |
| | 2013 | Retrospective cohort study | 91 | SVF + PRP | 26.62 ± 0.32 months | SVF is safe; no tumor formation; self-limited tendonitis and swelling | [37] |
| | 2014 | Case-series | 21 | SVF + PRP | 8.5 months | Improved joint function; Decreased pain score; Increased Lysholm score; Improved MRI findings; No serious side effects | [38] |
| | 2015 | Comparative study | 30 | SVF | 3, 12, 24 months | Improved clinical outcomes after 2-year follow-up | [77] |
| | 2015 | Case-series | 30 | SVF + PRP | 24 months | Improved clinical results and cartilage status under second-look arthroscopic analysis | [40] |
| | 2015 | Multi-center case-control study | 1114 | SVF | 17.2 months | Improved pain score and functional status | [42] |
| | 2016 | Case report | 3 | SVF + PRP + HA + ECM | 3.5 months | Improved FRI, ROM and VAS | [43] |
| | 2016 | Case report | 6 | SVF | 12 months | Improved pain, functional status; no MRI evidence of cartilage regeneration | [44] |
| | 2017 | Clinical trial | 10 | SVF + PRP | 3 months | Reduced WOMAC score; Improved cartilage thickness; safety of treatment | [35] |
| PRP therapy | 2012 | Case-series | 50 | PRP | 12 months | Improved pain, clinical scores and quality of life | [51] |
| | 2013 | Prospective cohort study | 22 | PRP | 12 months | Improved pain, functional and clinical scores | [78] |
| | 2013 | Randomized controlled trial | 78 | PRP | 6 months | Improved WOMAC score | [52] |
| | 2014 | Systematic review and meta-analysis | 1543 | PRP vs. HA | 6 to 24 months | Improved function; more effective than HA | [53] |
| | 2017 | Meta-analysis | 1069 | PRP | Variable | Similar pain relief and functional improvement at 6 months; better improvements for PRP at 12 months; PRP is safe | [54] |
| | 2018 | Meta-analysis | 1520 | PRP vs. HA | 6, 12 months | Similar effectiveness between PRP and HA | [55] |
| | 2018 | Randomized clinical trial | 89 | PRP vs. HA | 3, 6 months | Better improvement in pain and functional status for PRP; Improved synovial hypertrophy and vascularity scores | [56] |
| | 2018 | Randomized clinical trial | 42 | PRP vs. PRL | 6 months | PRP is more effective than PRL regarding pain, stiffness and functional limitations | [57] |
| | 2018 | Pilot study | 132 | Prior MP injection vs. PRP alone | 1, 3, 6, 12 months | Better clinical outcomes in prior MP injection | [58] |
| Stem cell therapy | 2013 | Double blinded controlled trial | 40 | Autologous Ad-MSCs | 6 months | Similar effectiveness in pain score compared to placebo | [70] |
| | 2014 | Double-blinded controlled trial | 46 | BM-MSCs | 12, 24 and 36 weeks | Significant clinical improvement after MSC treatment | [66] |
| | 2014 | Double-blinded controlled trial | 55 | Allogenic MSCs | 12 months | Meniscus regeneration and improved pain | [79] |
| | 2014 | Clinical trial | 18 | Ad-MSCs | 6 months | Safety; Improved WOMAC score; decreased cartilage defect | [71] |
| | 2015 | Double-blinded controlled trial | 30 | Allogenic BM-MSC | 12 months | Significant improvements in functional indices; more convenient than autologous MSCs | [67] |
| | 2016 | Clinical trial | 60 | BM-MSCs | 1, 3, 6, 12 months | Reduced pain in patients and repaired damaged cartilage in rats | [68] |
| | 2016 | Phase I/II multicenter randomized clinical trial | 30 | Autologous BM-MSCs | 12 months | Safety, clinical and functional improvement | [69] |
| | 2016 | Phase I Dose-Escalation Trial | 18 | Ad-MSCs | 6 months | Safety; Significant improvements in pain and function | [80] |

SVF: stromal vascular fraction; PRP: platelet-rich plasma; HA: hyaluronic acid; MRI: magnetic resonance imaging; VAS: visual analog scale; ECM: extracellular matrix; MSC: mesenchymal stem cell; ADSC: adipose-derived stem cell; WOMAC: Western Ontario and McMaster Universities osteoarthritis; FRI: functional rating index; ROM: range of motion; BM: bone-marrow; MP: methyl prednisone.

PRP in patients who experienced arthroscopic debridement and micro-fractures made significant improvement in disease activity and symptoms [51]. A total of 78 patients with bilateral OA enrolled into the trial and randomized into four intervention groups; single PRP injection, two PRP injections 3e weeks apart, single saline injection and placebo. Those who received two PRP injections had comparable effects regarding primary and secondary outcomes with single dose group. However, PRP had better results than saline in these patients [52]. According to a meta-analysis of five randomized controlled trials in 2014, which compared PRP with HA, PRP had more prolonged and better effectiveness than HA in patients who have degenerative knee OA [53]. In two recent similar meta-analyses, most of the studies have reported comparable outcomes between PRP and HA, however, the authors suggested that a large multicenter randomized trial is needed to determine the efficacy of PRP in OA patients [54,55]. The randomized clinical trial by Ahmad et al. have shown the efficacy of both PRP and HA regarding the reduced pain and improved functional status in patients. The authors suggested that even PRP made better results than HA. This trial has not been included in aforementioned recent meta-analyses and it does not have larger sample size than other included studies [56]. Very recent evidence compared the reducing effect of PRP and prolotherapy on pain and symptoms in patients with knee OA. An irritant solution like hypertonic dextrose is often injected in prolotherapy to stimulate proliferation of cells at the damaged site. In case of enthesitis, prolotherapy is more effective than PRP, however, findings from Rahimzadeh et al. study has reported that PRP is even more effective over time in treatment of OA patients. The authors have pointed out that the lack of control group, small sample size and lack of morphological assessments are among their study limitations [57]. As reported in a recent study, intra-articular injection of corticosteroids prior to PRP injection resulted in better outcomes in patients with mild to moderate OA [58].

Aside from studies including patients with knee OA, some randomized trials are also existed about other affected tissues like hip and ankle. To date, four studies compare PRP with HA in hip OA, which have conflicting results. This confliction has now been solved by a meta-analysis in 2018, which reported that PRP could reduce pain score at 2 months follow-up. However, this finding has not been approved at later months [59]. Just one study has pointed to the efficacy of PRP in patients with ankle OA, which again has shown that PRP can significantly reduce pain in these patients [60] (Figure 1).

Although unusual side effects have been reported for PRP, they are often mild and self-limited. Pain, allergic reactions and a grade of inflammatory response can be observed at the site of injection. If the aseptic conditions have not been considered during injection, infection can also be a serious side effect. Mild to moderate arthralgia is the most frequent event that often reported in patients, which can be resolved over time. Arthroscopic findings may report the hypertrophy of the cartilage tissue in some studies [61].

### Stem cells

Regeneration of the damaged cartilage is the main aim of therapy in degenerative osteoarticular diseases. There are a limited quantity of mesenchymal stem cells (MSCs) in normal joint fluid, which can differentiate into chondrocyte that further makes the new cartilage. However, this newly formed cartilage is fragile and easily destroyed by any minimal stress

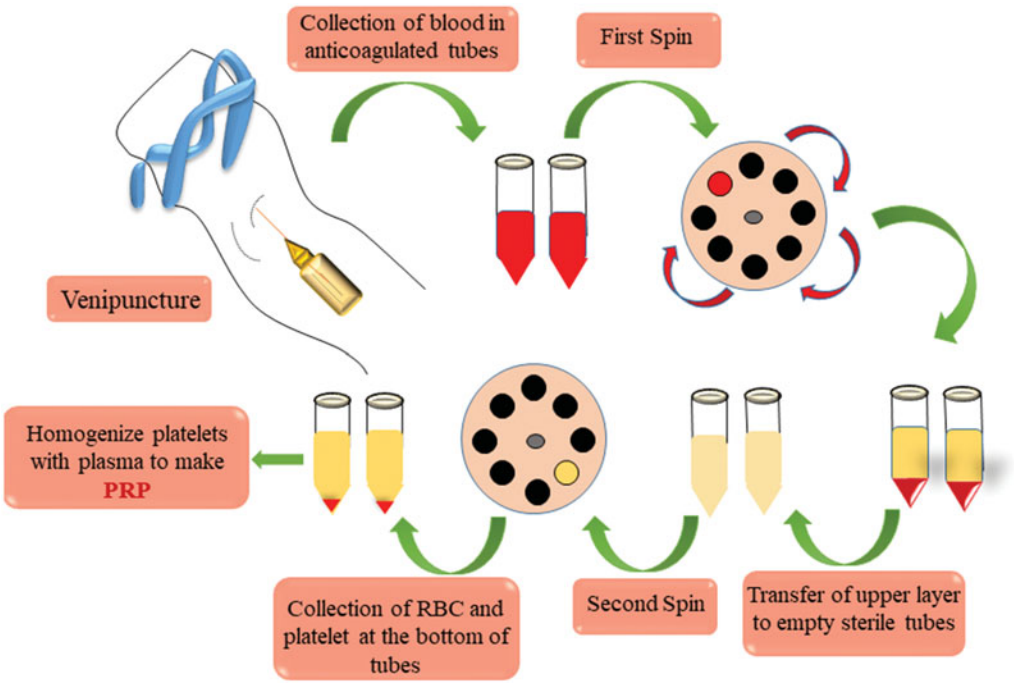


Figure 1. Steps for PRP preparation. The first step is to collect around 20 mL of whole blood in anticoagulated vacutainer tubes. Then, place the blood-filled tubes on a specially prepared centrifuge to spin at 600 ×g for 7 minutes. This results in formation of two phases, which the upper phase should be transferred to empty sterile tubes. In the next spinning stage, tubes are centrifuged at 2000 ×g for 5 minutes. First RBCs and then platelets are deposited at the bottom of the tubes and one can homogenize the platelet layer to available plasma to make PRP.

on the joint. Therefore, the need still exists for the application of exogenous MSCs, which have several advantages include lack of need for biopsy and ease of injection in the therapeutic field of OA.

Due to immunomodulatory and regenerative capacities of MSCs, some clinical trials have addressed their use in cartilage repair. There are different protocols for MSC therapy in patients. In most of the cases, cells are isolated and culture-expanded prior to injection; however, in other cases, cells can be harvested at one stage and finally injected. SVF-derived MSCs are the best example for the latter protocol. Other formulations in this group include stem cells derived from lipoaspirate, aspirated concentrate of bone marrow (BM) and microfragmentation, which is a non-enzymatic approach for isolation of BM vascular niche [62]. In 2012, Koh et al. isolated stem cells from infrapatellar fat pad (IFP) and then injected them to knees of OA patients underwent arthroscopic debridement. This approach was considered safe, reduced pain levels and improved function after one-year follow-up in patients. Evidence suggests that those cells isolated from IFP source have high chondrogenic potential than other cells [34].

The local microenvironment of culture media can affect MSC differentiation and *in vitro* expansion of these cells may change the properties of injected MSCs. However, limited number of MSCs are available in one-stage harvest protocol [63]. Therefore, most research, as well as our discussion in this review, have been focused on culture-expanded cells. In 2008, Centeno et al. reported that the use of autologous culture-expanded bone marrow-derived stem cells (BM-SCs) could improve pain and ROM in individuals with degenerative joint disease [64]. In a prospective unblinded controlled trial by Wong et al., injection of autologous cultured BM-SCs in patients underwent high tibial osteotomy and microfracture resulted in better scores for primary and secondary outcomes, as indicated by International Knee Documentation Committee (IKDC), Tegner and Lysholm clinical scores [65]. Another study by Aghdami et al. indicated similar improvement of clinical scores in patients with moderate to severe knee OA who were followed-up for 9 months. The authors conducted a double-blinded placebo-controlled study, in which the control group received carrier media as placebo. Decreased subchondral oedema in some patients, as well as increased cartilage thickness in the treatment group, is another important observation in that study [66]. Vega et al. conducted another study in 30 patients who were not satisfied with traditional treatments. The authors were divided patients into two treatment groups: intra-articular injection of BM-SC or HA and followed them until 1-year. Symptom improvement was more obvious in BM-SC-receiving individuals and the damaged area in cartilage had significant reduction as seen in clinical imaging. Another finding in this study was that the allogenic MSC is better than autologous regarding the ease of use. Moreover, expansion of allogenic cells is cheaper than autologous. Immune rejection is the main limiting issue while working with these cells [67]. Gupta et al. have also tried allogenic MSCs at different doses to be injected in knees and found that 25-million-cell dose can effectively reduce pain; however, no changes have been observed in imaging analysis [68]. In 2016, Espinosa et al. conducted a multicenter randomized controlled trial, in which individuals were randomly selected to receive autologous cultured BM-SCs or HA to assign either in treatment or control groups, respectively. All the participants were followed-up for 12 months, and the treatment group achieved good results especially in high-dose condition. Furthermore, radiographic evaluation has revealed reduced joint space only in control group. Joint damage was also decreased upon treatment [69].

Regarding studies on non-expanded stem cells, March et al. have observed that adipose-derived MSCs (Ad-MSCs) can reduce pain and improve symptoms in patients with knee OA [70]. One year later, Jo et al. decided to find the more appropriate strategy for treatment of generalized cartilage loss in OA. The study comprised two phases, which phase one included three dose-escalation cohorts and phase two began when patients received high doses of Ad-MSCs. Again, the primary and secondary outcomes, as well as the size of cartilage defect, have been improved in high-dose treatment group. In addition, histologic analysis has shown that hyaline-like cartilage regeneration was responsible for this improvement [71]. In a very recent study in 2018, a combination of in-vitro expanded Ad-MSCs with cell culture supernatant, known as progenza, was administered to patients with symptomatic knee OA. According to the findings, progenza was well-tolerated and induced significant improvements in patients. The authors claimed that any potential effect of progenza on disease modification warrants further studies [72].

## Limitations

There are some limitations regarding the application of PRP, SVF and stem cells in patients with OA. Most of the studies around the efficacy of PRP are case studies and preclinical investigations and few clinical trials are available in case of OA [73]. In addition, several protocols concerning the production of PRP exist; however, there is no consensus in methods that help us to select the gold standard. Some studies have declared that developing an antibody against bovine thrombin can initiate the activation of platelets [73]. Another issue that makes confusion is the dosage schedule of PRP in different studies [74]. SVF has many advantages that have been confirmed both *in vivo* and *in vitro* and those stem cells derived from SVF should fulfil the requirements of good manufacturing practices (GMPs) for clinical use. Other issues might be resolved before its application in clinics. What is the exact mechanism of action of ADSCs? How long do these cells stay in the joint to exert its local effects and which control group is more suitable to compare with SVF? Does SVF provide similar results if the source of adipose tissue obtained from obese individuals? Aside from the potential ability of MSCs in OA treatment, some limitations have been reported when using these cells. The availability of autologous cells might be scarce and tissue selection is important to minimize morbidity in patients. In addition, more

randomized clinical trials are required to better understand their positive impacts on patients with OA [75].

## Future perspectives and conclusion

The significant clinical burden of OA in populations highlights the importance of finding the most effective strategy for treatment. Cell-based therapies and regenerative medicine have provided effective results in the affected patients. PRP can reduce pain and improve functional status in the knee OA; however, imaging techniques have not pointed to any direct effect on cartilage. In addition, there is no calibration regarding the collection method and optimal treatment dosage of PRP in studies. Although SVF provides better quality of lives in OA patients, this kind of treatment is slightly aggressive to be used in humans. The efficacy of MSCs in cartilage repair is well established in animal, preclinical and phase I or II clinical studies. Other stem cell-based approaches such as the use of embryonic stem cells (ES) and induced pluripotent stem cells (iPS) are currently under investigation in animals. It has been reported that different tissue sources are available for iPS generation and using iPS is less invasive than MSC in regenerative medicine [76]. Finally, the efficacy of such treatments, as well as new stem cell-based approaches in OA, need to be confirmed by further controlled long-termed studies.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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Systematic Review

# Intra-articular Mesenchymal Stem Cells in Osteoarthritis of the Knee: A Systematic Review of Clinical Outcomes and Evidence of Cartilage Repair

Chul-Won Ha, M.D., Ph.D., Yong-Beom Park, M.D., Ph.D., Seong Hwan Kim, M.D., Ph.D., and Han-Jun Lee, M.D., Ph.D.

**Purpose:** To provide a systematic review of the clinical literature reporting the efficacy of mesenchymal stem cells (MSCs) in terms of clinical outcomes including pain and function and cartilage repair in patients with osteoarthritis. **Methods:** We systematically reviewed any studies investigating clinical outcomes and cartilage repair after the clinical application of cell populations containing MSCs in human subjects with knee osteoarthritis through MEDLINE, EMBASE, the Cochrane Library, CINAHL, Web of Science, and Scopus. Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed. Studies with a level of evidence of IV or V were excluded. Methodological quality was assessed using the Modified Coleman Methodology Score. Clinical outcomes were assessed using clinical scores, and cartilage repair was assessed using magnetic resonance imaging and second-look arthroscopy findings. **Results:** A total of 17 studies that met the criteria of 50 full-text studies were included in this review, with 6 randomized controlled trials, 8 prospective observational studies, and 3 retrospective case-control studies. Among 17 studies, 8 studies used bone marrow—derived MSCs, 6 used adipose tissue—derived stromal vascular fraction, 2 used adipose tissue—derived MSCs, and 1 used umbilical cord blood—derived MSCs. All studies except 2 reported significantly better clinical outcomes in the MSC group or improved clinical outcomes at final follow-up. In terms of cartilage repair, 9 of 11 studies reported improvement of the cartilage state on magnetic resonance imaging, and 6 of 7 studies reported repaired tissue on second-look arthroscopy. The mean Modified Coleman Methodology Score was 55.5 ± 15.5 (range, 28-74). **Conclusions:** Intra-articular MSCs provide improvements in pain and function in knee osteoarthritis at short-term follow-up (<28 months) in many cases. Some efficacy has been shown of MSCs for cartilage repair in osteoarthritis; however, the evidence of efficacy of intra-articular MSCs on both clinical outcomes and cartilage repair remains limited. **Level of Evidence:** Level III; systematic review of level I, II, and III studies.

*See commentary on page 289*

*From the Department of Orthopedic Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine (C-W.H.), Seoul, Republic of Korea; and the Department of Orthopedic Surgery, Chung-Ang University Hospital, Chung-Ang University College of Medicine (Y-B.P., S.H.K., H-J.L.), Seoul, Republic of Korea.*

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*Address correspondence to Yong-Beom Park, M.D., Ph.D., Department of Orthopedic Surgery, Chung-Ang University Hospital, Chung-Ang University College of Medicine, 102 Heukseok-ro, Dongjak-gu, Seoul 06973, Republic of Korea. E-mail: whybe1122@gmail.com*



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Articular cartilage has a limited capacity for spontaneous healing; therefore, any damage from trauma or degeneration ultimately progresses to osteoarthritis.[1] The current treatment approach to osteoarthritic cartilage defects is mainly palliative. A limited number of studies have reported that microfracture has led to improvements in pain and function in patients with osteoarthritis[2,3]; however, microfracture is understood to be most appropriate for small-sized lesions <2 to 4 cm and to deteriorate within a few years.[4,5] Although autologous chondrocyte implantation has been associated with improved structural and functional outcomes in young patients with focal chondral defects at long-term follow-up,[6-8] this technique is less optimal in elderly patients because of senescence or dedifferentiation of the proliferated chondrocytes.[9] Abrasion arthroplasty can be a valid treatment for cartilage lesions, but particularly for young patients

with small lesion.[10] Osteochondral autograft transfer (OAT) offers the advantage of restoring cartilage tissue as well as subchondral bony tissue but is limited to a small lesion and has donor site morbidity[11]; hence, there is no optimal cartilage repair method for patients with osteoarthritis. Mesenchymal stem cells (MSCs) have garnered significant attention in the field of regenerative medicine because of their self-renewal properties, multilineage differentiation potential, and immunomodulatory capacity.[12] In addition, recent studies supported the enhanced healing process of the host through the paracrine action of MSCs.[13-15] In light of successful preclinical studies on cartilage repair using MSCs,[16-18] the clinical application of MSCs for cartilage repair has been increasing. Many human tissues, including bone marrow, adipose tissue, umbilical cord blood, and synovium, are well-known sources of MSCs.[19]

Although some recent studies reported the clinical benefits of intra-articular MSCs in the treatment of osteoarthritis,[20-22] the clinical efficacy of MSCs in cartilage repair or cartilage protection in osteoarthritis has not been established. In addition, there is little consensus as which cell source, type of cell population, or delivery method should be used; therefore, the purpose of this study was to provide a systematic review of the clinical literature reporting the efficacy of MSCs in terms of clinical outcomes including pain and function and cartilage repair in patients with osteoarthritis. We hypothesized that the intra-articular MSCs would enhance clinical outcomes and allow for cartilage repair in patients with knee osteoarthritis.

## Methods

### Data and Literature Sources

This systematic review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[23] A literature search was undertaken in MEDLINE, EMBASE, the Cochrane Library, CINAHL, Web of Science, and Scopus. The date was restricted to all published studies until March 31, 2017. The search was conducted on April 30, 2017. The search specifics were: ("mesenchymal stem cell" OR "mesenchymal stromal cell") AND ("restoration of cartilage" OR "reproduce cartilage" OR cartilage) AND (human or clinical) NOT animal. A manual search for additional eligible studies that were not found by the automated search was performed using the reference lists of the included studies and relevant review articles. Identified articles were then assessed individually for inclusion. Abstracts and titles were screened for their relevance; then, the full text of the selected studies was reviewed for inclusion.

### Study Selection

Studies presented in the English language that assessed clinical outcomes and/or cartilage repair following the administration of a cell population containing MSCs in human knees with osteoarthritis with a level of evidence (LOE) of I, II, or III were eligible. The title and abstract of each publication were independently screened by 2 authors (C-W.H., Y-B.P.) for eligibility. Subsequently, the same 2 authors individually performed the full-text analysis. Disagreements regarding the inclusion of a given study were resolved by consensus or consultation with the other author (H-J.L.).

### Assessment of Literature Quality

LOE assessment of all included studies was performed by 2 authors (Y-B.P., S.H.K.) based on previously published criteria.[24] The methodological quality was also assessed by 2 authors (Y-B.P., S.H.K.) based on the Modified Coleman Methodology Score (MCMS).[25] The MCMS grades cartilage-related studies based on the following 11 criteria: study size, mean follow-up period, number of different surgical procedures, type of study, descriptions of the surgical procedure, descriptions of postoperative rehabilitation, inclusion of magnetic resonance imaging (MRI) outcomes, inclusion of histological outcomes, outcome criteria, procedure for assessing clinical outcomes, and descriptions of the subject selection process. The MCMS ranges from 0 to 100 for the grading of study quality as follows: a score $>85$ = excellent, between 70 and 84 = good, between 55 and 69 = fair, and $<55$ = poor.

### Assessment of Risk of Bias

Risk of bias was assessed using the Cochrane Collaboration's risk of bias tool by 2 authors (Y-B.P., S.H.K.) independently.[26] The following factors were assessed: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias. According to these items, each of included studies was scored as to be at low, unclear, or high risk of bias. Disagreements were resolved by discussion and assessed by kappa value.

### Grading of the Quality of the Evidence

The quality of the evidence was determined using the guidelines of the grading of recommendations, assessment, development and evaluation (GRADE) working group by 2 independent authors (Y-B.P., S.H.K.).[27] The grades of evidence definitions were the following categories: (1) high, defined as further research is unlikely to change confidence in the estimate of effect; (2) moderate, defined as further research is likely to have an important effect on confidence in the estimate of effect and may change the estimate; (3) low, defined as further research is very likely to have an important effect on our confidence in the estimate of effect and is

likely to change the estimate; and (4) very low, defined as any estimate of effect is very uncertain. Disagreements were resolved by discussion and assessed by kappa value.

### Data Extraction

Two authors (C-W.H., Y-B.P.) independently recorded data from each study on the study design, number of cases, concomitant treatment, source site, source (autologous or allogeneic), delivery methods, culture expansion, cell type, number of cells, alignment, activity level, postoperative activity protocol, surgical indication, number of surgeons and facilities, Kellgren-Lawrence grade, age, sex (female/male), body mass index, location, lesion size, follow-up, clinical outcomes, and cartilage repair evaluation using a predefined data extraction form. The identity of the cell populations was determined based on a consensus statement about nomenclature by the International Society of Cellular Therapy.[28] Cell populations were classified as bone marrow–derived MSCs (BM-MSCs), adipose-derived mesenchymal stem cells (ASCs), adipose-derived stromal vascular fraction (ADSVF), and umbilical cord blood–derived MCSs (UCB-MSCs).

## Results

After the selection process, 17 of 50 studies were included.[20-22,29-42] The selection process for the studies is shown as a flow diagram in Fig 1. The 17 studies included 499 knees with osteoarthritis. The mean age was 57.3 years. The Kellgren-Lawrence grade varied from grade 1 to 4. The mean follow-up period was 20 months (range, 6-84 months). Among these 17 studies, 6 were randomized controlled trials, 8 were prospective observational studies, and 3 were retrospective case-control studies.

### LOE and Quality of Evidence

There were 6 studies with LOE I, 8 with LOE II, and 3 with LOE III (Table 1). No studies were deemed excellent, whereas 9 (53.0%) were of poor quality (Table 1). The mean MCMS was 55.5 ± 15.5 (range, 28-74). Further details regarding the LOE and MCMS are shown in Table 2.

### Assessment of Risk of Bias

The results of assessment of risk of bias on included studies are summarized in Figure 2. All studies using autologous cells, which needed additional processing to obtain MSCs, were rated as having a high risk of performance or detection bias.[22,29-31,33-42] Moreover, all studies designed as an observational study or case-control study were rated as having a high risk of selection or performance bias because these design studies could not perform randomization.[21,22,29-31,33-36,39,40] The studies by Koh et al.[37] and Wakitani et al.[41] did not clearly report clinical outcomes or report specific scores completely and thus were rated as having an additional high risk of attrition and reporting bias. The studies of Vega et al.,[20] Koh et al.,[37] and Emadedin et al.[31] reported some clinical or image outcomes without specific scores; thus, the reporting bias for this study was rated as high. The number of included cases in the studies of Davatchi et al.,[30] Orozco et al.,[39] Emadedin et al.,[31] and Park et al.[21] was too small and were therefore rated as high in other bias. Moreover, the studies of Bui et al.,[29] Koh et al.,[36,37] Wakitani et al.,[41] Wong et al.,[42] Kim et al.,[34,35] and Park et al.[21] performed additional procedures including platelet-rich plasma (PRP) injection, high tibial osteotomy (HTO) or microfracture and thus were also rated as high in other bias. The interrater agreement according to the kappa value ranged from 0.73 to 0.86, which referred as good to excellent agreement.

### GRADE Evidence Quality of Each Outcome

GRADE evidence quality of each outcome is summarized in Appendix Table 1 (available at www.arthroscopyjournal.org). Five outcome categories were evaluated that are frequently used clinically. There were 1 of high quality, 6 of moderate quality,[22,32,33,38,40,42] 5 of low quality,[21,34,35,37,41] and 5 of very low quality[29-31,36,39] regarding final grade of evidence for each study. The final grade of evidence in outcomes of visual analog scale, Western Ontario and McMaster Universities Osteoarthritis Index, Lysholm and Tegner, International Knee Documentation Committee (IKDC), Knee Society Score, and Hospital for Specific Surgery (HSS) scores were low or very low because of the heterogeneity of included studies, however. The quality of study design showed limitations because many prospective observational studies and any other evidence of studies, such as case-control study, were included in this review. The interrater agreement of the final grade of evidence according to the kappa value was found ranged as 0.82 to 0.89, which is considered excellent agreement.

### Identity of the Cell Population, Cell Source, and Delivery Method

The study design, identity of the cell population, cell source site, cell source, delivery method, number of cells, alignment, activity level, postoperative activity protocol, surgical indication, and number of surgeons and facilities are summarized in Table 2 and Appendix Table 2. In terms of the cell population identity, 8 studies used BM-MSCs, 2 used ASCs, 6 used ADSVF, and 1 used UCB-MSCs. With regard to cell source, 14 studies used autologous cells, whereas 3 used allogeneic cells. With terms of delivery method, 7 studies delivered cells using 2-stage injection (direct injection of autologous cells after culture expansion), 2 used direct

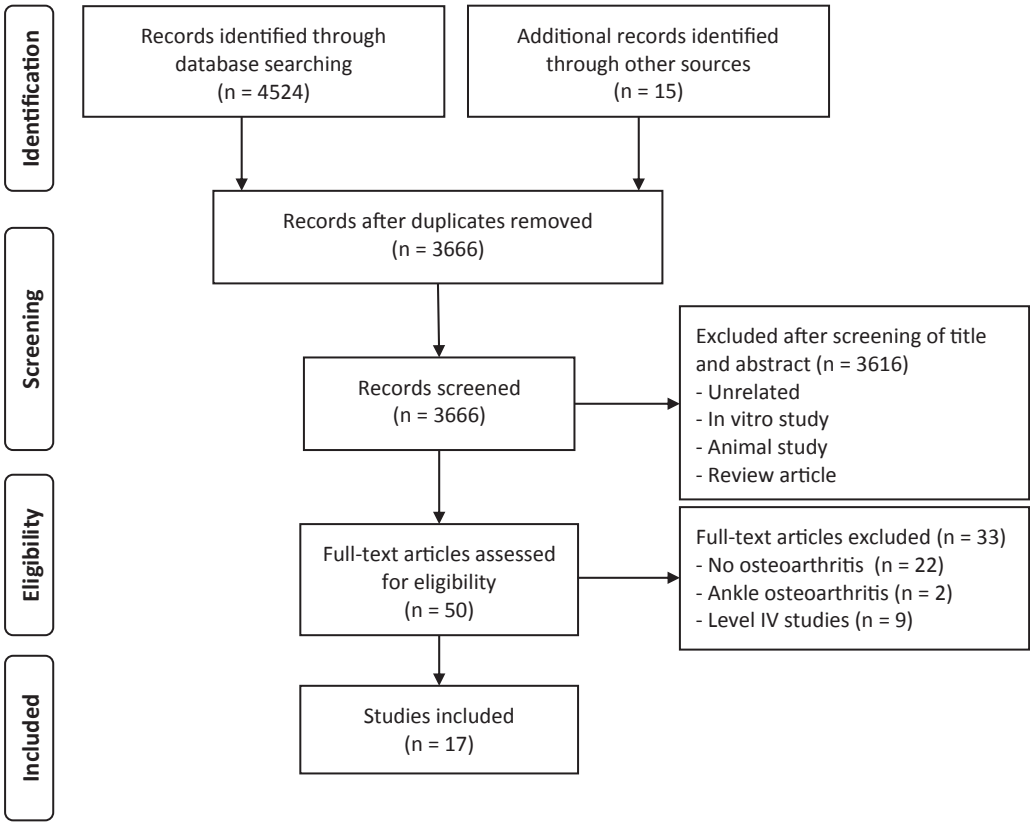


**Fig 1.** Flow diagram of the selection process for articles.

injection without arthroscopic guidance, 2 used direct injection under arthroscopic guidance (direct injection of autologous cells without culture expansion), 2 used direct injection both with and without arthroscopic guidance (direct injection of autologous cells without culture expansion), 2 used 1-stage injection (direct injection of culture-expanded allogeneic cells), 1 used 2-stage implantation (implantation through an arthrotomy of autologous cells after culture expansion), and 1 used 1-stage implantation (implantation through an arthrotomy of culture-expanded allogeneic cells).

**Table 1.** The Level and Quality of Evidence of the Clinical Studies

| | Study, n (%) |
|---|---|
| Level of evidence | |
| I | 6 (35.3) |
| II | 8 (47.1) |
| III | 3 (17.6) |
| Quality of evidence* | |
| Excellent | 0 (0.0) |
| Good | 4 (23.5) |
| Fair | 4 (23.5) |
| Poor | 9 (53.0) |

*The quality of evidence was classified according to the Modified Coleman Methodology Score (0-100): >85 = excellent, between 70 and 84 = good, between 55 and 69 = fair, and <55 = poor.

Among 17 studies, 9 involved concomitant treatments including HTO, PRP injection, microfracture, multiple drilling, or hyaluronic acid injection.

## Clinical Outcomes

Clinical outcomes are summarized in Table 3. Among 17 studies, 15 reported improvements in clinical outcomes, whereas 2 reported no improvement or no difference. Among 7 studies involving comparison with a control group, 4 studies reported better clinical outcomes in the MSC group,[20,37,38,42] 2 reported no difference,[32,41] and 1 reported no difference at final follow-up, with poor baseline outcomes in the MSC group.[36] All 8 prospective observational studies reported improved clinical outcomes at final follow-up. One study compared the intra-articular injection of autologous ADSVF with PRP to the intra-articular injection of autologous ADSVF under arthroscopy with a fibrin scaffold.[35] Significant improvements were shown in both groups, and there were significant differences in the IKDC scores at final follow-up (55.8 in injection vs 64.8 in arthroscopy, $P = .049$). The authors concluded that injection with fibrin under arthroscopy was a superior method for treating osteoarthritis. In a study that evaluated the effect of a fibrin scaffold on ADSVF therapy for osteoarthritis,[34] IKDC scores and the Tegner

**Table 2.** Details of Studies on Osteoarthritis Using MSCs

| Author(yr) | LOE | MCMS | Study Design | No. of Cases (Study/Control) | Concomitant Treatment | Source Site | Source | Delivery Method | Culture Expansion | Entity of Cells | No. of Cells |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Wakitani (2002) | I | 54 | RCT | 24 (12/12) | HTO | BM | Autologous | 2-stage implantation | 20 d | BM—MSCs | $1.3 \times 10^7$ |
| Davachi (2011) | II | 39 | POS | 4 | None | BM | Autologous | 2-stage injection | 4-5 wk | BM—MSCs | $0.8\text{-}0.9 \times 10^7$ |
| Emadedin (2012) | II | 50 | POS | 6 | None | BM | Autologous | 2-stage injection | 7 d 2 passages | BM—MSCs | $2\text{-}2.4 \times 10^7$ |
| Wong (2013) | I | 73 | RCT | 56 (28/28) | HTO, microfracture | BM | Autologous | 2-stage injection | 22 ds Passage | BM—MSCs | $1.4 \times 10^7$ |
| Orozco (2013) | II | 50 | POS | 12 | None | BM | Autologous | 2-stage injection | 22 d | BM—MSCs | $4 \times 10^7$ |
| Vega (2015) | I | 74 | RCT | 30 (15/15) | Control: HA | BM | Allogeneic | 1-stage injection | 22 d | BM—MSCs | $4 \times 10^7$ |
| Gupta (2016) | I | 73 | RCT | 60 (40/20) | HA injection | BM | Allogeneic | 1-stage injection | 21 d | BM—MSCs | $2.5\text{-}15 \times 10^7$ |
| Lamo-Espinosa (2016) | I | 65 | RCT | 30 (20/10) | HA | BM | Autologous | 2-stage injection | 3-4 wk | BM—MSCs | $1, 10 \times 10^7$ |
| Jo (2014) | II | 69 | POS | 18 | None | Adipose | Autologous | 2-stage injection | 21 d | ASCs | $1.0, 5.0, 10.0 \times 10^7$ |
| Pers (2016) | II | 69 | POS | 18 | None | Adipose | Autologous | 2-stage injection | 14 d | ASCs | $0.2, 1, 5 \times 10^7$ |
| Koh (2012) | III | 28 | Case control | 50 (25/25) | PRP | Adipose | Autologous | Direct injection | No | ADSVF | $0.12\text{-}0.23 \times 10^7$ |
| Bui (2014) | II | 47 | POS | 21 | PRP | Adipose | Autologous | Direct injection | No | ADSVF | NS |
| Koh (2014) | I | 72 | RCT | 44 (23/21) | HTO, PRP | Adipose | Autologous | Injection under arthroscopy, direct injection | No | ADSVF | $4.83 \times 10^7$ |
| Kim (2014) | III | 34 | Case control | 56 (17 fibrin, 39 no fibrin) | None | Adipose | Autologous | Injection under arthroscopy | No | ADSVF | $4.2 \times 10^7$ |
| Kim (2015) | III | 34 | Case control | 40 (20 injection, 20 surgery) | PRP in injection, fibrin in surgery | Adipose | Autologous | Direct injection, injection under arthroscopy | No | ADSVF | $4.0 \times 10^6$ (MSCs) |
| Kim (2016) | II | 62 | POS | 24 | None | Adipose | Autologous | Injection under arthroscopy | No | ADSVF | $4.9 \times 10^7$ |
| Park (2016) | II | 50 | POS | 6 | Multiple drilling ($5 \times 5$ mm) | Umbilical cord blood | Allogeneic | 1-stage implantation | 6 Passage | UCB-MSCs | $1.15\text{-}2.00 \times 10^7$ |

ADSVF, adipose-derived stromal vascular fraction; ASCs, adipose-derived mesenchymal stem cells; BM, bone marrow; BM-MSCs, bone marrow–derived mesenchymal stem cells; HA, hyaluronic acid; HTO, high tibial osteotomy; LOE, level of evidence; MCMS, Modified Coleman Methodology Score; NS, not specified; POS, prospective observational study; PRP, platelet-rich plasma; RCT, randomized controlled trial; UCB-MSCs, umbilical cord blood–derived mesenchymal stem cells.

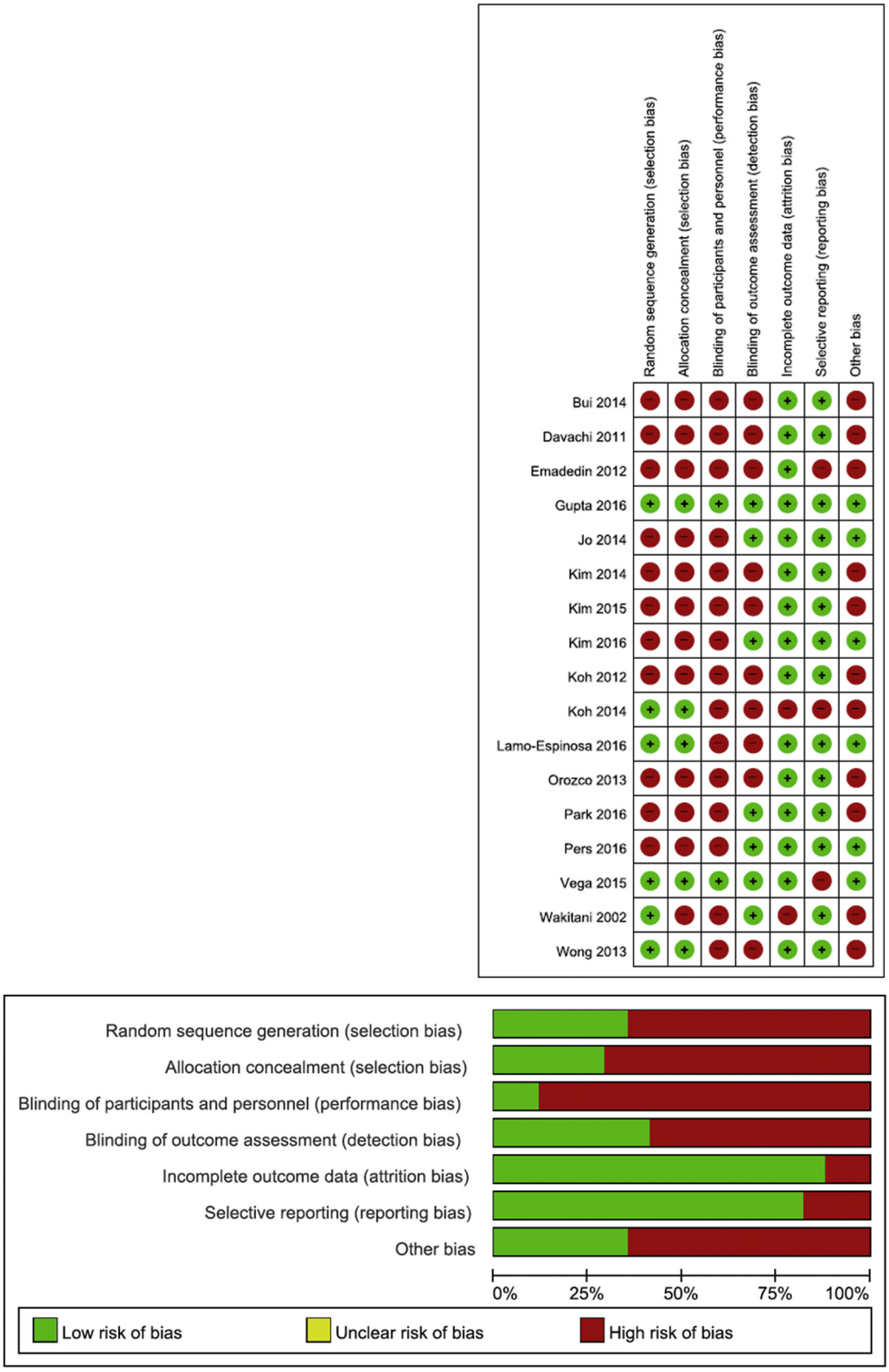


**Fig 2.** Risk of bias of included studies. Green circle, low risk; red circles, high risk.

activity scale showed significant improvement, but there was no significant difference directly associated with the use of a fibrin scaffold.

## Cartilage Repair Evaluation

In terms of cartilage repair, MRI was used in 11 studies and second-look arthroscopy was used in 7 (Table 4).

**Table 3.** Clinical Outcomes of Studies on Osteoarthritis Using MSCs

| Author (yr) | K-L Grade | Age* | Sex (F/M) | No. of Cases (Study/Control) | BMI* | Location | Lesion* ($cm^2$) | F/U* (mo) | Clinical Outcome | Description |
|---|---|---|---|---|---|---|---|---|---|---|
| Wakitani (2002) | Alback stages 1 and 2 | 63 | 15/9 | 24 (12/12) | NS | MFC/MTP | NS | 16 | HSS | 81.3 vs 79.2<br>No significant difference |
| Davachi (2011) | NS | 58 | 2/2 | 4 | 30.3 | NS | NS | 12 | Pain VAS, walking time, number of stairs | Pain, walking time, and number of stairs to climb improved |
| Emadedin (2012) | 4 | 55 | 6/0 | 6 | 31.6 | NS | NS | 12 | Pain VAS, WOMAC, walking distance | All outcomes improved |
| Wong (2013) | NS | 51 | 29/27 | 56 (28/28) | 23.9 (median) | Medial comp. | 5.0 (median) | 24 | IKDC, Lysholm, Tegner | All outcomes improved<br>Better scores in the MSC group* |
| Orozco (2013) | 2-4 | 49 | 6/6 | 12 | NS | NS | NS | 12 | VAS, WOMAC, SF-36 | All outcomes improved |
| Vega (2015) | 2-4 | 57 | 19/11 | 30 (15/15) | NS | NS | NS | 12 | VAS, WOMAC, Lequesne, SF-12 | All outcomes improved<br>Better improvement in the MSC group* |
| Gupta (2016) | 2-3 | 56 | 45/15 | 60 (40/20) | 27.8 | NS | NS | 12 | VAS, ICOAP, WOMAC | No significant differences in all groups |
| Lamo-Espinosa (2016) | 2-4 | 61 | 11/19 | 30 (20/10) | 28.4 | NS | NS | 12 | VAS, WOMAC | All outcomes improved<br>Better improvement in the MSC group*<br>Much improvement in the high-dose group |
| Jo (2014) | 3-4 | 62 | 15/3 | 18 | 26.3 | All comp. | 4.9 | 6 | VAS, KSS, WOMAC | Significant improvements mostly in the high-dose group |
| Pers (2016) | 3-4 | 65 | 10/8 | 18 | 27.6 | NS | NS | 6 | VAS, WOMAC, KOOS, SAS, PGA, SF-36 | All outcomes improved<br>Significant improvement in the low-dose group only |
| Koh (2012) | 2-4 | 54 | 34/16 | 50 (25/25) | NS | NS | NS | 16 (12-18) | VAS, Lysholm, Tegner | All outcomes improved<br>Poorer preoperative scores in the MSC group* |
| Bui (2014) | 2-3 | NS | NS | 21 | NS | NS | NS | 8.5 | VAS, Lysholm | All outcomes improved |
| Koh (2014) | <3 | 53 | 33/11 | 44 (23/21) | 25.2 | NS | NS | 24 | VAS, Lysholm, KOOS | All outcomes improved<br>Better VAS, KOOS-pain and sport in the MSC group* |
| Kim (2014) | 1-2 | 57 | 32/22 | 56 (17 fibrin, 39 no fibrin) | 26.6 | NS | 5.7 | 28 (24-34) | IKDC, Tegner | All outcomes improved<br>No difference between groups |
| Kim (2015) | 1-2 | 59 | 26/14 | 40 (20 injection, 20 surgery) | 26.8 | MFC, LFC, trochlea | 5.6 | 28 (24-42) | IKDC, Tegner | All outcomes improved<br>Better scores in the surgery group* |
| Kim (2016) | 1-2 | 58 | 15/9 | 24 | 26.6 | NS | 6.2 | 28 (24-34) | IKDC, Tegner | All outcomes improved |
| Park (2016) | NS | 59 | 4/2 | 6 | 26.4 | MFC/LFC | 5.9 | 84 | VAS, IKDC | All outcomes improved |

Comp., compartment; F/U, follow-up; HSS, Hospital for Specific Surgery; ICOAP, intermittent and constant osteoarthritis pain; IKDC, International Knee Documentation Committee; K-L, Kellgren-Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome Score; LFC, lateral femoral condyle; MFC, medial femoral condyle; MSC, mesenchymal stem cell; MTP, medial tibial plateau; NS, not specified; PGA, Patient Global Assessment; SAS, Short Arthritis Assessment Scale; SF-12. Short Form-12; SF-36, Short Form-36; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

*Data presented as mean.

**Table 4.** Evaluation of Cartilage Repair of Studies on Osteoarthritis Using MSCs

| Author (yr) | | MRI | Description | Second-Look Arthroscopy | Descriptions | Histologic Analysis | Description |
|---|---|---|---|---|---|---|---|
| Wakitani (2002) | | No | | 42-wk Arthroscopic score | Whitish repair tissue, softer than normal 10.4 vs 8.0* | Wakitani score | Hyaline-like cartilage 5.0 vs 2.7* |
| Davachi (2011) | | No | | No | | No | |
| Emadedin (2012) | | 6 mo | Cartilage thickness increase, extent of tissue repair | No | | No | |
| Wong (2013) | | 12 mo | MOCART score 62.3 vs 43.2* | No | | No | |
| Orozco (2013) | | 6, 12 mo | T2 mapping: poor cartilage area decreased (27%), quality improvement (11/12) | No | | No | |
| Vega (2015) | | 12 mo | T2 mapping: poor cartilage area decreased,* cartilage quality improved in the MSC group* | No | | No | |
| Gupta (2016) | | 6, 12 mo | WORMS: no significant change in score in all groups | No | | No | |
| Lamo-Espinosa (2016) | RCT | 6, 12 mo | WORMS: slight improvement only in high-dose group | No | | No | |
| Jo (2014) | | 3, 6 mo | Gradual regeneration over time<br>Decreased cartilage defect in the high-dose group* | 6 mo | White smooth surface<br>Improved ICRS grade* | ICRSII score | Hyaline-like cartilage, ICRS 21-52* |
| Pers (2016) | | 3-4 mo | dGEMRIC, $T_{1rho}$<br>3 of 6: possible improvement | 3 mo<br>11 of 18 | Severe OA | PS100, CD 34, Ki67 stain | Only 1: stem cell–grafted cartilage |
| Koh (2012) | | No | | No | | No | |
| Bui (2014) | | 6 mo | Partly regenerated cartilage | No | | No | |
| Koh (2014) | | No | | 19.8 mo (14-24) | Better ICRS grade in the MSC group* | No | |
| Kim (2014) | | No | | 12.3 mo (9-16) | Better ICRS grade with fibrin scaffold* | No | |
| Kim (2015) | | No | | 12.4 mo (10-15) | Better ICRS grade in the surgery group* | No | |
| Kim (2016) | | 24 mo | MOAKS: improvement in size and thickness of cartilage loss,* MOCART: 69.8 | No | | No | |
| Park (2016) | | 3 yr | dGEMRIC<br>Relative delta R1 index: 1.44 | 12 wk<br>1 yr | White smooth surface<br>Improved ICRS grade | 2 of 6 | Hyaline-like cartilage |

dGEMRIC, delayed gadolinium-enhanced magnetic resonance imaging of cartilage; ICRS, International Cartilage Repair Society; MOAKS, MRI Osteoarthritis Knee Score; MOCART, Magnetic Resonance Observation of Cartilage Repair Tissue; MSC, mesenchymal stem cell; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

*Indicates statistically significant difference between groups.

Based on MRI evaluation, 9 studies reported improvements in cartilage status, whereas 2 studies reported little or no improvement.[32,40] Among 4 comparative studies that used MRI evaluation, 2 reported significantly high Whole-Organ Magnetic Resonance Imaging Score (WORMS) scores for cartilage quality in the MSC group,[20,42] whereas 1 reported no significant difference in WORMS scores.[32] The other study reported improved WORMS scores for all groups at 6 months, which was deteriorated in the control and low-dose groups but maintained in the high-dose group at 12 months.[37] Among 7 prospective observational studies that used MRI evaluation, 6 reported improvements in cartilage repair, whereas 1 reported improvements on delayed gadolinium-enhanced MRI of cartilage and $T_{1rho}$ in 3 of 6 patients.[40]

On second-look arthroscopy, 6 studies reported improved cartilage status, whereas 1 reported that all patients showed signs of severe osteoarthritis (Osteoarthritis Research Society International histologic grade $> 3$).[40] In the 2 comparative studies that used second-look arthroscopy, improved arthroscopic scores or International Cartilage Repair Society cartilage grades were observed in the MSC group.[37,41] Histologic analysis was performed in 4 studies. Although 3 studies reported that histology showed hyaline-like cartilage, the remaining study reported that osteoarthritic chondrocytes were observed and that stem cell grafting on the cartilage surface was observed in only 1 of 11 cases.[40]

## Discussion

The principle findings of this study showed that intra-articular MSCs for the treatment of knee osteoarthritis had limited evidence for clinical outcomes and cartilage repair. Clinical outcomes such as pain and function were improved after the application of intra-articular MSCs at short-term follow-up in many cases. Several studies reported improved cartilage state after MSCs application; however, in randomized controlled trials, there were controversial results in clinical outcomes and cartilage repair. In addition, concomitant treatments were performed in several studies. Further high-quality studies with long-term follow-up are required to validate the clinical efficacy of MSC therapy in knee osteoarthritis.

This study showed that MSCs were very often associated with favorable clinical outcomes in osteoarthritis in terms of pain and function. Several assessment tools for pain and function were used to evaluate clinical outcomes, which involved patient-reported surveys assessing pain, functional level, activity level, and health status. Fifteen studies reported improvements in clinical outcomes or significantly better clinical outcomes in the MSC group, whereas 2 studies reported no benefit on clinical outcomes.[32,41] One study reported that there was no significant difference in clinical outcomes among all groups.[31] The other study reported that the improvement of the HSS score was higher in the MSC group from baseline to 16-month follow-up (16.3 vs 12.9), although the HSS scores at final follow-up were not significantly different (81.3 in the MSC group vs 79.2 in the control group). In several studies, HTO was performed at the time of surgery, which has been known to be effective in cases of knee osteoarthritis with varus deformity.[43] In addition, a recent review study reported that cartilage repair procedures in conjunction with HTO provided reliable functional improvement at mid- and long-term follow-ups and were associated with the potential for delayed or prevented knee arthroplasty surgery.[44] Some studies included in this review used PRP to enhance cartilage repair[29,35-37]; however, PRP has only shown pain relief and functional improvement in knee osteoarthritis at 1 year postinjection.[45] Overall, the follow-up period of the studies included in this review was short (mean, 20 months; range, 12-84 months). Most studies had follow-up periods <24 months, and only 1 study had a mid-term follow-up of 84 months.[21] Long-term studies without adjuvant treatments are required to evaluate the impact of MSCs in knee osteoarthritis.

The efficacy of MSCs on cartilage repair remains unclear in this review. Among 11 studies, 9 studies reported improved cartilage status on MRI evaluation; however, 3 randomized controlled trials without adjuvant treatment showed different results.[20,32,38] One study reported that improved cartilage quality was observed in the MSC group.[20] Another study reported that the MSC group showed no significant change from baseline to final follow-up and that there was no difference between groups in terms of the WORMS score.[32] The third study reported that, despite improved WORMS scores at 6 months in all groups, the scores were worse than baseline in the control and low-dose groups at 12 months and were maintained only in the high-dose group.[38] The remaining 3 randomized controlled trials showed improved cartilage status in the MSC group on either MRI evaluation at 12 months[42] or second-look arthroscopy at 10 and 20 months.[37,41] In all of those studies, however, HTO was performed at the time of MSC therapy. The efficacy of cartilage repair procedures with concomitant HTO is controversial. Some studies of HTO plus cartilage repair procedures showed good cartilage repair rates of >80%[46] and a higher incidence of a smooth cartilage surface compared with HTO without cartilage repair procedure.[47] Other studies, however, reported that HTO without a combined cartilage repair procedure was associated with the repair of degenerated articular cartilage.[48,49] In addition, a study comparing HTO plus

cartilage repair procedures and HTO alone reported no difference in cartilage repair between the 2 groups[50]; therefore, we believe that well-designed, long-term studies of MSC therapy without adjuvant treatments are necessary to accurately assess the efficacy of MSCs on cartilage repair in knee osteoarthritis. Moreover, further studies also need to determine the durability and quality of the repaired cartilage tissue and the association between the extent of cartilage repair and clinical improvement.

Because the existing clinical studies on MSCs have all used various types of cell populations, delivery methods, and adjuvant treatments, it was difficult to draw conclusions as to the effectiveness of MSCs on clinical outcomes and cartilage repair in knee osteoarthritis. The types of cell populations used in MSC therapy for knee osteoarthritis in the studies included in this review were BM-MSCs, ASCs, ADSVF, and UCB-MSCs. First, the various types of cell populations may lead to different clinical outcomes and degrees of cartilage repair because of variable chondrogenic differentiation potential and immunomodulatory capacity.[51-53] In addition, some studies erroneously used the term ASCs interchangeably with ADSVF, but the latter contains only a small amount of MSCs.[29,33-37] ADSVF is a pellet of cells derived from the centrifugation of lipoaspirates, which are heterogeneous cells containing pericytes, endothelial cells, smooth muscle cells, fibroblasts, and macrophages, along with a small fraction of ASCs.[28,54] Using the correct terminology is extremely critical to prevent confusion in interpreting the results of a given stem cell—based therapy and to correctly assess the scientific rationale for MSC therapy.[55] Regarding delivery methods, both surgical implantation and intra-articular injection have been used for MSC therapy in knee osteoarthritis. Osteoarthritis is a joint disease involving articular cartilage degeneration, synovial hypertrophy, and inflammation; therefore, it appears logical that MSCs be locally administered into the joint. As mentioned previously, several adjuvant treatments including HTO, PRP, hyaluronic acid injection, and arthroscopic debridement were performed in conjunction with MSC therapy, and HTO itself may improve pain, function, and degenerated cartilage status in knee osteoarthritis with varus deformity. Biological treatments such as PRP have gained attention because of their minimal invasiveness and lower cost,[56] and the application of PRP in knee osteoarthritis showed improvements in pain and function over a short period (12 months).[45] Hyaluronic acid is also recommended in knee osteoarthritis for short-term improvements in pain and function outcomes,[57] but, to date, only limited evidence regarding the clinical benefit of MSCs for knee osteoarthritis has been reported. Clearly, many aspects of MSC therapy still require to be optimized and standardized.

### Limitations

Several limitations needs to be addressed. First, some outcome assessment tools were used to evaluate clinical outcomes; therefore, it was difficult to assess quantitatively using specific outcome as a primary outcome. Second, different cell populations, cell sources, and delivery methods were used in the included studies. This heterogeneity could induce different clinical outcomes and cartilage repair. Finally, several adjuvant treatments including HTO that could affect clinical outcome and cartilage repair were used in several studies. Because of this, we did not perform a quantitative analysis of the studies reviewed, which limits the conclusions made by this systematic review.

## Conclusions

Intra-articular MSCs provide improvements in pain and function in knee osteoarthritis at short-term follow-up in many cases. Some efficacy has been shown of MSCs for cartilage repair in osteoarthritis; however, the evidence of efficacy of intra-articular MSCs on both clinical outcomes and cartilage repair remains limited.

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REVIEW ARTICLE OPEN

# Effectiveness of mesenchymal stem cells for treating patients with knee osteoarthritis: a meta-analysis toward the establishment of effective regenerative rehabilitation

Hirotaka Iijima[1,2,3], Takuya Isho[2,4], Hiroshi Kuroki[2], Masaki Takahashi[1] and Tomoki Aoyama[2]

This systematic review with a meta-analysis aimed to summarize the current evidence of the effectiveness of mesenchymal stem cell (MSC) treatment for knee osteoarthritis (OA) and to examine whether rehabilitation is an effect modifier of the effect estimate of MSC treatment. A literature search yielded 659 studies, of which 35 studies met the inclusion criteria ($n = 2385$ patients; mean age: 36.0–74.5 years). The meta-analysis results suggested that MSC treatment through intra-articular injection or arthroscopic implantation significantly improved knee pain (standardized mean difference [SMD]: −1.45, 95% confidence interval [CI]: −1.94, −0.96), self-reported physical function (SMD: 1.50, 95% CI: 1.09, 1.92), and cartilage quality (SMD: −1.99; 95% CI: −3.51, −0.47). However, the MSC treatment efficacy on cartilage volume was limited (SMD: 0.49; 95% CI: −0.19, 1.16). Minor adverse events (knee pain or swelling) were reported with a wide-ranging prevalence of 2–60%; however, no severe adverse events occurred. The evidence for these outcomes was "very low" to "low" according to the Grades of Recommendation, Assessment, Development and Evaluation system because of the poor study design, high risk of bias, large heterogeneity, and wide 95% CI of the effects estimate. Performing rehabilitation was significantly associated with better SMD for self-reported physical function (regression coefficient: 0.881, 95% CI: 0.049, 1.712; $P = 0.039$). We suggest that more high quality randomized controlled trials with consideration of the potential rehabilitation-driven clinical benefit would be needed to facilitate the foundation of effective MSC treatment and regenerative rehabilitation for patients with knee OA.

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## INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis.[1] OA ultimately results in cartilage degeneration, chronic knee pain, and disability. In 2010, knee OA was the 11th leading cause of disability worldwide, with increasing incidence over the last 2 decades.[2] Current treatments have little impact on the progressive degeneration of articular cartilage; therefore, developing effective and financially viable disease-modifying therapies is a critical medical priority.

Mesenchymal stem cells (MSCs) have emerged as a cell type with great potential for cell-based articular cartilage repair in patients with knee OA.[3] Clinical trials that investigate the effects of MSC treatments in patients with knee OA have recently begun emerging,[4] and results of clinical studies are continuously reported.[5,6] Several meta-analyses summarize the effects of MSC treatment in patients with knee OA;[7–10] these studies contribute to the establishment of effective cell-based therapies for degenerative cartilage disease. However, some of these systematic reviews included patients with focal cartilage lesions[8–10] or focused on pain and physical function as treatment outcomes,[7,9,10] with a large heterogeneity and lack of evaluation of bias risk.[7–9] As knee pain would be discordant with articular cartilage status, understanding the effects of MSC treatment against OA joint degeneration and exploring the mechanisms underlying symptom-modifying MSC treatment are important. In addition, confidence in the effects estimate from meta-analysis depends on the quality of the included studies and analytical process,[11] as the former can be evaluated using the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) approach.[12] However, no meta-analysis has examined the effects of MSCs on knee OA considering the GRADE approach.

Physical factors such as rehabilitation programs are potential effect modifiers that were not well addressed in previous meta-analyses.[7–10] Physical factors regulate MSC differentiation and tissue development, pointing to a potential therapeutic strategy for enhancing the MSCs injected or implanted into the knee joint,[13,14] such as the recently proposed new field "regenerative rehabilitation".[15] Regenerative rehabilitation is defined as the integration of principles and approaches from the fields of rehabilitation science and regenerative medicine.[16] The efficacy of regenerative medicine may be enhanced when coupled with mechanical input. Weight-bearing might influence the structural outcome in the postoperative phase of autologous chondrocyte implantation in adults with cartilage defects.[17,18] Thus, further investigation of the effects of MSC treatment in patients with knee OA and the potential role of rehabilitation (i.e., regenerative rehabilitation) as an effect modifier would be of interest.

[1]Department of System Design Engineering, Keio University, Yokohama, Japan; [2]Department of Physical Therapy, Human Health Sciences, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [3]Japan Society for the Promotion of Science, Tokyo, Japan and [4]Rehabilitation Center, Fujioka General Hospital, Gunma, Japan
Correspondence: Hirotaka Iijima (iijima.hirotaka.4m@yt.sd.keio.ac.jp)
Hirotaka Iijima and Takuya Isho contributed equally to this work.

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Potential adverse effects have a considerable impact on patient adherence to MSC treatment. To achieve a balanced perspective, a systematic review should consider the aspects of adverse events relevant to MSC treatment.[19] Randomized controlled trials (RCTs) would be insufficient to provide evidence of benefits and harms; thus, non-RCT, such as prospective cohort studies with long-term follow up periods should be included.[19] However, no systematic reviews have investigated adverse events after MSC treatment, even though previous systematic reviews included both RCTs and non-RCTs.[7–9] Thus, the purpose of this systematic review was (i) to examine the literature on the effects of MSCs in patients with knee OA in the clinical setting and to summarize the current evidence for their potential benefits and harms, and (ii) to examine whether rehabilitation is an effect modifier of effect estimate of MSC treatment. This study would provide a framework for a future high quality study with the aim of developing effective cell-based regenerative rehabilitation in patients with knee OA.

## RESULTS

eFigure 1 shows a flow chart of the study selection. The database search yielded 659 studies, of which 31 met the eligibility criteria. With the citation index, 4 additional studies were found in accordance with the pre-specified inclusion criteria provided in eMethod 1; in total, 35 studies were used in the meta-analysis.

### Study characteristics

Table 1 shows the characteristics of the included studies. Of 35 studies, 21 (60.0%)[20–40] had a single-arm prospective design, 7 (20.0%)[6,41–46] had a quasi-experimental design, and the remaining 7 (20.0%)[5,47–52] were RCTs. From the 35 studies, 2385 patients treated with MSC therapy were included. The mean age across 35 articles was 56.7 ± 6.78 years (36.0–74.5 years). In the 30 studies that reported sex ($n = 1975$ patients), 1119 patients (56.7%) were female. Twenty-nine studies (82.9%)[5,6,20,23–35,37–48,50] reported the radiographic severity of knee OA (i.e., Kellgren/Lawrence [K/L] grade); however, the eligibility criteria of disease severity differed between studies. The final follow-up period was 3–60 months. Fourteen studies (40.0%)[5,6,20,23–27,33,38,40,42,46,47] reported funding sources (eTable 1). Of the 35 studies, 25 (73.5%)[5,6,20–27,31–42,45,46,49] and 2 (5.7%)[47,50] used autologous and allogeneic MSC intra-articular injection, respectively. The other studies used arthroscopic autologous MSC implantation,[28–30,43,44] or a combination of these procedures with high tibial osteotomy.[48,51,52] The rehabilitation program included patients' education in the pre-MSC treatment phase, gradual increase in weight-bearing using crutches, use of physical therapy modalities, range of motion exercise, and muscle strength exercise (eTable 2). Notably, none of the included studies stratified for the presence of rehabilitation.

### Risk of bias within studies

A summary of the Downs and Black scale for assessing bias risk is shown in eTable 3. The mean score for all 35 studies was 6.1 ± 2.1 (range, 3–12); 5.5 ± 1.6 for single-arm prospective studies; 6.3 ± 1.0 for quasi-experimental studies; and 7.9 ± 3.2 for RCT. Only two studies[47,50] received a score of 1, for blinding of participants and assessors who measured key outcomes and concealed randomization of patients. The main differences between RCTs and non-RCTs included the reporting of patients' recruitment and adequate adjustment for confounders, which is important for assessing the external and internal validities of studies.

### Outcome measures

*Self-reported knee pain*. Nineteen studies with 27 data sets ($n = 318$) reported MSC treatment effects on knee pain by using the visual analog scale (VAS) pain score (Fig. 1). The mean follow-up period in these studies was 14.0 ± 12.9 months. The baseline VAS pain score in these studies was 60.2 ± 13.8 mm. Considering all 19 studies, the pooled standardized mean difference (SMD) on the VAS knee pain was −1.45 (95% confidence interval [CI]: −1.94, −0.96; $P < 0.001$). This statistical value implies a mean difference of 27.6 mm (95% CI: 13.4, 41.9 mm). However, effects estimates were highly heterogeneous among studies ($I^2 = 84\%$). Stratification for donor type (i.e., autologous vs. allogeneic) did not much improve the heterogeneity, but the pooled SMD in autologous MSC was likely to have a larger pain relief effects than those in allogeneic MSC. A meta-regression analysis indicated that a higher score of the Downs and Black scale (i.e., low risk of bias) is significantly associated with a higher (i.e., lower effect) SMD (eTable 4). Among the subitems of the Down and Black scale and SMD, clear patients' recruitment site was significantly associated with a higher SMD (eTable 5). Rehabilitation (i.e., using physical therapy modalities, range of motion exercise, or muscle strength exercise at least one time) was not an effect modifier of SMD (regression coefficient: 0.451, 95% CI: −1.909, 2.811; $P = 0.696$). Small-study effects were visually observed by two independent reviewers (eFigure 2), and the Egger's regression test was positive for significant evidence of publication bias ($P = 0.016$). By using the trim-and-fill method, the adjusted SMD was −0.93 (95% CI: −1.29, −0.56; $P < 0.001$).

To address the possibility that effect estimates on VAS pain score and heterogeneity change if only RCTs were included in the meta-analysis, we performed a sensitivity analysis (Fig. 2). Three RCT studies with 7 data sets ($n = 75$) were included, and the follow-up period of all these studies was 12.0 months. The baseline VAS pain score of these studies was 60.4 ± 9.2 mm. Including only RCTs attenuated the pain relief effects (pooled SMD: −0.67, 95% CI: −1.28, −0.05; $P = 0.030$). This statistical value implies a mean difference of 18.1 mm (95% CI: 1.35, 34.8 mm). However, effects estimates were still highly heterogeneous among the studies ($I^2 = 68\%$). Stratification for donor type slightly improved the heterogeneity, and the pooled SMD in autologous MSC was likely to have larger pain relief effects than those in allogeneic MSC. A meta-regression analysis indicated that a higher score in the Downs and Black scale and younger age were significantly associated with higher (i.e., lower effect) SMDs (eTable 6), and blinding of participants and assessors, valid outcome measures, and concealed allocation were significantly associated with higher SMDs (eTable 7). As all the included RCTs did not report a rehabilitation program, the regression coefficient could not be calculated. No small-study effect was visually observed by two independent reviewers (eFigure 3).

*Self-reported physical function*. Nineteen studies with 29 data sets ($n = 528$) reported MSC treatment effects on self-reported physical function by using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) functional, International Knee Documentation Committee (IKDC), and Lysholm scores (Fig. 3). The mean follow-up period in these studies was 17.0 ± 10.8 months. Considering all 19 studies, the pooled SMD on the self-reported physical function was 1.50 (95% CI: 1.09, 1.92; $P < 0.001$). This statistical value implies a mean difference of 14.7 (95% CI: 9.39, 20.0) in the WOMAC functional outcome (0–100 points); 26.0 (95% CI: 23.1, 28.9) in the IKDC (0–100 points); and 24.1 (95% CI: 19.0, 29.2) in the Lysholm score (0–100 points). However, effects estimates were highly heterogeneous among the studies ($I^2 = 86\%$). Pooled SMD in autologous MSC was likely to have a larger functional improvement effects than those in allogeneic MSC. A meta-regression analysis indicated that implantation technique (compared to injection), lower Downs and Black scale score, presence of rehabilitation, and absence of funding source were significant factors associated with higher (i.e., higher effect) SMDs (eTable 8), and blinding of participants, unblinding of assessors, unclear patients' recruitment site, non-randomization and non-concealed allocation were significant factors associated

**Table 1.** Summary of included studies

| Author | Subject population | KL grade | Treatment | Donor | Outcomes | Follow-up | Funding |
|---|---|---|---|---|---|---|---|
| Single-arm, prospective follow-up studies | | | | | | | |
| Bui 2014[20] (Vietnam) | $N = 21$ | II–III | SVF injection + PRP | Auto | Lysholm score, VAS pain, MRI | 1, 3, 6 M | X |
| Centeno 2008a[21] (Unites states) | $N = 1$ (age: 36 y; M) | – | BD-MSC injection ($4.56 \times 10^7$ cells) | Auto | VAS pain, MRI (cartilage and meniscus volumes) | 1, 3 M | – |
| Centeno 2008b[22] (Unites states) | $N = 1$ (46 y; M) | – | BD-MSC injection ($2.24 \times 10^7$ cells) | Auto | VAS pain, functional rating index, ROM, MRI evaluation (cartilage and meniscus volumes) | 1, 3, 6 M | – |
| Davatchi 2011[23] (Iran) | $N = 4$ (age: 57.8 ± 5.0 y; 50% F) | II–III | BD-MSC injection ($8–9 \times 10^6$ cells) | Auto | VAS pain, ROM | 6 M | X |
| Davatchi 2016[24] (Iran) | $N = 4$ (age: 57.8 ± 5.0 y; 50% F) | II–III | BD-MSC injection ($8–9 \times 10^6$ cells) | Auto | VAS pain, ROM | 60 M | X |
| Emadedin 2012[25] (Iran) | $N = 6$ (age: 53.8 ± 8.9 y; 100% F) | IV | BD-MSC injection ($2.0–2.4 \times 10^7$ cells) | Auto | VAS pain, WOMAC, ROM, MRI evaluation | 2 W; 1, 2, 6, 12 M | X |
| Emadedin 2015[26] (Iran) | $N = 6$ (age: 53.8 ± 8.9 y; 100% F) | IV | BD-MSC injection ($2.0–2.4 \times 10^7$ cells) | Auto | VAS pain, WOMAC, MRI evaluation | 2, 6, 12, 30 M | X |
| Fodor 2016[27] (Unites states) | $N = 6$ patients 8 knees (age: 59.0 ± 7.3 y; 83.3% F) | I ($N = 2$) II ($N = 2$) III ($N = 4$) | SVF injection | Auto | VAS pain, WOMAC, ROM, TUG, MRI evaluation | 3, 12 M | X |
| Kim 2015c[28] (Korea) | $N = 49$ patients, 55 knees (age: 58.1 ± 8.9 y; 52.7% F) | I–II | AD-MSC implantation ($4.3 \times 10^6$ cells) + AD | Auto | IKDC, Tegner activity scale | 26.7 M | – |
| Kim 2016[29] (Korea) | $N = 20$ patients, 24 knees (age: 57.9 ± 5.9 y; 45.0% F) | I–II | AD-MSC implantation ($4.4 \times 10^6$ cells) + AD | Auto | IKDC, Tegner activity scale, MRI evaluation (MOCART and MOAKS) | 27.9 M | – |
| Koh 2013[32] (Korea) | $N = 18$ (age: 54.6 ± 7.8 y; 66.7% F) | III–IV | AD-MSC injection ($1.18 \times 10^6$ cells) + PRP | Auto | WOMAC, Lysholm score, VAS pain, MRI evaluation (WORMS) | 24.3 M | – |
| Koh 2014a[30] (Korea) | $N = 35$ patients, 37 knees (age: 57.4 ± 5.7 y; 60.0% F) | I–II | AD-MSC implantation ($3.8 \times 10^6$ cells) + AD | Auto | IKDC, Tegner activity scale, arthroscopic evaluation (ICRS grade) | 26.5 M | – |
| Koh 2015[31] (Korea) | $N = 30$ (age: 70.3 [65–80] y; 83.3% F) | II–III | AD-SVF ($4.2 \times 10^7$ cells) injection + PRP + AD | Auto | Lysholm, KOOS, VAS pain, K/L grade, arthroscopic evaluation | 3, 12, 24 M | – |
| Michalek 2015[33] (Czech Republic) | $N = 1114$ (age: 62.0 [19–94] y; 47.8% F) | II–IV | AD-SVF injection ($1.6 \times 10^6$ cells) + PRP | Auto | Modified KOOS, X-ray, MRI evaluation | 17.2 M | X |
| Orozco 2013[34] (Spain) | $N = 12$ (age: 49.0 ± 17.3 y; 50.0% F) | II ($N = 4$) III ($N = 3$) IV ($N = 5$) | BD-MSC injection ($4.0 \times 10^7$ cells) | Auto | VAS pain, Lequesne index, WOMAC, PCI, SF-36 | 3, 6, 12 M | – |
| Orozco 2014[35] (Spain) | $N = 12$ (age: 49.0 ± 17.3 y: 50.0% F) | II–IV | BD-MSC injection ($4.0 \times 10^7$ cells) | Auto | VAS pain score, Lequesne index, WOMAC, PCI | 3, 6, 12, 24 M | – |
| Pak 2011[36] (Korea) | $N = 2$ (age: 74.5 ± 6.4 y; 100% F) | – | AD-MSC injection + HA + PRP + $CaCl_2$ + dexamethasone | Auto | VAS pain, ROM, MRI evaluation | 3 M | – |
| Sampson 2016[37] (Unites states) | $N = 125$ (age: 57.0 [23–79] y; 100% F) | III–IV | BMC injection + PRP | Auto | VAS, global patients satisfaction survey | 4.8 M | – |
| Soler Rich 2015[39] (Spain) | $N = 50$ (age: 57.8 ± 14.1 y; 40.0%F) | II–IV | BD-MSC injectio ($4.0 \times 10^7$ cells) | Auto | VAS, Lequesne score, WOMAC, MRI evaluation T2 mapping, PCI) | 0, 6, 12 M | – |
| Soler 2016[38] (Spain) | $N = 15$ (age: 51.1 ± 10.3 y; 60.0% F) | II ($N = 9$) III ($N = 6$) | BD-MSC injection ($4.1 \times 10^7$ cells) | Auto | VAS, Lequesne score, WOMAC, SF-36, MRI evaluation (T2 mapping) | 1 W; 3, 6, 12, 48 M | X |
| Trajune 2013[40] (Thailand) | $N = 5$ (age: 57.2 ± 1.92 y; 80.0% F) | II | AAPBSC injection + GFAP concentrate + HA + MCS | Auto | WOMAC, KOOS | 1, 6 M | X |

**Table 1** continued

| Author | Subject population | KL grade | Treatment | Donor | Outcomes | Follow-up | Funding |
|---|---|---|---|---|---|---|---|
| Quasi-experimental studies | | | | | | | |
| Centeno 2014[41] (Unites states) | I: $N = 518$ (age $54.3 \pm 14.1$ y) C: $N = 163$ (age $59.9 \pm 10.3$ y) | I: I ($N = 223$) II ($N = 145$) III/IV ($N = 102$) C: I ($N = 69$) II ($N = 58$) III/IV ($N = 39$) | I: BMC injection + PRP with adipose fat graft C: BMC injection + PRP | Auto | Improvement rating scale, LEFS, NPS | 1, 3, 6, 12 M | – |
| Jo 2014[42] (Korea) | I-a: Low dose, $N = 3$ (age: $63.0 \pm 8.6$ y; 66.7% F) I-b: Mid dose, $N = 3$ (age: $65.0 \pm 6.6$ y; 100% F) I-c: High dose, $N = 12$ (age: $61.0 \pm 6.2$ y; 83.3% F) | I-a: III ($N = 2$) IV ($N = 1$) I-b: III ($N = 2$) IV ($N = 1$) I-c: III ($N = 8$) IV ($N = 4$) | AD-MSC injection (I-a: $1.0 \times 10^7$, I-b: $5.0 \times 10^7$, I-c: $1.0 \times 10^8$ cells) | Auto | WOMAC, VAS pain, KSS, MRI evaluation (defect size and cartilage volume), arthroscopic evaluation (defect size and ICRS grade), biopsy | 1, 2, 3, 6 M | X |
| Kim 2015a[43] (Korea) | I: $N = 17$ patients, 17 knees (age: $57.7 \pm 5.8$ y; 52.9% F) C: $N = 37$ patients, 39 knees (age: $57.5 \pm 5.9$ y; 62.2% F) | I–II | I: AD-MSC implantation with fibrin glue ($3.9 \times 10^6$ cells) + AD C: AD-MSC implantation ($3.9 \times 10^6$ cells) + AD | Auto | IKDC, Tegner activity scale, arthroscopic evaluation (ICRS grade) | 28.6 M | – |
| Kim 2015b[44] (Korea) | I: $N = 20$ (age: $59.1 \pm 3.5$ y; 65.0% F) C: $N = 20$ (age: $59.4 \pm 3.1$ y; 65.0% F) | I–II | I: AD-MSC implantation ($4.0 \times 10^6$ cells) + AD C: AD-MSC injection ($4.0 \times 10^6$ cells) + PRP | Auto | IKDC, Tegner activity scale, arthroscopic evaluation (ICRS grade) | 28.6 M | – |
| Koh 2012[45] (Korea) | I: $N = 25$ (age: $54.2 \pm 9.3$ y; 68.0% F) C: $N = 25$ (age: $54.4 \pm 11.3$ y; 68.0% F) | I: $3.3 \pm 0.8$ C: $2.7 \pm 0.7$ | I: AD-MSC injection ($1.89 \times 10^6$ cells) + PRP C: PRP | Auto | Lysholm, Tegner activity scale, VAS pain | 3, 16.4 M | – |
| Nguyen 2017[46] (Vietnam) | I: $N = 15$ (age: $58.6 \pm 6.5$ y; 80.0% F) C: $N = 15$ (age: $58.2 \pm 5.7$ y; 80.0% F) | I: II ($N = 4$) III/IV ($N = 11$) C: II ($N = 5$) III/IV ($N = 10$) | I: AD-SVF injection ($1.89 \times 10^6$ cells) + AM + PRP C: AM + PRP | Auto | WOMAC, modified VAS pain, Lysholm, MRI | 1, 6, 12, 18 M | X |
| Pers 2016[6] (France) | I-a: Low dose, $N = 6$ (age: $63.2 \pm 4.1$ y; 50.0% F) I-b: Mid dose, $N = 6$ (age: $65.5 \pm 8.1$ y; 50.0% F) I-c: High dose, $N = 6$ (age: $65.2 \pm 2.3$ y; 66.7% F) | I-a: III ($N = 2$) IV ($N = 41$) I-b III ($N = 1$) IV ($N = 5$) I-c III ($N = 0$) IV ($N = 6$) | AD-SVF injection (I-a: $2 \times 10^6$, I-b: $10 \times 10^6$, I-c: $50 \times 10^6$ cells) | Auto | WOMAC, Global knee pain, PGA, KOOS, SAS, SF-36, MRI evaluation | 1 W; 3, 6 M | X |
| Randomized controlled trials | | | | | | | |
| Gupta 2016[47] (India) | Cohort 1: I-a (Low dose): $N = 10$ (age: $58.1 \pm 8.2$ y; 70.0% F) I-b (Mid dose): $N = 10$ (age: $57.3 \pm 9.5$ y; 80.0% F) C-a: $N = 10$ (age: $54.9 \pm 8.3$ y; 100.0% F) Cohort 2: I-c (High dose): $N = 10$ (age: $55.0 \pm 6.7$ y; 80.0% F) I-d (Very high dose): $N = 10$ (age: $54.0 \pm 6.7$ y; 50.0% F) C-b: $N = 10$ (age: $56.7 \pm 5.2$ y; 70.0% F) | I-a: II ($N = 4$) III ($N = 6$) I-b: II ($N = 1$) III ($N = 9$) C-a: II ($N = 3$) III ($N = 7$) I-c: II ($N = 1$) III ($N = 9$) I-d: II ($N = 3$) III ($N = 7$) C-b: II ($N = 2$) III ($N = 8$) | I: BD-MSC injection (I-a: $25 \times 10^6$, I-b: $50 \times 10^6$, I-c: $75 \times 10^6$ cells, I-d: $150 \times 10^6$ cells) + HA C: HA | Allo | VAS, WOMAC, ICOAP, X-ray, MRI (WORMS) | 12 M | X |
| Koh 2014b[48] (Korea) | I: $N = 21$ (age: $54.2 \pm 2.9$ y; 76.2% F) C: $N = 23$ (age: $52.3 \pm 4.9$ y; 73.9% F) | I: II ($N = 0$) III ($N = 9$) IV ($N = 12$) C: II ($N = 1$) III ($N = 11$) IV ($N = 11$) | I: HTO + AD-MSC implantation + PRP C: HTO + PRP | Auto | Lysholm, KOOS, VAS pain, FTA, arthroscopic evaluation (Kanamiya grade) | 24.4 M | – |
| Lamo-Espinosa 2016[5] (Spain) | I-a (Low dose): $N = 10$ (age: 65.9 [IQR: 59.5, 70.6] y; 60.0% F) I-b (High dose): $N = 10$ (age: 57.8 [IQR: 55.0, 60.8] y; 20.0% F) C: $N = 10$ (age: 60.3 [IQR: 55.1, 61.1] y; 30.0% F) | I-a: II ($N = 1$) III ($N = 2$) IV ($N = 7$) I-b: II ($N = 3$) III ($N = 3$) IV ($N = 4$) C: II ($N = 4$) III ($N = 2$) IV ($N = 4$) | I: BD-MSC injection (Low dose: $1 \times 10^7$ cells; High dose: $1 \times 10^8$ cells) + HA C: HA | Auto | VAS, WOMAC, ROM, X-ray, MRI (WORMS) | 3, 6, 12 M | X |
| Varma 2010[49] (India) | I: $N = 25$ (age: $50.7 \pm 5.4$ y) C: $N = 25$ (age: $48.2 \pm 5.1$ y) | – | I: BMC injection + AD C: AD | Auto | VAS pain, OAOS | 1, 2, 3, 6 M | – |

**Table 1** continued

| Author | Subject population | KL grade | Treatment | Donor | Outcomes | Follow-up | Funding |
|---|---|---|---|---|---|---|---|
| Vega 2015[50] (Spain) | I: $N = 15$ (age: 56.6 ± 9.6 y; 60.0% F) C: $N = 23$ (age: 57.3 ± 9.4 y; 66.7% F) | I: II ($N = 6$) III ($N = 6$) IV ($N = 3$) C: II ($N = 7$) III ($N = 5$) IV ($N = 3$) | I: BD-MSC injection ($4.0 \times 10^7$ cells) C: HA | Allo | VAS pain, WOMAC, Lequesne algofunctional indices, SF-12, MRI evaluation (T2 mapping, PCI) | 1 W; 3, 6, 12 M | – |
| Wakitani 2002[51] (Japan) | $N = 24$ (I: $N = 12$; C: $N = 12$) (age: 63.0 [49–70] y; 62.5% F) | – | I: HTO + BD-MSC implantation ($1.0 \times 10^7$ cells) C: HTO + cell free collagen gel-sheet implantation | Auto | Hospital for special surgery knee-rating scale, arthroscopic and histological assessment | 16 M | – |
| Wong 2013[52] (Singapore) | I: $N = 28$ (age: 53.0 [36–54] y; 54.0% F) C: $N = 28$ (age: 49.0 [24–54] y; 50.0% F) | – | I: HTO + BD-MSC implantation ($1.5 \times 10^7$ cells) C: HTO | Auto | IKDC, Lysholm, Tegner activity scale, MRI evaluation (MOCART) | 6, 12, 24 M | – |

*AAPBSC* autologous activated peripheral blood stem cells, *AD* arthroscopic debridement, *AD-MSC* adipose tissue derived mesenchymal stem (stromal) cells, *AD-SVF* adipose tissue derived stromal vascular fraction, *AM* arthroscopic microfracture, *BD-MSC* bone marrow derived mesenchymal stem (stromal) cell, *BMC* bone marrow concentrate, *FTA* femorotibial angle, *GFAP* growth factor addition/preservation, *HA* hyaluronic acid, *HTO* high tibial osteotomy, *ICOAP* intermittent and constant osteoarthritis pain, *ICRS* international cartilage repair society, *IKDC* international knee documentation committee, *IQR* interquartile range, *K/L grade* Kellgren/Lawrence grade, *KOOS* knee osteoarthritis outcome score, *KSS* knee society score, *LEFS* lower extremity functional questionnaire, *MCS* microdrilling mesenchymal cell stimulation, *MOAKS* MRI osteoarthritis knee score, *MOCART* magnetic resonance observation of cartilage repair tissue, *MRI* magnetic resonance image, *NPS* numeric pain scale, *OAOS* osteoarthritis outcome score, *PCI* poor cartilage index, *PGA* patient global assessment, *PRP* platelet-rich plasma, *ROM* range of motion, *SAS* short arthritis assessment scale, *SF-12* short form-12 health survey, *SF-36* short form-36 health survey, *SVF* stromal vascular fraction, *TUG* timed up and go, *VAS* visual analog scale, *WOMAC* Western Ontario and McMaster Universities Osteoarthritis Index, *WORMS* whole-organ magnetic resonance imaging score. *X* indicates presence of funding.

with higher SMDs. (eTable 9). Notably, performing rehabilitation was a significant effect modifier of SMD (regression coefficient: 0.881, 95% CI: 0.049, 1.712; $P = 0.039$). No small-study effect was visually observed by two independent reviewers (eFigure 4), and the Egger's regression test was negative for significant evidence of publication bias ($P = 0.516$).

As in the VAS pain score, we performed a sensitivity analysis (Fig. 4) and included only RCTs into the meta-analysis for self-reported physical function ($n = 60$). We found that including only RCTs in the meta-analysis attenuated the effects of MSC in improving WOMAC functional score (pooled SMD: 0.53, 95% CI: 0.07, 0.99; $P = 0.020$). The follow-up period in all these studies was 12.0 months. Heterogeneity was much improved because of using a single outcome measure ($I^2 = 33\%$). Stratification for donor type improved the heterogeneity, and pooled SMD in autologous MSC was likely to have a larger functional improvement effects than those in allogeneic MSC. All the included RCTs did not perform rehabilitation. No small-study effect was visually observed by two independent reviewers (eFigure 5).

*MRI findings in articular cartilage*. Two studies with 4 data sets ($n = 20$) reported the MSC treatment effect on cartilage volume, evaluated using magnetic resonance imaging (MRI; Fig. 5a). The mean follow-up period of these studies was 5.3 ± 1.5 months. In these analyses, two single case reports from the same authors[21,22] were combined, as these case reports included patients with a similar clinical status. The pooled SMD on the cartilage volume was 0.49 (95% CI: −0.19, 1.16; $P = 0.160$), a non-significant small effect size. Excluding the combined two case reports resulted in similar results (pooled SMD: 0.51, 95% CI: −0.23, 1.26; $P = 0.180$).

The 5 other studies with 7 data sets ($n = 95$) reported MSC treatment effects on cartilage quality by using the poor cartilage index (PCI), dGEMERIC index, and T2 mapping values, evaluated using MRI (Fig. 5b). The mean follow-up period in these studies was 16.3 ± 15.4 months. The pooled SMD on the cartilage quality was −1.99 (95% CI: −3.51, −0.47; $P < 0.001$), a significantly heightened effect size (SMD ≥ 0.8), with high heterogeneity ($I^2 = 91\%$). When the pooled SMD was evaluated in each outcome measure, it became higher in the PCI but became insignificant in the dGEMERIC index, and heterogeneity improved markedly. A meta-regression analysis indicated that the presence of funding source was a significant factor associated with a higher (i.e., lower effect) SMD (eTable 10). No small-study effect was visually observed from funnel plots by two independent reviewers (eFigures 6 and 7).

## Adverse events

Of 35 studies, 17 (48.6%) reported adverse events related to MSC treatment. Adverse events included knee pain or swelling. eFigure 8 summarizes the event rates with their 95% CIs. Owing to the large clinical and statistical heterogeneity among the studies, we did not pool the adverse event rates. In 10 studies that reported timing of adverse event,[5,6,31,32,34,37–39,45,50] knee pain or swelling occurred within 1 week after MSC treatment; these symptoms were treatable with pain medication.

## Summary of quality of evidence

Table 2 shows a summary of evidence according to the GRADE approach.[12] The effects estimate was downgraded in all outcome measures. None of these effects estimates were upgraded. Each meta-analysis scored 1 (very low) or 2 (low) with the GRADE approach, indicating very little (i.e., the true effect is likely to be substantially different from the effect estimate) or limited (i.e., the true effect may be substantially different from the effect estimate) confidences of the effects estimate.[12]

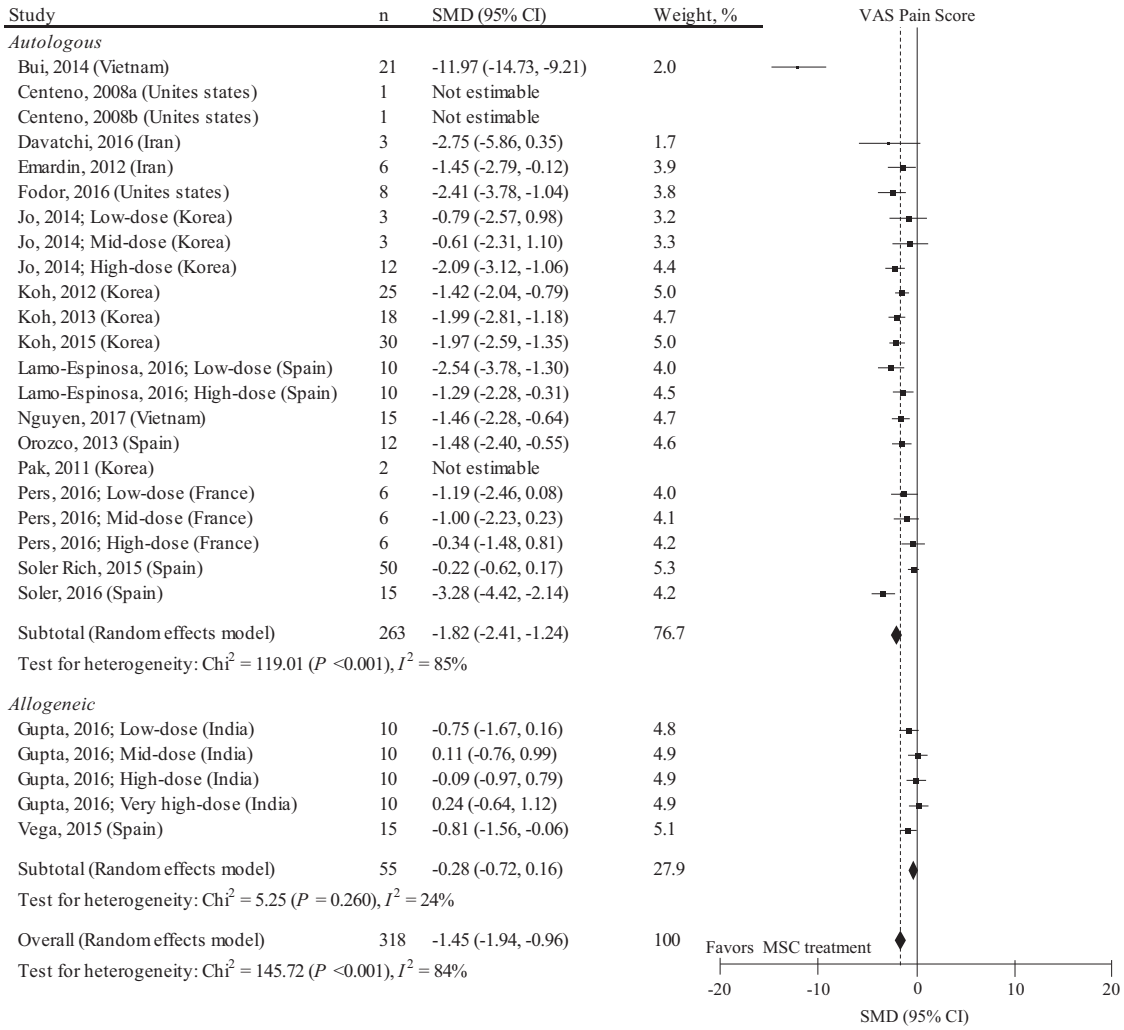


**Fig. 1** SMD and 95% CI for the VAS pain score between pre and post MSC treatment at final follow-up ($n = 318$). The diamond represents the pooled SMD using the DerSimonian-Laird method. The vertical line at 0 represents no difference. MSC treatment was effective in improving VAS pain score (pooled SMD: −1.45, 95% CI: −1.94, −0.96; $P < 0.001$). SMDs were highly heterogeneous among studies ($I^2$: 84%; $P < 0.001$)

## DISCUSSION

This systematic review and meta-analysis found that MSC treatment significantly improved knee pain and self-reported physical function in patients with knee OA. While MSC treatment has an insignificant tendency to improve cartilage volume, MSC treatment significantly improved cartilage quality. However, these data should be interpreted with caution because the quality of evidence was "very low" to "low" according to the GRADE approach because of the poor study design, high risk of bias, large heterogeneity, and wide 95% CI of the pooled SMD. Sensitivity analyses showed that these GRADE ratings were comparable even if we only included RCTs in the meta-analysis; therefore, the true effect is likely to be substantially different from the effects estimate.[12] Detail information about rehabilitation was lacking, but rehabilitation was a significant effect modifier of MSC treatment on self-reported physical function. We suggest that more high quality RCTs with stratification for rehabilitation are needed to facilitate a foundation of effective MSC therapy and regenerative rehabilitation.

The search strategies used in this study provide a more comprehensive assessment of relevant articles by adding new findings to the recent meta-analysis for the clinical efficacy of MSCs transplantation for knee OA and focal cartilage defect up to a maximum 24 months follow-up.[10] Indeed, the current meta-analysis further added 28 non-RCTs and 4 RCTs to the previous meta-analysis,[10] which enable us to examine the latest evidence of both benefits and harms of MSCs treatment on degenerative knee OA with a longer follow-up period that cannot be adequately determined by reviewing only RCTs.[19]

We found that the pooled effect size on the VAS pain score exceeded the effects of nonsteroidal anti-inflammatory drugs and corticosteroid injections,[53,54] consistent with previous meta-analyses.[7,9,10] The mean differences after intervention were ≥10% for both pain and self-reported physical function,[55] exceeding the minimum for clinically important differences, and meeting the responder criteria of the Outcome Measures in Rheumatology Clinical Trials and Osteoarthritis Research Society International. However, we found a large heterogeneity among studies, which was partly explained by the level of risk of bias, cell donor type, and study design. Including only RCTs, which has a lower risk of bias than non-RCTs, in the meta-analysis attenuated the effects of MSC treatment in improving knee pain and self-reported physical function, supporting this interpretation. The observed effects from RCTs had a wide 95% CI, and clinical action would differ if the true SMD was the upper or lower boundary of the 95% CI. This suggests the need for a larger number of RCTs to elucidate whether MSC treatment can provide clinical benefit to patients with knee OA.

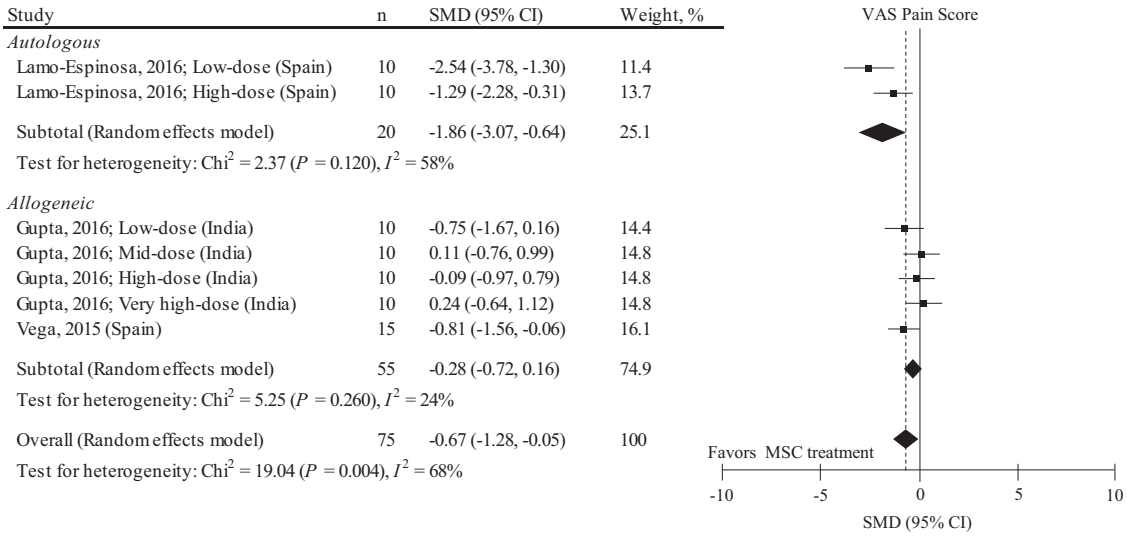


**Fig. 2** Results of sensitivity analysis representing SMD and 95% CI for the VAS pain score between pre and post MSC treatment at final follow-up in 3 RCTs with 7 data sets ($n = 75$). The diamond represents the pooled SMD using the DerSimonian–Laird method. The vertical line at 0 represents no difference. Including only RCTs attenuates the pain relief effects (pooled SMD: −0.67, 95% CI: −1.28, −0.05; $P = 0.030$) compared to those shown in Fig. 1. SMDs were highly heterogeneous among studies ($I^2$: 68%; $P = 0.004$)

The strength of this meta-analysis is that we estimated pooled SMD for structural outcomes of articular cartilage evaluated by MRI. This effect estimate was based on only 2 non-RCTs with 4 data sets, raising the need for high quality RCTs for examination of the structural modifying effects of MSC treatment. We found a discrepancy between MSC efficacy on cartilage quality and MSC efficacy on cartilage quantity (volume). While MSC treatment improved cartilage quality, it did not significantly improve cartilage volume. Although these results should be interpreted cautiously because the studies that evaluated cartilage quality differed from that evaluated cartilage volume, we found that MSC treatment may have a limited therapeutic effect on cartilage volume. Three of these 4 data sets were based on data from patients with severe knee OA (K/L grade ≥3), which may cause limited efficacy in improving cartilage volume. Furthermore, the mean follow-up period in these studies was within 6 months, which might be too short to show a biological effect. One high quality study[42] found that MSC injection particularly improved knee pain when a relatively large number of MSCs was used, but a significant increase in cartilage volume did not accompany this pain reduction, indicating that improved knee pain is not necessarily attributable to increased cartilage volume. Although this meta-analysis only included outcome measures for articular cartilage, some included studies found that MSC treatment improved subchondral bone edema[25,26,46] and meniscus thickness,[36] which are predictors of knee pain severity.[56] Improved knee pain after autologous chondrocyte implantation on cartilage defects moderately correlated with bone edema, but not the cartilage structure evaluated using MRI.[17] Further studies that investigate the mechanism of pain reduction after MSC treatment in patients with knee OA would be of interest.

Physical factors regulate MSC differentiation and tissue development, pointing to a potential therapeutic strategy for enhancing the MSCs injected into the knee joint.[13,14] Weight-bearing might influence the structural outcome evaluated by MRI in the postoperative phase of autologous chondrocyte implantation.[17,18] The mean follow-up period after MSC treatment was 3–60 months in the included studies, which includes some rehabilitation and physical activity programs in the post-MSC treatment phase. These post-MSC rehabilitations might affect the effects of cell-based therapy. Indeed, the presence of rehabilitation was a significant effect modifier of SMD on self-reported physical function. Although the presence of a rehabilitation program was not a significant effect modifier of the estimated effect on VAS pain score, rehabilitation does not necessarily have no impact; the lack of statistical power due to a small number of studies in the meta-analysis[19] and the lack of details of rehabilitation program in each article may explain this absence. As physiological stimulation such as moderate level exercise,[57] ultrasound irradiation,[58] and mechanical loading after joint distraction[59] may enhance cartilage regeneration after MSC injection in a preclinical study, applying exogenous stimulation may be one strategy for enhancing the injected MSCs. This point is particularly important because the lower boundary of the 95% CI of SMD on knee pain and physical function corresponds to the lower effect size in the meta-analysis of RCTs. As all the included RCTs did not report (perform) rehabilitation and none of the included non-RCTs stratified for rehabilitation program, investigating the effects of rehabilitation on the SMD of MSC treatment would be of interest in future studies. Rehabilitation programs was differed among the included studies; thus, this review highlights the need for a standardized rehabilitation program that encompasses at least weight-bearing schedule, range of motion exercise, and muscle strength exercise, which would influence the therapeutic effect of MSCs to facilitate further comparisons among studies. The implementation of longitudinal activity-based questionnaires might help address this question.

We observed a large heterogeneity of adverse event rates among the included studies; this observation limits our ability to summarize the adverse event rate. The causes of heterogeneity in this study are unclear. Detailed reports on adverse events are sparse, which may have contributed to the heterogeneity. Nevertheless, we found only minor adverse events (knee pain/swelling) after MSC treatment, indicating that benefits may outweigh harms of MSC treatment of knee OA. These findings can be achieved by reviewing the data from both non-RCTs and RCTs, which is the strength of the present meta-analysis. Most adverse events occurred within 1 week following MSC treatment. Conversely, pain or swelling that persists for more than 1 week should be interpreted as a rare and potentially severe adverse event that might contribute to arthrogenic muscle inhibition.[60] Close attention to adverse events may be key to the clinical success in optimizing post-MSC treatment of knee OA.

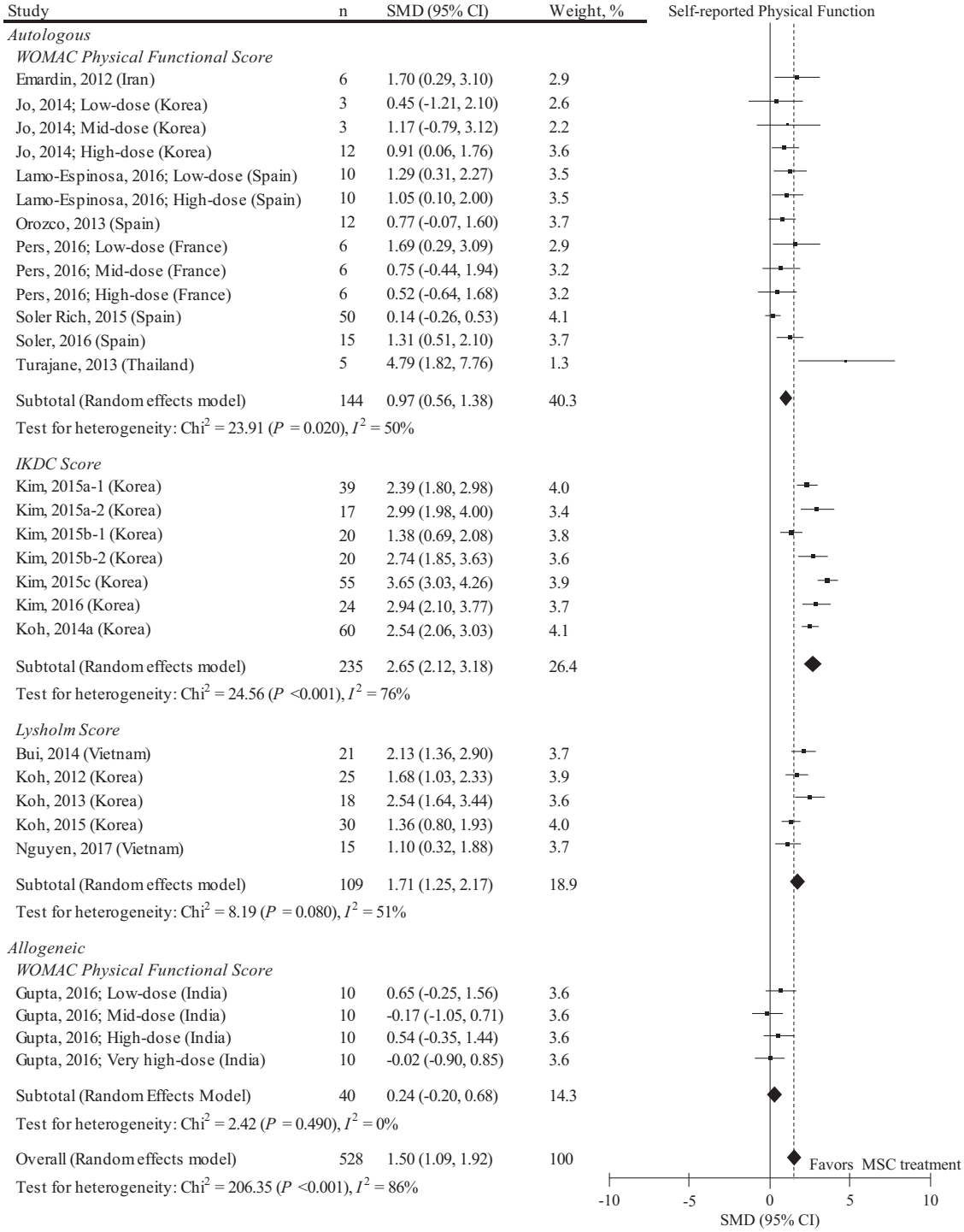


**Fig. 3** SMD and 95% CI for the self-reported physical functional outcome between pre and post MSC treatment at final follow-up. The diamond represents the pooled SMD using the DerSimonian-Laird method. The vertical line at 0 represents no difference. MSC treatment was effective in improving self-reported physical function (pooled SMD: 1.50, 95% CI: 1.09, 1.92; $P < 0.001$). SMDs were highly heterogeneous among studies ($I^2$: 86%; $P < 0.001$)

Autologous MSCs are a widely selected source to minimize the immune response and an excellent therapeutic option for treating OA. Most included trials used autologous MSCs to eliminate immune rejection, while 2 of 35 articles attempted to investigate the potential application of allogeneic MSCs.[47,50] No observed severe adverse event indicates the safety of allogeneic MSCs for applying knee OA. The present meta-analysis revealed that the therapeutic effects of VAS pain score and self-reported physical function were likely higher in autologous than in allogeneic MSCs. However, direct comparisons of the therapeutic effects between autologous and allogeneic MSCs are difficult because these are based on data from different studies. Moreover, two of the studies

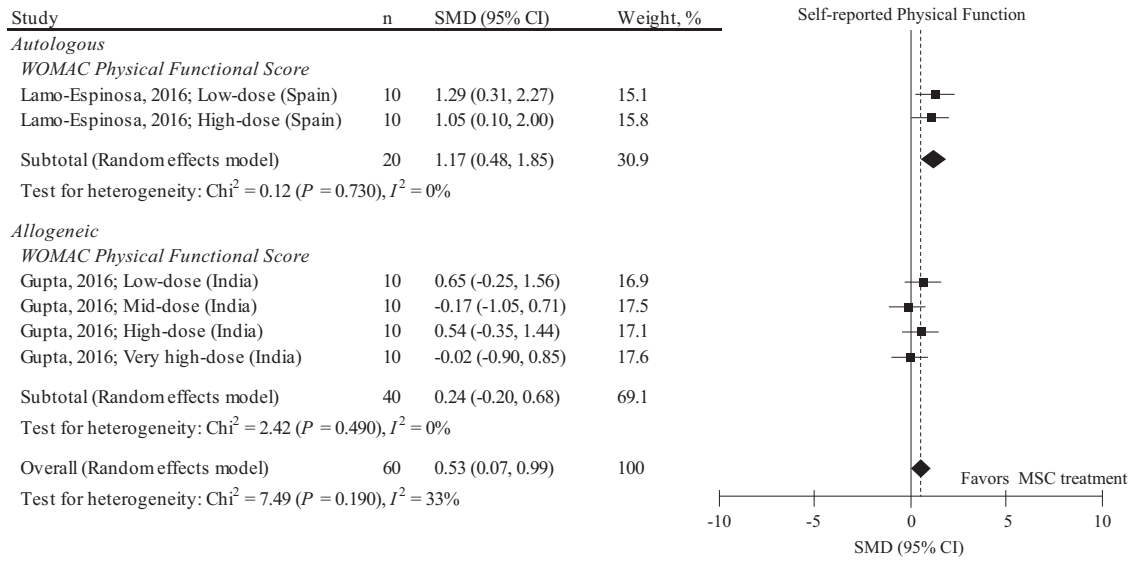


**Fig. 4** Results of sensitivity analysis representing SMD and 95% CI for the self-reported physical function (WOMAC physical functional score) between pre and post MSC treatment at final follow-up in 2 RCTs with 6 data sets ($n = 60$). The diamond represents the pooled SMD using the DerSimonian-Laird method. The vertical line at 0 represents no difference. Including only RCTs attenuates the effects of MSC in improving WOMAC functional score (pooled SMD: 0.53, 95% CI: 0.07, 0.99; $P = 0.020$) compared to those shown in Fig. 3

of allogeneic MSCs were RCTs, which had lower risks of bias than those of autologous MSCs, which might have contributed to the lower therapeutic effect. Thus, direct comparison between autologous and allogeneic MSCs in the same trial would be of interest.

This systematic review included patients with knee OA diagnosed either radiographically or clinically, and excluded those with a focal cartilage defect. Thus, the observed effect of MSCs on clinical outcomes may not hold true in patients with focal cartilage defects. As knees with OA have diffuse cartilage loss rather than an isolated cartilage lesion, several researchers have sought to assess the effect of inter-articular MSC injections rather than implantation to a focal lesion. Whereas MSC implantation on focal cartilage defects in both preclinical and clinical studies is effective in cartilage repair, the cartilage repair effects of intra-articular injection is controversial.[61] We found that the type of treatment was a strong effect modifier of MSC treatment on physical function. It should be highlighted that 2 studies failed to detect a clear dose-response relationship between injected MSC and cartilage volume[42] and cartilage quality;[6] thereby no effects estimates were upgraded in the GRADE approach. Mamidi *et al.* recently suggested that investigating post-transplanted MSC behavior and how to enhance the potency of the transplanted MSCs are the major challenges to be directly solved in future research.[4] We could not address post-injected MSC behavior in the diseased microenvironment; investigating the kinematics of injected MSCs is needed to enhance their disease-modifying effects.

The present study has some limitations. First, this meta-analysis included non-RCTs with 3 case reports. As non-RCTs would have greater bias and more confounders than RCTs, evaluating MSC efficacy using only RCTs might be preferable.[19] Thus, we performed a sensitivity analysis and calculated the effect estimate based on RCTs. Meta-analyses that include non-RCTs can provide evidence of effects that are difficult to detect using a RCT, such as long-term effects and adverse events. Evaluating the beneficial and harmful effects of MSC treatment would be needed to make decisions about the clinical utility of MSC treatment. As discussed previously, as no RCTs have performed rehabilitation, the present meta-analysis, which included non-RCTs, could shed light on the importance of rehabilitation as a new strategy for enhancing functional improvement after MSC treatment and would set a basis for future high quality RCTs. Second, this meta-analysis included 35 studies, but few studies were available for use in the meta-analysis of structural outcomes. This dearth is attributable to the absence of a standard system for evaluating cartilage regeneration. Many studies that use MRI to evaluate cartilage regeneration are only qualitative;[20,25–27,33,36] using validated imaging outcomes would be integral for scientifically validating cell-based therapies and precipitously advancing efficacy.[62] Third, the pooled SMD included the effects of cointervention such as PRP with injected or implanted MSC. PRP improves knee pain and physical function in patients with knee OA,[63] and has a similar effect to MSC injection;[45] the pooled SMD might be attributed to the cointervention. Nevertheless, we confirmed that use of PRP was not a significant predictor of the pooled SMD (data not shown). Fourth, many studies included in this meta-analysis were performed by the same group of investigators.[28–32,43–45,48] Thus, caution is required when interpreting the effect estimate, and further studies from different investigators are needed to elucidate the effects of MSCs on knee OA. Finally, a protocol for this systematic review has not been registered. However, protocol registration was not associated with outcome reporting bias in the meta-analysis,[64] and the outcome measures were extracted according to the highest rank on the pain and functional outcome hierarchy, determined a priori.[65,66]

In conclusion, MSC treatment improves knee pain, physical function, and cartilage quality, without any severe adverse events. However, evidence for these outcomes that are considered critical for clinical decision making was “very low” to “low” according to the GRADE system because of the poor study design, high risk of bias, large heterogeneity, and wide 95% CI of the effects estimate. These GRADE ratings were similar even if only high quality RCTs were included in the meta-analysis. Detail information about rehabilitation is lacking; therefore, the role of rehabilitation in MSC treatment in patients with knee OA is unclear. However, rehabilitation was a significant effect modifier of better MSC treatment on self-reported physical function, supporting a concept of the newly born field, regenerative rehabilitation. Integration of rehabilitation into MSC-based therapy may be beneficial at least in improving physical function. These findings would help researchers and clinicians in designing future high quality clinical trials.

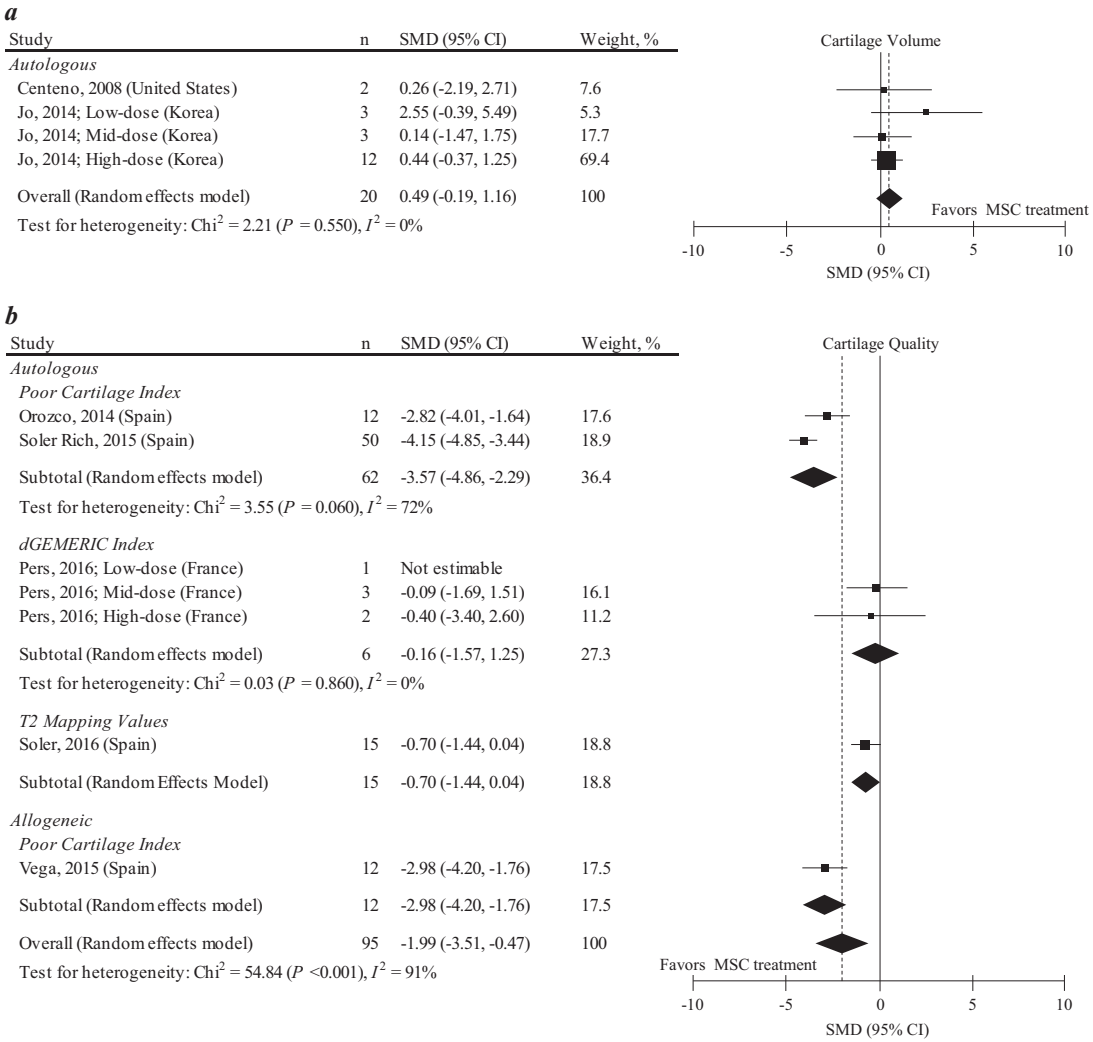


**Fig. 5** SMD and 95% CI for cartilage volume (**a**) and cartilage quality (**b**) between pre and post MSC treatment at final follow-up. The diamond represents the pooled effect size using the DerSimonian-Laird method. The vertical line at 0 represents no difference. While MSC treatment has a non-significant tendency to improve cartilage volume (pooled SMD: 0.49, 95% CI: −0.19, 1.16; $P = 0.160$), MSC treatment was effective in improving cartilage quality (pooled SMD: −1.99, 95% CI: −3.51, −0.47; $P < 0.001$). SMDs for cartilage quality were highly heterogeneous among studies ($I^2$: 91%; $P < 0.001$)

## METHODS

This study was conducted in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement,[67] PRISMA protocols (PRISMA-P),[68] meta-analysis of observational studies in epidemiology (MOOSE) checklist,[69] and Cochrane handbook for systematic reviews of interventions.[19] A detailed protocol for this systematic review has not been previously published and registered.

### Literature search and study selection

The electronic databases of PubMed, Physiotherapy Evidence Database (PEDro), Cumulative Index to Nursing and Allied Health Literature (CINAHL), and Cochrane Central Register of Controlled Trials were used. Searches used combined key terms, including "osteoarthritis, knee," "transplantation," "stem cells," and "stromal cells," using Medical Subject Headings terms. A database search strategy and determining inclusion are provided in the eMethods 1 and 2.

### Outcome measures and data extraction

The primary outcomes in this review were (i) pain, (ii) self-reported physical function, (iii) structural outcomes of articular cartilage evaluated using MRI, and (iv) adverse events relevant to MSC treatment. Two reviewers independently extracted the data regarding authors, country, study design (single-arm, prospective follow-up studies, quasi-experimental studies, and RCTs), subject population, K/L grade, treatment, cell donor type, outcome measures, follow-up period, rehabilitation program, and funding sources using standardized data forms. When an article reported outcomes using multiple pain and functional scales, we used only the scale with the highest rank on the pain and functional outcome hierarchy, in accordance with previous recommendations[65,66] and meta-analyses[70] (eMethod 3).

### Data analysis

Percent agreement of duplicate study removal and interrater reliability of title/abstract and full-text screening between the two reviewers were evaluated. For the meta-analysis, pooled estimates and 95% CIs for SMDs for changes in outcomes were calculated using the DerSimonian-Laird method.[71] The SMD was calculated for paired samples using the within-patient change for patients treated with MSC divided by the pooled standard deviation (SD). Formulae for calculating the pooled SD and pooled SMD are

shown in eMethod 5. The meta-analyses were performed using Review Manager Version 5.3 (Nordic Cochrane Center, Cochrane Collaboration, Copenhagen, Denmark). We used a forest plot to represent the meta-analysis results in accordance with a previous study.[72] The size of the SMD was interpreted using Cohen's d[73] (<0.5: small effect size, 0.5–0.8: moderate effect size, and ≥0.8: large effect size). As a clinical frame of reference, a small effect is equivalent to the effect of non-steroidal anti-inflammatory drugs on knee pain in OA trials.[53] A moderate effect is equivalent to the effect of corticosteroid injections on knee pain.[54] When mean and SD values were not directly reported in an article, they were calculated from other available data, if possible (eMethod 6). To test for publication bias, we used a funnel plot and Egger's test,[74] where publication bias is the tendency for positive trials to be published and the tendency for negative or null trials to not be published. We interpreted *P*-values of <0.10 to indicate the existence of publication bias, as practiced by a previous study.[74] When studies are relatively few, the power of the test is too low to distinguish chance from real asymmetry; we tested for publication bias only when least 10 studies were included in the meta-analysis,[19] and if present, adjustment was planned using a trim-and-fill method.[75] As SMD would be difficult to interpret in a clinical context, the mean differences in pain and functional outcomes were also calculated and compared with minimum clinically important difference (eMethod 7). Furthermore, we performed prespecified sensitivity analyses to provide pooled SMD with 95% CI by using the data from RCTs only.

Study heterogeneity was assessed using the $I^2$ statistic and Q statistic.[76] If $I^2$ was ≥50, random effects meta-regression was performed using the certain parameters selected a priori including the presence of rehabilitation, defined when patients were treated using physical therapy modalities, range of motion exercise, or muscle strength exercise at least one time after MSC treatment (eMethod 8). Adverse events were evaluated in each study, and adverse event rates were calculated from the numbers of events and sample sizes by using the Comprehensive Meta-Analysis software (Biostat, Inc., Englewood, NJ, USA). All other statistical analyses were performed using JMP Pro 12.2 (SAS Institute, Cary, NC, USA).

## Additional methods

Additional methods for assessment of risk of bias and GRADE approach are provided in eMethods in the Supplement.

## Data availability

Data available on request from the authors.

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## AUTHORS CONTRIBUTIONS

All authors met following criteria: substantial contributions to the conception and design of the study, acquisition of data, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; final approval of the version to be submitted; and accountability for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The specific contributions of the authors are as follows: Conception and design of the study: H. I., T. I., and T. A. Analysis and interpretation of the data: H. I., T. I., H. K., and T. A. Drafting of the article: H. I., T. I., M. T., and T. A. Critical revision of the article for important intellectual content: H. I., T. I., H. K., and T. A. Final approval of the article: H. I., T. I., H. K., M. T., and T. A. Statistical expertize: H. I. and T. I. Obtaining of funding: H. I. Collection and assembly of data: H. I. and T. I.

**Table 2.** Summary of body of evidence according to the GRADE's approach

| Outcome | SMD (95% CI) | Study design | Sample size | Downs and black scale | Heterogeneity | Effect of rehab. | Level of evidence (GRADE) |
|---|---|---|---|---|---|---|---|
| VAS pain score | −1.45 (−1.94, −0.96) | 12 × Within-subject repeated design 8 × Quasi-experimental design 7 × RCT | *n* = 318 | 7.2 ± 2.6 (7 [4–12]) points | $I^2$ = 84% | Unclear | ⊕ ⊖ ⊖ ⊖ Very low[a,b,d] |
| VAS pain score (Trim-and-fill) | −0.93 (−1.29, −0.56) | | | | | | ⊕ ⊖ ⊖ ⊖ Very low[a,b] |
| VAS pain score (sensitivity analysis) | −0.67 (−1.28, −0.05) | 7 × RCT | *n* = 75 | 10.9 ± 2.0 (12 [8–12]) points | $I^2$ = 68% | Unclear | ⊕ ⊖ ⊖ ⊖ Very low[b,c,d] |
| Self-reported physical function | 1.50 (1.09, 1.92) | 11 × Within-subject repeated design 12 × Quasi-experimental design 6 × RCT | *n* = 528 | 7.2 ± 2.0 (7 [4–12]) points | $I^2$ = 86% | Significant effect modifier[e] | ⊕ ⊖ ⊖ ⊖ Very low[a,b] |
| Self-reported physical function (sensitivity analysis) | 0.53 (0.07, 0.99) | 6 × RCT | *n* = 60 | 10.7 ± 2.1 (12 [8–12]) points | $I^2$ = 33% | Unclear | ⊕ ⊕ ⊖ ⊖ Low[c,d] |
| Cartilage volume | 0.49 (−0.19, 1.16) | 1 × Within-subject repeated design 3 × Quasi-experimental design | *n* = 20 | 6.3 ± 1.5 (7 [4–7]) points | $I^2$ = 0% | Unclear | ⊕ ⊖ ⊖ ⊖ Very low[a,c,d] |
| Cartilage quality | −1.99 (−3.51, −0.47) | 3 × Within-subject repeated design 3 × Quasi-experimental design 1 × RCT | *n* = 95 | 7.4 ± 2.1 (7 [5–12]) points | $I^2$ = 91% | Unclear | ⊕ ⊖ ⊖ ⊖ Very low[a,b,c,d] |

[a]Downgraded for risk of bias (most of included studies scored less than 8 points on the Downs and Black scale) [b]Downgraded for inconsistency (results were highly heterogeneous across included studies) [c]Downgraded for imprecision (clinical action would differ if true SMD is the upper or the lower boundary of the 95% CI) [d]Downgraded for publication bias (Egger's regression test was positive or unable to determine because of a few included studies [<10 data set]) [e]Presence of rehabilitation (physical therapy modalities, range of motion exercise, or muscle strength exercise) is a significant effect modifier on the SMD for self-reported physical function (regression coefficient: 0.881, 95% CI: 0.049, 1.712 *P* = 0.039; see eTable 8 in the Supplementary Materials)

## ADDITIONAL INFORMATION

**Supplementary information** accompanies the paper on the *npj Regenerative Medicine* website (https://doi.org/10.1038/s41536-018-0041-8).

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# Cartilage Regeneration in Humans with Adipose Tissue-Derived Stem Cells and Adipose Stromal Vascular Fraction Cells: Updated Status

**Jaewoo Pak [1,†], Jung Hun Lee [2,†], Natalie Pak [1], Yoon Pak [3], Kwang Seung Park [2], Jeong Ho Jeon [2], Byeong Chul Jeong [2] and Sang Hee Lee [2,*]**

1 Mipro Medical Clinic, 32-3 Chungdamdong, Gangnamgu, Seoul 06068, Korea; jaewoopak88@gmail.com (J.P.); chxnlxs@gmail.com (N.P.)
2 National Leading Research Laboratory, Department of Biological Sciences, Myongji University, 116 Myongjiro, Yongin, Gyeonggido 17058, Korea; topmanlv@hanmail.net (J.H.L.); ryduses@naver.com (K.S.P.); jeonjh961245@gmail.com (J.H.J.); bcjeong@mju.ac.kr (B.C.J.)
3 First Medical Center, 11841 South St., Cerritos, CA 90703, USA; yoonpak79@gmail.com
* Correspondence: sangheelee@mju.ac.kr; Tel.: +82-31-330-6195; Fax: +82-31-335-8249
† These two authors contributed equally to this work.

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**Abstract:** Adipose tissue-derived stem cells (ASCs) in the form of stromal vascular fraction (SVF) and cultured expansion have been applied in clinical settings in some countries to treat osteoarthritis (OA) of knees, one of the most common debilitating, incurable disorders. Since the first report of successful cartilage-like tissue regeneration with autologous adipose SVF containing ASCs, there has been a gradual increase in the number of publications confirming such results. Thus far, most of the reports have been limited to treatments of OA of knees. Recently, successful applications of adipose SVF in treating OA of ankles and hips have been reported. In addition, several groups have reported modified methods of applying adipose SVF, such as combining bone marrow stimulation with adipose SVF or adding additional extracellular matrix (ECM) in treating OA. Here, we present an updated, systematic review of clinical effectiveness and safety in treating OA of knees, ankles, and one hip since 2016 using ASCs in the form of adipose SVF or in cultured expansion, along with a description and suggestion of potential biological mechanisms of cartilage regeneration.

**Keywords:** adipose tissue-derived stem cells; stromal vascular fraction; human cartilage regeneration; osteoarthritis

## 1. Introduction

Current medical therapies for degenerative joint disease (DJD) are limited only to symptomatic treatments. Nonsteroidal anti-inflammatory drugs (NSAIDs), hyaluronic acid (HA) joint injections, physical therapy, steroid injections, and even arthroscopic lavage provide only symptomatic relief without addressing the underlying causes of osteoarthritis (OA). Although cartilage regeneration is not the "cure-all" remedy for OA, it can be considered to be a form of curative therapy. When these medical therapies fail, arthroplasty for knee (TKR) or arthroplasty for hip (THR) is the only alternative option of treatment available. However, these surgical measures carry relatively high risks of morbidity and mortality [1,2]. In total, 5.6% of the patients who have received these surgeries experience complications [3,4]. Furthermore, the possibility of adverse outcomes and the finite lifespan of the implanted prostheses necessitating repeated surgical procedures are additional potential limitations of the surgery [5].

Mesenchymal stem cells (MSCs) exist in various human tissues, such as bone marrow and adipose tissue matrix [6–8]. These MSCs obtained from adipose tissue matrix are referred to as adipose tissue-derived stem cells (ASCs), which have the capability to differentiate into various tissues originated from the mesoderm, including cartilage [9–11]. ASCs have been used in animals and human patients for cartilage regeneration [12,13]. In 2011, Pak, for the first time, successfully treated two human OA patients using a mixture of autologous adipose stromal vascular fraction (SVF) containing ASCs, platelet-rich plasma (PRP), and hyaluronic acid (HA). This mixture was introduced into the diseased knee via percutaneous intra-articular injection [14]. Since then, numerous studies have been published showing similar results [15].

In this review, we present an updated status of the comprehensive and systematic review of publications since 2016 involving the treatment of human OA patients using either autologous adipose SVF cells or culture-expanded ASCs. Also, we will try to ascertain potential biological mechanisms of action of these MSCs in cartilage regeneration.

## 2. ASCs in the Form of Adipose SVF and Cultured Expansion

First, a liposuction needs to be performed to obtain adipose SVF containing ASCs. The adipose tissue procured from the liposuction is referred to as the lipoaspirate. In order to extract ASCs and extracellular matrix (ECM), the lipoaspirate is mixed with collagenase, homogenized, and digested [16–18]. Afterwards, the collagenase in the mixture is removed by the dilution method of using normal saline solution and centrifugation in a sterile fashion. After removal of the collagenase, the final volume that is injected into the joint is referred to as adipose SVF, containing several cell and tissue types, including ASCs, ECM, fibroblasts, white blood cells (WBCs), red blood cells (RBCs), and so forth. The ASCs in adipose SVF can further be isolated and culture-expanded [16–18]. The process of preparing autologous adipose SVF is considered to be a medical procedure in Korea when it is performed by a physician within a medical facility as a single surgical procedure in the same day with minimal manipulations [19]. On the contrary, culture-expanded stem cells are usually processed in a laboratory and are classified as a pharmaceutical product in Korea [19].

## 3. Potential Biological Mechanisms of Cartilage Regeneration by MSCs

Chondroblasts and chondrocytes are the major cellular components of cartilage tissue, along with the ECM, which makes up the most of the cartilage matrix [20]. The chondroblasts are developed from MSCs, while the ECM is produced by chondroblasts and chondrocytes [20,21]. As chondroblasts mature into chondrocytes, they secrete extracellular matrix, trapping themselves within it. Inside the ECM, chondrocytes further divide into groups of 2–4 cells, forming ECM-covered lacunae [20,21]. The ECM of cartilage is composed of proteoglycan molecules, which are cross-linked and contain fixed negative charges. Proteoglycans, as a component with such a specialized structure, enables the ECM to withstand various different forces [21]. Chondroblasts and chondrocytes in the cartilage tissue maintain the specialized functions of the ECM by regulating synthesis and degradation [20,21].

In OA/DJD, joints become diseased by a variety of factors damaging chondroblasts, chondrocytes, and the ECM, which in turn, causes degradation of the cartilage tissue, resulting in loss of structure and function [22,23]. Such disruption of the tissue is induced by oxidative stress, inflammatory factors, and mitochondrial dysfunction [24]. Mitochondrial dysfunctions have been linked with the pathophysiology of OA/DJD, in which chondrocytes and chondroblasts are found to have reduced mitochondrial functions due to decreased mitochondrial electron transport chain (ETC) proteins [25,26]. ETC proteins are essential for ATP production [25]. Reduction in ETC proteins results in decreased mitochondrial activity, leading to diminished ATP production and thus a decline in the availability of adenosine in the extracellular space [25].

Adenosine in the extracellular space prevents OA phenotypic changes [27]. Extracellular adenosine is derived mainly from the hydrolysis of ATP by the actions of ectoenzymes CD39 and CD73, and mediates its effects via activation of G-protein-coupled receptors (A1R, A2AR, A2BR, and A3R) [27]. Thus, the reduction in ATP leads to decreased availability of adenosine in the extracellular space, resulting in OA phenotypic changes by stimulating expression of matrix metalloproteinases (MMPs), as shown by the following animal study. Mice lacking the A2A adenosine receptor (A2AR) or ecto-5′-nucleotidase, an enzyme that converts extracellular AMP to adenosine, developed spontaneous OA. On the other hand, replacing adenosine by intra-articular injection prevented development of OA [27]. Hence, it can be concluded that the negative factors such as aging, inflammation, and oxidative stress can disrupt the homeostasis of the cartilage matrix and lead to degradation of the cartilage and apoptosis of chondrocytes/chondroblasts, mediated by the lack of adenosine in the extracellular space [28–31].

MSCs can differentiate into chondroblasts and chondrocytes [21]. In the case of OA/DJD, MSCs can differentiate into chondrocytes, resulting in improvement in joint functions and pain [9–11]. Such potential therapeutic function of MSCs can be explained by two possible mechanisms of action: (1) direct adherence and incorporation of MSCs into the host tissue for growth and differentiation and/or (2) trophic effects resulting from the secretome of MSCs. Although the actual true mechanism of action of cartilage regeneration by MSCs is not yet clear, the current evidence is pointing in the direction of both the potential mechanisms working together in harmony [32].

### 3.1. Direct Engraftment

Stem cells have a "homing" effect [33–35]. When introduced into a host, stem cells may be able to migrate to the target tissue by interacting with various chemokine receptors, such as CXCR4, integrins, selectins, vascular cell adhesion molecule-1, and so forth [36–39]. CXCR4, being present on a subpopulation of MSCs, is one of the numerous chemokine receptors involved in MSC migration [36]. Although this is not yet clear, homing is presumed to be significantly dependent on CXCR4 having a binding affinity toward stromal derived factor-1 [36]. Integrins are another family of cell surface molecules associated with cell migration through not-yet-understood pathways. MSCs usually migrate to an infarcted myocardium; however, when integrins are neutralized, the homing of MSCs to the infarcted myocardium is abolished [40]. This is just one example of chemokine receptors being involved in stem cell migration.

After migration via the homing mechanism, MSCs need to attach to and migrate across endothelial cells (ECs) to enter the target tissue. Rüster et al. [37] demonstrated that MSCs, like leukocytes, bind to ECs and migrate by extending podia, followed by rolling and adhesion on the EC. They also showed that the binding and rolling of MSCs were mediated by the P-selectin adhesion molecule, in addition to very late antigen-4 (VLA-4), vascular cell adhesion molecule 1 (VCAM-1), and proteolytic enzymes [37,41].

In 2008, a group in Japan published a report of meniscus cartilage regeneration in rats [42]. The group isolated MSCs from the synovium of the rats, which were inflicted with meniscus damage. Then, the MSCs were introduced into joints of the rats by percutaneous intra-articular injection. After the joint injection, the stem cells migrated to the site of meniscus injury, adhered to the site, and regenerated cartilage, filling the meniscal defect.

In 2017, a group in Korea transplanted umbilical cord-blood-derived (UCB) MSCs along with HA into a rabbit joint to repair articular cartilage defects [43]. They showed that the UCB-MSCs adhered to the site and repaired the defects by regenerating cartilage that had similar cellular architecture and collagen arrangement to the normal cartilage tissue.

These two groups showed that injected MSCs have the ability to attach at the site of damage and repair the host cartilage by regeneration. Furthermore, the first group showed that the MSCs could actually migrate and adhere to the site of damage for tissue regeneration. Although the MSCs introduced definitely attached at the site of injury, the possibility of these MSCs being actually

incorporated into the host tissue to transform into the host chondroblasts and/or chondrocytes is not clear.

In the same year, a group in Germany described a "cell tracking system" using a transgenic donor and corresponding immune-competent recipient mouse [32]. Using this method, the group showed that MSCs regenerate cartilage through "non-progenitor" mechanisms [32]. These findings clearly indicated that the adherence of MSC at the site of cartilage defects was necessary; but the attached MSCs just orchestrated the regeneration process instead of transforming themselves into new chondroblasts and chondrocytes in the host tissue.

The above finding was further confirmed by a human clinical trial by de Windt et al. [44]. This group transplanted, via intra-articular injection, allogeneic MSCs and autologous chondrons into knees with cartilage defects. On second-look arthroscopies, the cartilage defects were filled with regenerated cartilage. Biopsies of the regenerated cartilage, however, failed to show any evidence of donor-derived DNA, proving that the transplanted allogeneic MSCs failed to transform into the host chondrocytes or chondroblast. Thus, it can be postulated that the engraftment of stem cells along with the trophic effects produced by MSCs coordinates the regeneration process [32,44].

### *3.2. Trophic Bioactive Factors*

MSCs secrete many different bioactive factors that can be categorized into three classes: (1) growth factors, (2) cytokines, and (3) extracellular vesicles [31,45–47]. These bioactive factors may have a variety of activities influencing the immune system, the apoptosis, and growth and differentiation of reparative progenitor cells [45,46,48,49]. Extracellular vesicles can be further divided into apoptotic bodies, microvesicles, and exosomes [50].

#### 3.2.1. Cytokines and Growth Factors

MSCs produce a variety of proinflammatory and anti-inflammatory factors. Some examples of anti-inflammatory factors are the hypoxia-inducible factors (HIF), basic fibroblastic growth factor (bFGF), tumor necrosis factor-alpha (TNF-α), transforming growth factor-β1 (TGFβ1), insulin-like growth factors (IGFs), vascular endothelial growth factor (VEGF), interleukin (IL) 13, IL10, IL18 binding protein (IL18BP), IL1 receptor antagonist (IL1RA), anti-apoptotic proteins, and others [51–60]. Some of the proinflammatory cytokines are IL-1beta (IL1β), IL6, IL8, IL9, and matrix metalloproteinase-3 (MMP-3), among others [53,54,58,59]. Thus, the final anti-inflammatory effects of MSCs are determined by the net effect of these cytokines interacting together. Among these cytokines, hypoxia-inducible factors (HIF) have been reported to promote chondrogenesis [56,60], and insulin-like growth factor-1 (IGF-1) to promote MSC proliferation and differentiation [52,55]. In addition to lowering the amount of inflammatory factors available in the diseased joint, MSCs may prevent the death of chondrocytes by improving the local microenvironment through the expression of antiapoptotic proteins and stimulating the production of inhibitor proteins of apoptosis [51]. Furthermore, MSCs inhibit the production of proapoptotic factors and stimulate the production of antiapoptotic factors [57]. All of these data support the speculation that a variety of growth factors and cytokines produced by MSCs act in concert to promote cartilage tissue regeneration.

#### 3.2.2. Extracellular Vesicles

Extracellular vesicles (EV) are "membrane vesicles that are released by a variety of cells into the extracellular space" and can be "divided into apoptotic bodies, exosomes, and microvesicles" [50,61,62]. When released from stem cells, they may contribute to the regeneration of cartilage via paracrine-like actions. These EVs transfer bioactive cytoplasmic components such as nucleic acids, mitochondria, lipids, and proteins from stem cells to recipient cells [63–67]. Among the subtypes of EVs, most of the available data concern exosomes, showing their significant regenerative properties.

Exosomes are generally referred to as "a specific class of extracellular vesicle characterized by a diameter of 40–150 nm and a density of 1.09–1.18 g/mL" [68]. After being originated from the endosomal system, they are released into the extracellular space [50,69,70]. While in the extracellular space, exosomes are internalized by host cells by fusion with the cell membrane or by phagocytosis, releasing their cytoplasmic contents into the recipient cells, potentially exerting regenerative effects by improving cellular cytoplasmic contents, decreasing death signals, and by immunomodulation [71–73].

MSCs are known to produce large amounts of exosomes carrying cargos rich in active glycolytic adenosine triphosphate (ATP)-generating enzymes, along with other cytoplasmic contents [31,46,47]. It is postulated that these enzymes and cytoplasmic contents in exosomes are transferred into the defective cells, for example, chondroblasts and chondrocytes in cartilage, and replenish the reduced mitochondrial ATP production in damaged cells for cellular proliferation and cartilage matrix production.

When cells are injured, ATP is released from the damaged cells into the extracellular space as an immune signal [74]. This extracellular ATP causes immune cells to migrate and accumulate at the site of damage and remove damaged, dying cells [75,76]. This extracellular ATP is hydrolyzed to adenosine monophosphate (AMP), which is converted to adenosine, a potent activator of signals mediated by AKT and ERK pathways [77,78]. The process of degradation of AMP to adenosine is catalyzed by CD73, also known as extracellular ecto-5′-nucleotidase, which is a sure marker of exosomes [79]. Exosomes, through the actions of CD73, may convert extracellular ATP to adenosine.

Adenosine, in turn, activates AKT and ERK signaling pathways, which have been implicated in cellular survival and proliferation [80]. The activated AKT signaling pathway influences many factors involved in apoptosis. In the nucleus, the AKT pathway inhibits transcription factors involved in the expression of cell death genes and enhances the transcription of antiapoptotic genes [81]. In addition, activation of the ERK signaling pathway leads to the phosphorylation of many agents involved in the regulation of cell proliferation. As an example, the ERK pathway is involved in the mitosis phase of the cell cycle by phosphorylating cyclin D complexes [82].

In OA/DJD, immune cells, including macrophages, produce inflammatory cytokines, causing cartilage matrix degradation and joint damage. Macrophages, however, can be further divided into M1 and M2 macrophages [83]. M1 macrophages produce IL6, which inhibits the chondrogenic differentiation of MSCs, while M2 macrophages produce anti-inflammatory IL10, which supports the survival of chondrocytes [22,83,84]. An increase in M2 macrophages was evident in injured immune-competent rats when treated with MSC exosomes [85]. M2 macrophages produce anti-inflammatory cytokines, such as TGF-β1 and IL10, and thus attenuate the effects of inflammatory cytokines such as TNF-α and IL1 [86]. This is an example of the immune-modulating effect of MSCs in cartilage regeneration.

## 4. PRP, HA, and ECM

Some of the studies reviewed in this article utilized either PRP, HA, and/or ECM with adipose SVF or culture-expanded ASCs. The potential rationale for using any one, or more, of these agents is to provide additional complementary effects for ASCs, to achieve better cartilage regeneration by providing scaffold material for stem cells to attach to and/or to stimulate the stem cells to grow and differentiate.

PRP can provide various growth factors which can stimulate the proliferation and differentiation of stem cells [87,88]. In addition to providing a variety of growth factors, PRP may also function like a scaffold material, necessary for stem cells to attach to at the site of cartilage damage after becoming a "curd-like" material by being activated with calcium chloride, thrombin, or collagen [88–91].

HA and ECM are two naturally occurring scaffold materials. Both HA and ECM have a high affinity for cartilage and provide an environment for stem cells to adhere and attach to the host tissue [92,93]. In addition, ECM secretes a variety of growth factors, which further enhances the stem cells' growth and differentiation [93].

## 5. Clinical Applications of ASCs in the Form of Adipose SVF and Culture-Expanded Cells

The main features of the clinical studies on ASC therapies for cartilage damage due to OA/DJD published since 2016 are summarized in Table 1.

### 5.1. Retrospective Cohort Study by Kim et al.

This is a retrospective cohort study looking at the short-term result of an adipose SVF injection combined with marrow stimulation while performing supramalleolar osteotomy (SMO) in 64 ankles with varus ankle OA [94]. The clinical outcomes and second-look arthroscopic outcomes of adipose SVF injection with marrow stimulation were superior compared to those of marrow stimulation alone when performing SMO.

As expected, this article shows better results with adipose SVF combined with bone marrow stimulation than bone marrow stimulation alone when performing the SMO surgical procedure. Although this study is interesting, it would have been more worthwhile if the study prospectively compared the effect of adipose SVF alone versus bone marrow stimulation alone, while performing SMO.

### 5.2. Case Series by Fodor and Paulseth

This is a safety and feasibility study of assessing the potential management of OA of eight knees of six human patients with the percutaneous intra-articular injection of autologous adipose SVF obtained by the collagenase digestion of adipose tissue [95]. The knees were injected with, on average, 14.1 million nucleated cells per knee.

After enzymatic digestion of the lipoaspirate with collagenase, on average, 14.1 million viable, nucleated SVF were injected via percutaneous intra-articular injection. Since 1% to 10% of the nucleated cells are ASCs, a maximum of 1.41 million stem cells were injected [17,96]. As shown by Jo et al., potentially a minimum of 10 million ASCs is needed for the joint to achieve adequate cartilage regeneration to be able to be seen in MRI studies [97].

### 5.3. A Phase 1 Dose Escalation Trial by Pers et al.

This is an open phase I clinical trial without a control group. The study was conducted in France and Germany for the evaluation of the safety of a dose-escalation protocol of the intra-articular injection of culture-expanded ASCs in patients with knee OA [98]. There was no correlation with symptom improvement and MRI findings.

This is a dose-escalation study using culture-expanded ASCs. As stem cells go through the culture expansion passages, cells lose the homing effect [34,35]. When injected, some of these stem cells may not migrate to the site of cartilage damage. Also, compared to the study published by Jo et al. in 2014, fewer numbers of stem cells were injected into the knee joint [15]. These two factors: (1) a potentially decreased homing effect and (2) a lower number of stem cells injected may have contributed to the results reported.

### 5.4. Placebo-Controlled Prospective Comparative Study by Nguyen et al.

This is a placebo-controlled randomized study comparing the clinical efficacy of arthroscopic microfracture (AM) with or without the addition of adipose SVF in 30 patients with OA [99].

This comparative study is additional piece of evidence showing the safety and efficacy of adipose SVF joint injections. AM, unlike ASCs, is an invasive procedure that does not regenerate cartilage. Probably, percutaneous injection of adipose SVF without any surgical procedure would be more beneficial for patients if it were to be applied in clinical settings. It would be worthwhile to design a clinical study comparing AM alone versus the percutaneous intra-articular injection of an autologous adipose SVF/PRP mixture.

### 5.5. Case Report by Pak et al.

This case report shows that the percutaneous intra-articular injection of autologous adipose SVF, ECM, HA, and PRP could regenerate cartilage-like tissue in a human hip OA patient [100]. Autologous adipose SVF and ECM were obtained by enzymatically digesting lipoaspirate with collagenase and then homogenizing the mixture. The adipose SVF containing ASCs and ECM was injected into a hip joint along with PRP and HA.

The amount of adipose tissue utilized in this clinical study was about 100 g, which may contain up to 200,000,000 nucleated cells. Of these 200,000,000 nucleated cells, the potential number of ASCs can be 1–10% [17,96]. Thus, a maximum of 20 million ASCs was injected percutaneously into the joint along with ECM and autologous PRP, both of which may release growth factors for stem cells to migrate and attach at the site of cartilage damage [88,89]. HA, being a scaffold material for stem cells, also may have assisted ASCs to regenerate cartilage [92,93].

**Table 1.** Clinical studies on treatments with adipose tissue-derived stem cells and adipose stromal vascular fraction cells for cartilage defects.

| Study (Year) | Intervention Treatment | Study Type | Number of Subjects/Age (Years) | Subject Characteristic | Concurrent Treatment | Follow-Up | Outcome Measures | Results |
|---|---|---|---|---|---|---|---|---|
| Kim et al. (2016) [94] | ASCs harvested from the patient's buttock ASC injection Arthroscopic marrow stimulation and SMO alone vs. arthroscopic marrow stimulation and SMO + ASCs ($4.0 \times 10^6$ stem cells) | Retrospective comparative study, level III | 62 patients (64 ankles)/51.8: 31 patients/33 ankles Marrow stimulation alone (Group I); 31 patients/31 ankles Marrow stimulation with ASCs injection (Group II) | Varus ankle OA | | 12.8 months | VAS, AOFAS | The mean VAS and AOFAS scores improved significantly for both groups. There were significant differences in the mean VAS and AOFAS scores between groups at the final follow-up. At second-look arthroscopy, there were significant differences in ICRS grades between groups |
| Fodor and Paulseth (2016) [95] | ASCs obtained through enzymatic disaggregation of lipoaspirate from the abdomen, flanks, or lateral thighs One intra-articularinjection of ASCs (14.1 million cells) | Case series, level IV | 6 patients (8 knees)/59 | OA knee | | 12 months | WOMAC, VAS, ROM, TUG, MRI | Improvement in WOMAC and VAS scores at 3 months and maintained at 1 year. ROM and TUG both improved from preoperative to 3 months. MRI showed no detectable structural differences |
| Pers et al. (2016) [98] | Autologous ASCs: one intra-articular injection, low dose ($2 \times 10^6$ cells) vs. medium dose ($10 \times 10^6$ cells) vs. high dose ($50 \times 10^6$ cells) | Cohort study, level III | 18/64.6: 6 low dose, 6 medium dose, 6 high dose | OA knee | | 6 months | VAS | Even the low-dose patients group experienced significant improvements in pain levels and function compared with the baseline |
| Nguyen et al. (2016) [99] | Autologous ASCs harvested from the abdomen isolated arthroscopic microfracture vs. arthroscopic microfracture + ASCs ($10^7$ ASCs cells/mL) suspended in PRP | Prospective comparative study, level II | 30 patients: 15 patients placebo group/58.2; 15 patients treatment group/58.6 | Knee OA (Kellgren–Lawrence grade II–III) | Arthroscopic microfracture and ASC injection | 18 months | WOMAC, Lysholm, VAS, Outerbridge classification, MRI | WOMAC, Lysholm, and VAS scores improved; Outerbridge classification, measured with MRI, showed non-differences between the two group, but Outerbridge scores increased in the placebo group over time and decreased in the treatment group |
| Pak et al. (2017) [100] | Autologous adipose SVF + ECM + PRP + HA | Case report | 1 patient | Hip OA | | 20 weeks | MRI, FRI, ROM, VAS | Along with MRI evidence, FRI, ROM, and VAS all improved |
| Song et al. (2018) [101] | Autologous culture-expanded ASCs were injected for the low-dose, mid-dose, and high-dose groups, providing three injections and followed up for 96 weeks. | Double-blind, randomized pilot study | 18 patients divided into three dose groups: the low-dose ($1 \times 10^7$), mid-dose ($2 \times 10^7$), and high-dose group ($5 \times 10^7$) cells | Knee OA | | 96 weeks | WOMAC, NRS-11 and SF-36, MRI | Along with MRI evidence, autologous ASCs improved WOMAC, NRS-11, and SF-36 results. The dosage of $5 \times 10^7$ adipose MSCs exhibited the highest improvement |
| Kim and Koh (2016) [102] | ASCs harvested from the patient's buttock ASCs injection along with arthroscopic marrow stimulation Arthroscopic marrow stimulation vs. ASCs ($4.1 \times 10^6$ stem cells) + marrow stimulation | Retrospective comparative study, level III | 49 patients/53.9: 23 ankles underwent marrow stimulation alone (Group 1), and 26 underwent marrow stimulation with ASC injection (Group 2). | Varus ankle OA | | 27.6 months 12.5 second-look arthroscopies | VAS, AOFAS, Second-look-arthroscopy | The mean VAS and AOFAS scores improved significantly for both groups. The VAS and AOFAS scores were significantly better in Group 2. Significant differences in ICRS grades between the groups |

**Table 1.** *Cont.*

| Study (Year) | Intervention Treatment | Study Type | Number of Subjects/Age (Years) | Subject Characteristic | Concurrent Treatment | Follow-Up | Outcome Measures | Results |
|---|---|---|---|---|---|---|---|---|
| Jo et al. (2017) [103] | Autologous ASCs isolated from abdominal subcutaneous fat by liposuction and culture-expanded autologous ASCs in normal saline were injected intra-articularly | Cohort study; level of evidence, 3. | 18 patients: 3 male/61.8; 15 female/66.6 | Knee OA | | 24 months | WOMAC, KSS, KOOS, VAS, MRI | WOMAC, KSS, KOOS, and VAS improved for up to 2 years regardless of the cell dosage. However, statistical significance was found mainly in the high-dose group. Clinical outcomes tended to deteriorate after 1 year in the low- and medium-dose groups, whereas those in the high-dose group plateaued until 2 years. The structural outcomes evaluated with MRI also showed similar trends. |
| Pak et al. (2016) [18] | Autologous adipose SVF + ECM | Case series | 3 patients: 2 female/60 and 87; 1 male/68 | Knee OA | | 6–22 weeks | MRI, FRI, ROM, VAS | Along with MRI evidence, FRI, ROM, and VAS all improved |
| Kuah et al. (2018) [104] | Culture-expanded ASCs with culture media supernatant (CMS) | Randomized, double-blind, placebo-controlled Study | 20 patients/40–65 | Knee OA | None | 12 months | MRI, VAS, WOMAC | VAS and WOMAC improved in ASC + CMS groups, but MRI deteriorated in placebo and high-dose ASC + CMS group; no change in low-dose ASC + CMS group |

ASC: adipose tissue-derived stem cells; SMO: supramalleolar osteotomy; OA: osteoarthritis; VAS: visual analogue scale; AOFAS: American Orthopaedic Foot & Ankle Society Score; ICRS: International Cartilage Repair Society; WOMAC: Western Ontario and McMaster Universities osteoarthritis index; ROM: range of motion; TUG: time up-and-go; MRI: magnetic resonance imaging; SVF: stromal vascular fraction; ECM: extracellular matrix; PRP: platelet-rich plasma; HA: hyaluronic acid; FRI: functional rate index; NRS-11: numerical pain rating scale; SF-36: short form-36; MSC: mesenchymal stem cell; KSS: Knee Society clinical rating system; KOOS: knee injury and osteoarthritis outcome score.

### 5.6. A Randomized, Double-Blinded Pilot Study by Song et al.

Eighteen patients with OA of knees were randomized into three different groups and received culture-expanded ASCs [101]. The dosage of $5 \times 10^7$ ASCs exhibited the highest improvement. The result of this study is consistent with the engraftment and trophic factor theory. When high numbers of MSCs are injected, increased numbers of MSCs can adhere to the site of damage, producing a greater amount of trophic factors for cartilage regeneration.

### 5.7. Retrospective Comparative Study by Kim and Koh

This study looked at the effect of adipose SVF combined with lateral sliding calcaneal osteotomy (LSCO) with bone marrow stimulation [102]. Although the mean VAS (visual analogue scale) and AOFAS (American Orthopaedic Foot & Ankle Society) scores and talar tilt angle radiology improved in both groups, the parameters were significantly better in the group with adipose SVF. ICRS (International Cartilage Repair Society) grades were very well correlated with clinical outcomes in both groups.

Again, as expected, this article shows better results with adipose SVF combined with bone marrow stimulation than bone marrow stimulation alone when performing the LSCO surgical procedure. Although this study is interesting, again, it would have been more worthwhile if the study were prospective, instead of retrospective, and compared the effect of adipose SVF alone versus bone marrow stimulation alone when performing LSCO.

### 5.8. Prospective Cohort Study by Jo et al.

This is a prospective cohort study involving 18 patients with OA of the knees [103]. Although clinical parameters improved for up to two years in all patients, the statistical significance was evident only in the high-dose group. Furthermore, clinical improvement deteriorated after one year in the low- and medium-dose groups, while the improvement reached the plateau in the high-dose group within the two years. The structural outcomes resulted in similar trends.

The result of this study is also consistent with the engraftment and trophic factor theory. When high numbers of MSCs are injected, increased numbers of MSCs can attach at the site of damage, producing a greater amount of trophic factors and regenerating a high volume of cartilage. With greater cartilage regeneration, the improvement of clinical symptoms may have persisted for a longer time duration.

### 5.9. Case Series by Pak et al.

This clinical case series showed that cartilage-like tissue can be regenerated in human knee OA joints by a percutaneous intra-articular injection of a mixture of autologous adipose SVF, ECM, HA, and PRP [18]. Adipose tissue was obtained from the abdominal origin and was minced to extract ECM. The lipoaspirate with ECM was then mixed with collagenase and incubated. The resulting adipose SVF with extra ECM was introduced into the knee joints of three Korean OA patients, along with HA and PRP, via percutaneous intra-articular injection. The knee joints were repeatedly injected with weekly injections of autologous PRP for three weeks. As a result, cartilage-like tissue regeneration was evident in all three patients' post-treatment MRIs, along with clinical outcome improvements in terms of ROM, VAS, and FRI. This study emphasized the addition of extracted ECM, which was injected with adipose SVF, HA, and autologous PRP. ECM, in addition to HA and PRP, may have enhanced the ability of ASCs to migrate and adhere to the site of cartilage damage.

### 5.10. Randomized, Double-Blind, Placebo-Controlled Study by Kuah et al.

This is a very well-designed study involving 20 knee OA patients with Kellgren–Lawrence (KL) grade 1–3 [104]. The patients were randomized into three groups: (1) a placebo group ($n = 4$), with only cell culture supernatants (CCS) injected, as a control; (2) a 3.9 million ASC group with CCS ($n = 8$);

(3) a 6.7 million ASC group with CCS ($n$ = 8). All patients received one single intra-articular injection and were followed for 12 months. All patients reported at least one adverse event (AE) after the injection. None were serious AEs, and no withdrawal due to AEs was reported. Statistically significant improvement was noted in terms of VAS in both ASCs groups, while VAS in the placebo group showed marginal improvement. In terms of cartilage regeneration, there was no deterioration in average cartilage volume in the 3.9 million ASC group, while cartilage loss was evident in the placebo group and 6.7 million ASC group. The authors concluded that a single intra-articular injection of ASCs with CCS to patients with symptomatic knee OA was safe.

However, it is difficult to accept the safety claim when 100% of participants experienced adverse events. MSCs are known to have anti-inflammatory effects [59], and numerous human studies, including a safety study reported by Pak et al., did not show 100% adverse events [91]. Thus, the cause of the 100% adverse events should be investigated. Furthermore, the MRI result showed a loss of cartilage volume in the placebo group and 6.5 million ASC group, while no loss of cartilage was evident in the 3.9 million group. It would be interesting to know the exact cause of the adverse events and its potential role in the loss of cartilage volume.

## 6. Discussion

With the accumulation of clinical data, potential mechanisms of action of MSC regeneration of cartilage tissue have been postulated. Although it is not yet clear, the mechanism involves the engraftment of stem cells and their trophic effects working together in harmony. MSCs secrete various bioactive factors: cytokines, growth factors, and extracellular vesicles, which include exosomes that transfer cytoplasmic contents from one cell to other recipient cells. Caplan first postulated that these bioactive factors have trophic effects, regenerating cartilage tissue via autocrine and paracrine fashions [10]. Later, other groups provided evidence that MSCs actually attach at the site of cartilage defects and regenerate cartilage.

In 2017, a German group was able to show that the attached MSCs disappeared after regenerating cartilage [32]. Thus, it can be postulated that after attaching at the site of injury, extracellular vesicles are released and transferred from the donor ASCs to the recipient chondroblasts and chondrocytes.

A safety study reported in 2013 involving the treatment of 91 patients with autologous adipose SVF described a couple of patients repeatedly receiving autologous adipose SVF into the identical knee joints [91]. The group showed that the symptoms of these patients did not correlate well with the number of autologous adipose SVF injections. Such results can be explained by the extracellular vesicle theory. When the second repeated procedure was performed, it can be assumed that there were fewer sites with damage for ASCs to attach. Since fewer cells were attached, fewer extracellular vesicles were available for the host cartilage tissue to regenerate. The result was relatively less improvement compared to the first treatment.

The extracellular vesicle theory may also explain the limited efficacy of the regeneration of cartilage with MSCs. Although the regeneration of cartilage has been documented in various publications, with more stem cells producing better results, none have shown the full amount of growth of cartilage to a normal, undegenerated state. This again can be attributed to the fact that there can only be a limited number of chondroblasts and chondrocytes in the damaged cartilage tissue to regenerate and to produce ECM for cartilage regeneration.

Adipose tissue is an excellent source of MSCs. One gram of adipose tissue may yield up to 2,000,000 nucleated cells, of which 1% to 10% is considered to be ASCs [17,96]. Based on these numbers, we can be certain that a sufficient number of ASCs can be provided to treat OA with an adequate amount of adipose tissue. Since a large number of MSCs attached at the site of injury may produce a huge quantity of trophic factors, it is only logical to assume that utilizing a great number of stem cells would produce better efficacy, as demonstrated by Pers et al., Song et al., and Jo et al. [98,101,103]. In such a sense, culture expansion of the stem cells may be able to produce better efficacy than autologous adipose SVF.

However, stem cells lose their homing effect with a higher number of passages during culture expansions [34,35]. Thus, culture-expanded stem cells with a high number of passages may need a surgical procedure to expose the cartilage lesion for direct application of the stem cells. Adipose SVF stem cells, on the contrary, should have relatively a strong homing effect. Cartilage tissue could be regenerated with percutaneous intra-articular injection of adipose SVF, probably due to the homing effect of stem cells leading them to adhere at the site of cartilage damage.

In addition to introducing a high number of stem cells, growth factors from PRP and ECM may also stimulate stem cells to grow within the joint for better cartilage regeneration. Centeno et al. used autologous platelet lysate to grow bone marrow-derived MSCs in vitro [88]. Pak et al. and other groups have used PRP or another form of platelet-derived materials to enhance stem cell growth in the joint [15,99,100]. Also, ECM and HA have the capability of providing a scaffold material for stem cells to attach at the site of cartilage lesion. Based on the above described presumptive mechanisms of action, MSCs should be able to have positive effects on all other joints of the body, including hips and ankles, as shown by Pak et al. [100] and Kim and Koh [102].

## 7. Method

We used the preferred reporting items for systematic review and meta-analysis (PRISMA) in our our review (Figure 1) [105]. We conducted a systematic literature search in the PubMed, Medline, Embase databases. We used the keywords as our search terms. We combined terms for selected indications (stem cell, osteoarthritis, and adipose). The literature search included all studies published in English between 2016 and 2018. We identified 227 references after removing duplicates. We independently assessed full-text articles for inclusion in our review. The criteria for the inclusion of studies in our review encompassed clinical studies on ASC injection conducted on humans for cartilage regeneration. Finally, we found 10 articles showing clinical studies on ASC treatments for cartilage defects (Figure 1).

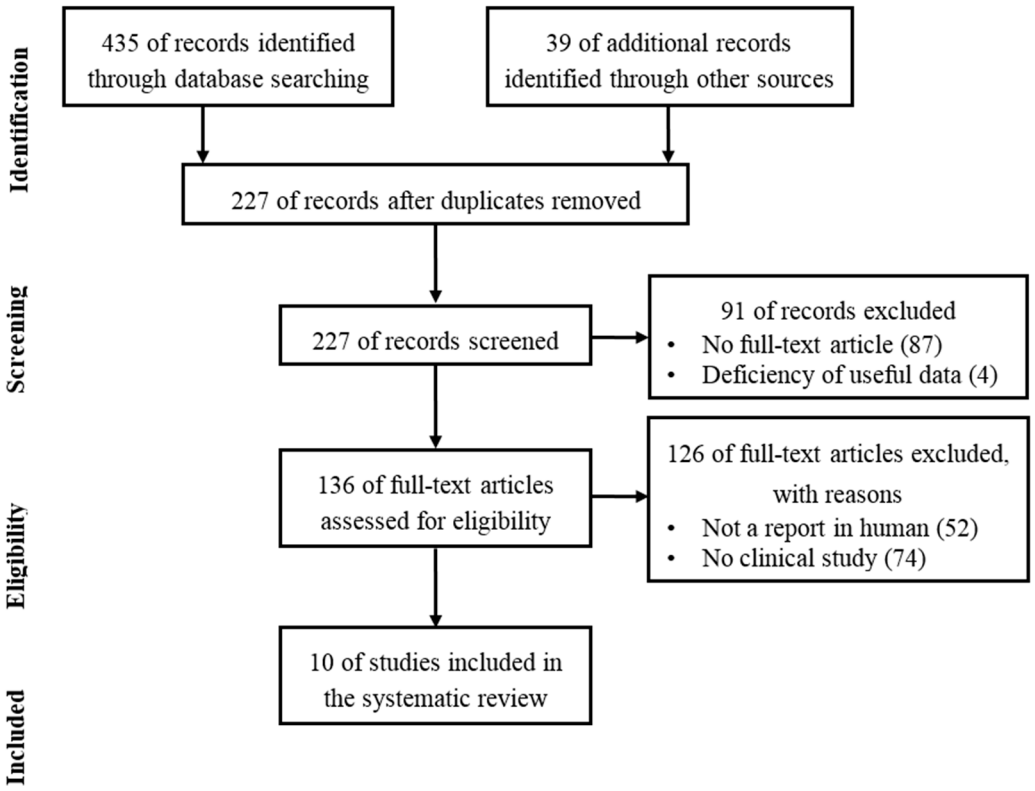


**Figure 1.** Literature selection process (PRISMA flow diagram).

## 8. Conclusions

At present, there is no cure for painful OA of knees, hips, and ankles. For these patients, treatment with ASCs, either in the form of adipose SVF cells or culture-expanded cells, can be an

## 8. Conclusions

At present, there is no cure for painful OA of knees, hips, and ankles. For these patients, treatment with ASCs, either in the form of adipose SVF cells or culture-expanded cells, can be an alternative option that has been slowly gaining evidence of being safe and efficacious. As data accumulates, the mechanisms of cartilage regeneration by ASCs/MSCs are being elucidated to involve both direct engraftment and trophic factors. Among the trophic factors, extracellular vesicles, especially exosomes, are gaining much attention.

ASC/MSC-based therapy, as with all other cell-based therapies, incurs significant operational efforts and costs as the therapy requires stringent manufacturing processes, storage, and delivery to patients in order to ensure the safety and optimal viability of the cells. Thus, isolating the potential trophic factors responsible for cartilage regeneration may help in overcoming these obstacles and possibly applying the therapy to the general patient population. For now, however, better-designed studies are needed to elucidate the true mechanism of action of the therapy and for the potential general application of these stem cells to treat OA/DJD by cartilage regeneration.

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RESEARCH ARTICLE

# Use of Autologous Adipose-derived Stromal Vascular Fraction Grafting in Treatment of Knee Osteoarthritis: A Safety and Efficacy Study

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Vinay Tantuway[1*], Ashish K. Sharma[2], Manoj H. Mehta[3], Raj Sharma[4], Piyush Mantry[4], Pankaj Mehto[5], Murtuza Rassiwala[6]

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## ABSTRACT

Autologous adipose-derived stromal vascular fraction (SVF) grafting done in a single surgical sitting was used to treat 201 osteoarthritic knees of grades II or III (as per Kellgren and Lawrence classification scale) under an IEC-approved protocol for its safety and efficacy study in Indians. The primary objective of this study was to determine if adipose-derived SVF can be safely used for intra-articular injection of the knee. The secondary objective of this study was to evaluate the efficacy of an intra-articular grafting of adipose-derived SVF for pain relief in osteoarthritic knees. SVF was obtained through lipoaspirated adipose tissue without using enzymes or chemicals or animal products which is grafted into the intra-articular space of effected joint. Patient pain data were obtained at per SVF grafting as well as post grafting at 3-, 6-, 9-,12- and 24-month follow up using Knee injury and Osteoarthritis Outcome Score (KOOS) to access statistical significance for the 5 subscales; pain, other symptoms, function in daily living (ADL), function in sport and recreation (Sport/Rec) and knee-related quality of life (QOL) to access improvisation.

Adipose-derived SVF of adipose tissue is a rich source of preadipocytes, Pericytes, endothelial progenitor cell, T cells, B cells, mast cells as well as adipose tissue macrophages obtained from loose connective tissue can significantly improve outcome of degenerative OA leading to a better QOL.

A total of 201 joints mainly knee OA were treated with autologous grafting of SVF done in a single surgical sitting. A total of 201 joints studied out of which 60 joints were followed up for 24 months, 107 joints followed for 12 months, 127 joints are followed for 9 months, 160 joints followed for 6 months and finally all 201 joints were followed for minimum 3 months for safety and efficacy. Modified KOOS Clinical Score was used to evaluate clinical effect and was based on pain, non-steroid analgesic usage, limping, extent of joint movement, and stiffness evaluation before and at pre-operative, 1, 6, 9, 12 and 24 months post-op after grafting. No side effects, systemic infection or cancer were associated with autologous grafting of SVF. There was a significant improvement from pre-op to post-op in all the followed patients. Average KOOS score improved from pre-operative 45.09 to post-operative 24 months average 80.27, which is a very significant improvement in all grades. All sub-scale parameters for pain, symptoms, activity of living and QOL showed significant improvement. Higher grade of OA was associated with comparatively slower healing. Autologous grafting of SVF in single surgical sitting is a novel and promising treatment approach for patients with degenerative OA. This treatment method was found to be minimal invasive, safe and cost-effective treatment modality for osteoarthritis.


**KEYWORDS** Autologous adipose derived stromal vascular fraction, SVF, Osteoarthritis, KOOS, Pericyte, Autologous grafting, Stromal vascular fraction

## INTRODUCTION

Osteoarthritis (OA) or degenerative joint disease is a common chronic, progressive musculoskeletal disorder characterized by gradual loss of articular cartilage. The disease most commonly affects the middle-aged and elderly, although it may begin earlier as a result of injury or overuse. It is often more painful in weight-bearing joints such as the knee and hip.

It can be caused by aging, heredity and injury from trauma or disease. OA is the most prevalent form of arthritis in the world. The CDC combined data from the National Health Interview Survey (NHIS) years 2010-2012, sample adult core components to estimate average annual arthritis prevalence in the civilian, and non-institutionalized US adult population aged 18 years or older. Overall, 22.7% (52.5 million) of adults reported doctor-diagnosed arthritis, with significantly higher age-adjusted prevalence in women (23.9%) than in men (18.6%). Arthritis prevalence increased with age[1].

[1]Associate Professor, Department of Orthopedics, Index Medical College Hospital & Research Centre, Indore, Madhya Pradesh, India
[2]Senior Consultant and Head, Sports Medicine & Joint Replacement Department, SDM Hospital, Jaipur , Rajasthan, India
[3]Knee and Shoulder Surgeon, S.A.A.I. Centre for Arthroscopy and Arthroplasty, Vadodara, Gujarat, India
[4]Department of SVF, Sahaj Hospitals, Indore, Madhya Pradesh, India
[5]Chief Physiotherapist, Sahaj Hospitals, Indore, Madhya Pradesh, India
[6]Trainee, Arthros Clinic, Sahaj Hospital, Indore, Madhya Pradesh, India
Corresponding author: *Dr. Vinay Tantuway
Associate Professor, Department of Orthopedics, Index Medical College Hospital & Research Centre, Indore, Madhya Pradesh, India
Email: vinayforever@gmail.com
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This figure is more alarming in India with a study saying we have over 180 million patients in India, and this figure will enhance in future. There is no blood test for the diagnosis of OA. The goal of treatment in OA is to reduce joint pain while improving and maintaining joint function[3]. The cartilage is a unique avascular, aneural tissue that has limited capacity of self-repair once damaged[4].

OA of weight-bearing joints is associated with chronic devastating pain, stiffness, decreasing range of motion and joint deformity, being one of the leading causes of decreased quality of life (QOL) and work limitations. Despite ongoing research, treatments to manage the disease remain symptomatic. Treatment generally involves a combination of lifestyle modification, analgesics, NSAIDs and joint injections with steroids or hyaluronic acid (lubricant). If pain becomes debilitating, joint replacement surgery may be used to improve the QOL, e.g. partial joint resurfacing (hip and shoulder), and total joint replacement (hip and knee). Total joint arthroplasty (TJA) is the mainstay of treatment for end-stage OA of the hip or knee. Unfortunately, TJA is relatively frequently associated with serious and life-threatening complications including increased risk of infection, thromboembolism, myocardial infarction, stroke, increased risk of death at 30 and 90 days after surgery, and the life-span of the prosthesis is limited[5–8]. Recently, it was shown that stromal vascular fraction (SVF) holds a great promise for their healing potential for cartilage damage[9]. Preclinical animal studies that utilize MSCs demonstrated safety and efficacy in treatment of OA, cartilage defects or other orthopedic conditions[10–13]. The grafting of autologous SVF derived from adipose tissue as a treatment option has been rapidly gaining momentum globally.

## REVIEW OF LITERATURE

The initial results were the safety and the WOMAC (Western Ontario and McMaster Universities Osteoarthritis index) at 6$^{th}$ month. Secondary outcomes comprised clinical, arthroscopic, radiological and histological assessments. There was no procedure-related adverse or side effect. The WOMAC scores improved at 6 months after injection in the high-dose group. The size of cartilage defect or lesions reduced while the volume of cartilage improved in the tibial and medial femoral condyles of the high-dose group. Arthroscopy displayed that the size of cartilage defect or lesions reduced in the medial tibial and medial femoral condyles of the high-dose group. Histology confirmed thick, hyaline-like cartilage regeneration. These outcomes showed that intra-articular injection of $1.0^3 \times 10^8$ AD MSCs into osteoarthritic knee improved function and reduced pain of knee joint without producing adverse effects and reduced cartilage defects or lesions by regeneration of hyaline-like articular cartilage[35].

Practically, all patients showed substantial enhancement in all clinical outcomes at the concluding follow-up examination or analysis. All experimental outcomes are significantly better at 2-year follow-up as compared to 12-month follow-up ($P < 0.05$). Among elderly patients aged >65 years, only five patients demonstrated deterioration of Kellgren-Lawrence grade for OA. On second-look arthroscopy, 87.5% of elderly patients (14/16) developed or retained cartilage status at least 2 years post-operatively. Besides, no one from the patients undertook total knee arthroplasty for the duration of this 2-year period. Adipose-derived cell therapy for elderly patients with knee OA was effective in cartilage healing, reducing pain, and improving function. Therefore, adipose-derived cell treatment appears to be a good option for OA treatment in elderly patients[36].

MSCs are well-known for their potential to regenerate articular cartilage for focal cartilage defect through surgical implantation. In this study, efficacy and safety of intra-articular injection of autologous adipose tissue derived AD-MSCs for knee OA of 18 patients and injected AD MSCs into the knee. The phase I study consists of three dose-escalation cohorts of three groups of 6 patients; the low-dose ($1.0 \times 10^7$ cells), mid-dose ($5.0 \times 10^7$) and high-dose ($1.0 \times 10^8$). The phase II 9 patients received the high dose. The chief outcomes were the safety and the WOMAC at 6 months. Secondary outcomes comprised clinical, arthroscopic, radiological and histological assessments. There was no adverse event found. The WOMAC score is better at 6 months in the high-dose group. The dimension of cartilage defect or lesions reduced although the volume of cartilage improved in the medial femoral and tibial condyles of the high-dose group. Arthroscopy revealed that the dimension of cartilage defect decreased in the medial femoral and medial tibial condyles. Histology proven thick, hyaline-like cartilage regeneration. These results revealed that intra-articular injection of $1.0 \times 10^8$ cells into the osteoarthritic knee improved function and reduced pain of the knee joint devoid of any adverse events and reduced cartilage defects or lesions by regeneration of hyaline-like articular cartilage[35].

In humans, the culture-expanded bone marrow-derived BM-MSCs used for cure of 339 patients with OA documented and more than 75% progress was reported in 41.4% and more than 50% progress was reported in 63.2% of patients. No severe side or adverse effects and no neoplastic complications were discovered at any cell re-implantation site in a mean follow-up 435 days[14]. We recommend that the collective use of autologous SVF and platelet-rich plasma will bring substantial benefits in the cure of OA.

SVF and other regenerative cells, can be effortlessly acquired from loose connective tissue that is connected with adipose tissue. Adipose tissue-derived MSCs are more genetically stable in a long-term culture, display a lower senescence ratio and higher proliferative capacity[13]. Bone marrow MSCs constitute only about

0.001–0.01% of all nucleated cells in bone marrow, whereas the amount of adipose tissue-derived MSCs is approximately 1000-fold greater when isolated from equivalent volume of tissue[13,15,16]. Adipose tissue can be easily obtained by standard lipoaspiration under local anaesthesia and isolated SVF cells contain 1–4% pericytes as well as other cell types involved in tissue regeneration such as vascular endothelial cells, pericytes, fibroblasts, macrophages and regulatory T lymphocytes[13,17–19]. SVF cells demonstrated anti-inflammatory and immunomodulatory effects and MSCs have the capacity to differentiate into connective tissue cells including cartilage, tendon and ligament[13,20].

Here, we evaluated safety and clinical efficacy of freshly isolated autologous grafting of SVF in single surgical sitting in patients with grade 2–4 degenerative OA. Based on previously published results from animal and human studies, we hypothesize that non-manipulated SVF cells freshly isolated from adipose tissue and administered to the close proximity or into the arthritic joint can demonstrate healing potential in patients with degenerative OA. Here, we present data from our study that demonstrate how practicing medicine with patient's own regenerative cells freshly.

## STATEMENT OF THE PROBLEM

A study to assess the safety and efficacy of autologous adipose tissue-derived SVF grafting done in a single surgical sitting in treatment of knee OA on pain and inflammation associated with OA of Knee.

## OBJECTIVES

- To find out the safety and efficacy of the autologous adipose-derived SVF in the treatment of OA.
- To define role of autologous adipose-derived SVF in the treatment of OA.
- To evaluate the difference in KOOS of patients before and after autologous grafting of SVF as per approved methodology.
- To find out the correlation between BMI of the patient and post-operative KOOS score.

### Hypotheses (H1)

There is a significant improvement on the pre- and post-op KOOS score in patients who underwent autologous adipose-derived SVF grafting in the treatment of OA.

### Hypotheses (H2)

There is significant correlation between BMI of the patients and their post-operative KOOS score after Autologous adipose-derived SVF injection in the treatment of osteoarthritis.

## OPERATIONAL DEFINITIONS

### Stromal vascular fraction

SVF derived from autologous adipose tissue extracted from mini-lipoaspiration under tumescent anaesthesia. In the vast majority of scientific literature, only the term adipose tissue is used, but the true source of SVF cells is not the adipose part but only the stromal, i.e. loose connective tissue; part of the fat obtained typically by lipoaspiration.

### Knee Injury & Osteoarthritis Outcome Score (KOOS) Score

KOOS scoring system was used to assess the outcome. The KOOS is a knee-specific instrument, developed to assess the patients' opinion about their knee and associated problems. The KOOS evaluates both short-term and long-term consequences of knee injury. It holds 42 items in 5 separately scored subscales; a. Pain, b. Other Symptoms, c. Function in Daily Living (ADL), d. Function in Sport and Recreation (Sport/Rec), and e. knee-related QOL[21].

## MATERIALS AND METHODS

A prospective experimental research design is adopted in this study. 201 joints of patients were included in the study who had OA. All the patients underwent autologous grafting of SVF. Out of which 60 joints of patients were followed for their improvement in their scores of (pain, symptoms, activity of living, QOL) as mentioned in schedule - Pre-SVF, after 1 week, 1, 3, 6, 9, 12 and 24 months of surgery and 201 joints are being studied of

**TABLE 1** Grade of osteoarthritis.

| Grades of osteoarthiritis | | |
|---|---|---|
| Grade | F | % |
| OA - 1 | 1 | 1.25 |
| OA - 2 | 14 | 17.50 |
| OA - 3 | 75 | 93.75 |
| OA - 4 | 11 | 13.75 |

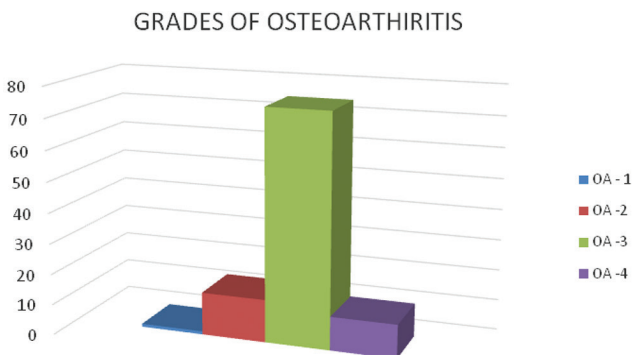


**Fig. 1** Grade of osteoarthritis.

**TABLE 2** BMI of patients.

| BMI of patient | | |
|---|---|---|
| BMI | F | % |
| 17 | 1 | 0.99 |
| 20 | 1 | 0.99 |
| 21 | 1 | 0.99 |
| 23 | 10 | 9.90 |
| 24 | 4 | 3.96 |
| 25 | 9 | 8.91 |
| 26 | 10 | 9.90 |
| 27 | 9 | 8.91 |
| 28 | 3 | 2.97 |
| 29 | 7 | 6.93 |
| 30 | 14 | 13.86 |
| 31 | 2 | 1.98 |
| 32 | 6 | 5.94 |
| 33 | 5 | 4.95 |
| 34 | 6 | 5.94 |
| 35 | 4 | 3.96 |
| 36 | 1 | 0.99 |
| 37 | 1 | 0.99 |
| 38 | 1 | 0.99 |
| 39 | 2 | 1.98 |
| 40 | 2 | 1.98 |
| 45 | 1 | 0.99 |
| 51 | 1 | 0.99 |
| Total | 101 | 100.00 |

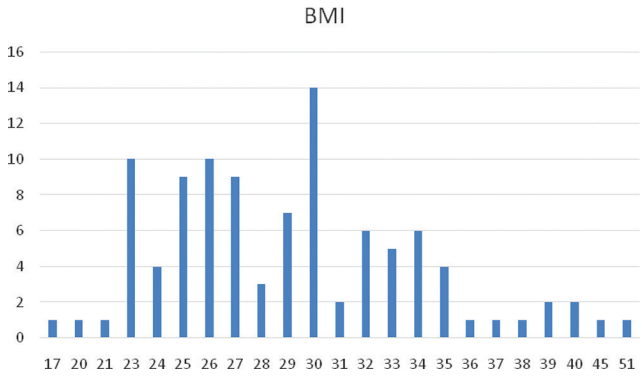


**Fig. 2** BMI of patients.

more than 3 months. Further study will be done with more number of patients with longer follow-up.

## SAMPLE SELECTION CRITERIA

### Inclusion criteria

History of idiopathic OA of knee was characterized by pain. Self-reported difficulty in at least one of the following

**TABLE 3** Age group of patients.

| Age group of patients | | |
|---|---|---|
| Range | F | % |
| 25-30 | 1 | 0.99 |
| 31-35 | 0 | 0.00 |
| 36-40 | 0 | 0.00 |
| 41-45 | 1 | 0.99 |
| 46-50 | 8 | 7.92 |
| 51-55 | 14 | 13.86 |
| 56-60 | 25 | 24.75 |
| 61-65 | 14 | 13.86 |
| 66-70 | 17 | 16.83 |
| 71-75 | 12 | 11.88 |
| 76-80 | 7 | 6.93 |
| 81-85 | 2 | 1.98 |
| TOTAL | 101 | 100.00 |

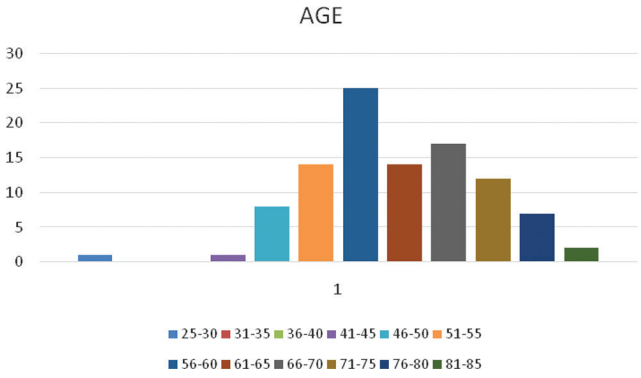


**Fig. 3** Age group of patients.

**TABLE 4** Gender of patients.

| Gender | | |
|---|---|---|
| Gender | F | % |
| Female | 60 | 59.41 |
| Male | 41 | 40.59 |
| Total | 101 | 101 |

activities attributed to knee pain: lifting and carrying groceries, walking 400 m, getting in and out of a chair or going up and down stairs. Patients with indication of OA, grades I, II, III and IV (Kellgren-Lawrence system) can be from degeneration or chronic injury (Table 1, Fig. 1). Patients range from 25 to 85 years of age (Table 3, Fig. 3). Patients must be able to comply with treatment plan, laboratory tests and periodic interviews. Patients must be with adequate renal function, creatinine ≤1.5 mg/dl, with adequate cardiac and respiratory function, and with adequate blood coagulation activity, PT(INR) <1.5, APTT. Patients must have adequate immune system function, with no known immunodeficiency disease, must have greater than 6 months knee pain on the index side (left or right knee).

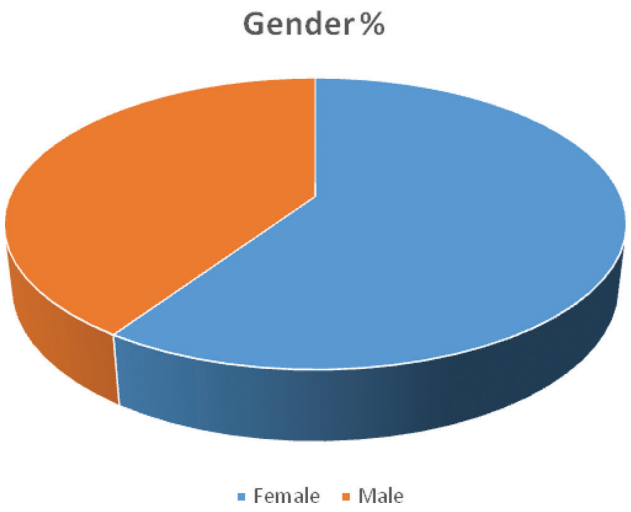


Fig. 4 Gender of patients.

Patients who have not received any intra articular steroids or hyaluronic acid within the last 3 months.

### Exclusion criteria

Active neoplastic disease in the previous 3 years. Knee deformity more than $15^0$ Varus or valgus. Prior or ongoing medical condition (e.g. concomitant illness, psychiatric condition, alcoholism, drug abuse), medical history, physical findings, ECG findings, or laboratory abnormality that, in the investigator's opinion could adversely affect the safety of the subject, makes it unlikely that the course of treatment or follow-up could be completed or could impair the assessment of the study results. History of surgery, including arthroscopy or major trauma to the study joint in the previous 12 months. Signs of active study joint inflammation including redness, warmth and/or, if qualifying with the OA of the knee, a large, bulging effusion of the study knee joint with loss of normal contour of the joint at the screening visit or at the baseline examination after the washout period. Infections in or around the knee. Patients with other conditions that cause pain or congenital or acquired diseases leading to significant knee deformities that may interfere with cell application or interpretation of results. Patients taking corticosteroid medicines or hyaluronic injection in the last 3 months. Patients with other known rheumatic or inflammatory disease such as Gout, hepatitis or syphilis or bleeding disorders. Positive hepatitis B surface antigen, hepatitis C antibody test, Antihuman Immunodeficiency Virus (HIV) antibody test or VDRL. Neoplasia and immunosuppression. For woman of child-bearing potential; +ve pregnancy test or breast-feeding. Age >90 or <18 years or legally dependent. Obesity with body mass index >30 (calculated as mass in kg/height in $m^2$). Congenital or acquired diseases leading to significant knee deformities that may interfere with cell application or interpretation of results.

## PATIENT INTAKE

Prior to scheduling, the patient is screened by the an Orthopedician. All inclusion and exclusion criteria are considered and patient intake is done at this time. Images are evaluated.

### Protocol

1. **Consent**
   a) Discussion and signing of consent forms: risks, benefits and alternatives of treatment are discussed.
   b) The patient understands that he/she is consenting to participate in a study and although agreeing to return to the clinic at designated intervals for follow-up visits, and to respond to the questionnaires, there is no obligation on their part to do so and participation is voluntary.

2. **Lipo aspiration (performed by surgeon on staff)**
   a) Patient prepared in a sterile manner.
   b) Pre-procedural antibiotics, anxiolytic and/or opioid pain medication administration if necessary.
   c) Stab incisions are made for cannula entry with #11 blade after local infiltration with 1% Lidocaine with epinephrine 1:100, 000.
   d) Areas to be treated are then infiltrated with the tumescent anaesthesia fluid with the following concentration of lidocaine and epinephrine using the infiltration cannula. (40 ml of lidocaine 2% without epinephrine plus 1 ml of epinephrine 1% are added to a 1000 ml bag of 0.9% normal saline.)
   e) 300–450 cc adipose tissue is aspirated into a sterile container containing sterile 0.9% normal saline and sodium bicarbonate.

3. **ACRU (Autologous Adipose tissue Cell Recovery Unit)**
   a) Take patient's adipose tissue (fat) that was harvested to lab area.
   b) Turn Class II Bio Hood "ON".
   c) Wipe down surface in hood with 70% alcohol.
   d) Take sample and divide into 50 ml tubes.
   e) The fat is processed in ACRU. Ultrasonic cavitation is used to separate fat and SVF.
   f) Then 50 ml tubes are centrifuged.
   g) You will see a pellet at the bottom of the tube. You will need to remove the top layer until you reach 5 ml. Do your best not to disturb the pellet.
   h) Take a 100-micron filter and screw it onto the 50 ml tube. Turn upside down and use pump to suck it through the filter.
   i) Now, we have the finished cells, cell and viability count is done in Muse cell flow cytometer.

4. **Intra-articular grafting**
   a) If the patient has OA in both knees, then both knees will be grafted, with the worst knee identified as the Index knee, which will be reported on.
   b) Area is prepared for grafting with chlorhexidine.
   c) Local anaesthetic (lidocaine 2%) given to skin and deep tissue as needed.
   d) Autologous SVF is grafted in single surgical sitting.

5. **Follow up**
   a) Patient is discharged when stable after observation and all post procedure instructions have been discussed.
   b) Patient is asked to report any side effects such as fever, pain and others.
   c) Patient is seen for follow up next day or within one week.
   d) Patients are interviewed by phone, email, or in person and asked to complete the KOOS questionnaires prior to initial treatment, at 1, 6, 9, 12 and at 24 months.

## Data analysis and interpretation

All patients underwent treatment with autologous SVF grafting as scheduled and no complications related to adipose tissue processing though there is no utilization of enzymes, chemical and culturing of cell and SVF cells counts were noticed. There were no serious side effects associated with SVF grafting. Other side effects related to the procedure consisted of local pain and swelling at the site of injection or lipoaspiration site, in few patients, there is bit bruising and pain on site for 2–3 days.

At this point, we should also clarify the terminology regarding the source of SVF. In the vast majority of scientific publications only the term adipose tissue is used, but the true source of SVF cells is not the adipose part but only the stromal (i.e. loose connective tissue) part of the fat obtained typically by lipoaspiration. We can demonstrate indirectly the healing potential of SVF grafting in OA using clinical examinations and symptom scoring as well as objective visualization of damaged joints by MRI and X-ray imaging. Since imaging was not the primary aim of this case control study, the follow-up X-ray and/or MRI examination was not performed in all patients.

A total of 201 joints mainly knee OA were treated with autologous grafting of SVF done in a single surgical sitting. A total of 201 joints studied out of which 60 joints were followed up for 24 months, 108 joints followed for 12 months, 128 joints are followed for 9 months, 160 joints followed for 6 months and finally all 201 joints were followed for minimum 3 month for safety and efficacy.

We have given grafted SVF to 201 Joints from May 2015 to June 2017. Most of the patients were in age group 51–60 years. i.e. 39 out of 101. As per sex distribution there was 60 female and 41 male in study (Table 4, Fig. 4). We studied 60 joints for 24 months, 110 joints for 12 Months, 160 joints for 6 months and 201 joints for 3 months. These all joints were grafted with SVF with minimal follow-up of 3 months. We are able to demonstrate safety with no serious side effects reported in 3 months of follow-up and clinical improvement in a vast majority of patients. Some patient's experienced local pain and swelling at the lioaspriation site, but those symptoms were lasting shortly and were well controlled with common analgesics.

BMI ranges from 17.3 to 51.42 in complete range of patients (Table 2, Fig. 2). 34 patients (34.34 %) had associated cardiovascular disease among which 02 patient has gone through bypass surgery, 14 patients (14.1%) had *Diabetes mellitus*, 02 patient (2.02%) had respiratory disorders , 02 patients (2.02%) had neurological disorder and 02 patient had other endocrine disorder (2.02%). 01 (1.25%) patient had OA grade I, 14 (17.5%) patients had grade II, 75 (93.75%) patients had grade III, 11 (13.75%) patients had grade IV OA (as per Kellgren-Lawrence classification).

KOOS scoring system was used to assess the outcome. The KOOS is a knee-specific instrument, developed to assess the patients' opinion about their knee and associated problems. The KOOS evaluates both short-term and long-term consequences of knee injury. It holds 42 items in 5 separately scored subscales; pain, other symptoms, function in daily living (ADL), function in sport and Rec- reaction (Sport/Rec), and knee-related QOL[27].

## DATA ANALYSIS AND INTERPRETATION

All patients underwent treatment with SVF cells as scheduled and no complications related to adipose tissue processing and SVF isolation was noticed. There were no serious side effects associated with autologous SVF grafting. Other side effects related to the procedure consisted of local pain and swelling at the site of injection, bruising at site of aspiration and mild headache found in few patients in initial days.

At this point, we should also clarify the terminology regarding the source of SVF. In the vast majority of scientific publications, only the term adipose tissue is used, but the true source of SVF is not the adipose part but only the stromal (i.e. loose connective tissue) part of the fat obtained typically by lipoaspiration. We can demonstrate indirectly the healing potential of SVF grafting in OA using clinical examinations and symptom scoring as well as objective visualization of damaged joints by MRI or X-ray imaging. Since imaging was not the primary aim of this case control study, the follow-up X-ray and/or MRI examination was not performed in all patients, since it is a short-term follow-up study. Thus, we are not able to draw any conclusion on the correlation between clinical improvement and imaging studies.

We have given grafted SVF to 201 joints from May 2015 to June 17 of one hundred one patients. Most of the patients were in age group 46–75 years. i.e. 90 out of 101 (range 29–84 years). As per sex distribution, there were 60 females and 41 males in study (Table 4, Fig 4). We stud-

**TABLE 5** Paired *t*-test compare the difference in KOOS Score 3 months.

| | Pairs | *t* | df |
|---|---|---|---|
| Pair 1 | Pre-op AVG-Post-op AVG 3 month | 12.7 | 200 |
| Pair 2 | Pre-op Pain-Post-op Pain 3 month | 12.31 | 200 |
| Pair 3 | Pre-op Symptom-Post of Symptom 3 month | 11.61 | 200 |
| Pair 4 | Pre-op AOL-Post-op AOL 3 month | 10.85 | 200 |
| Pair 5 | Pre-op QOL-Post of QOL 3 month | 11.32 | 200 |
| Pair 6 | Pre-op Sport-Post of Sport 3 month | 3.521 | 200 |

**TABLE 6** Paired *t*-test compare the difference in KOOS Score 6 months.

| | Pairs | *t* | df |
|---|---|---|---|
| Pair 1 | Pre-op AVG-Post-op AVG 6 month | 12.98 | 159 |
| Pair 2 | Pre-op Pain-Post-op Pain 6 month | 11.99 | 159 |
| Pair 3 | Pre-op Symptom-Post of Symptom 6 month | 14.07 | 159 |
| Pair 4 | Pre-op AOL-Post-op AOL 6 month | 10.16 | 159 |
| Pair 5 | Pre-op QOL-Post of QOL 6 month | 11.25 | 159 |
| Pair 6 | Pre-op Sport-Post of Sport 6 month | 6.728 | 159 |

**TABLE 7** Paired *t*-test compare the difference in KOOS Score 9 months.

| | Pairs | *t* | df |
|---|---|---|---|
| Pair 1 | Pre-op AVG-Post-op AVG 9 month | 12.89 | 127 |
| Pair 2 | Pre-op Pain-Post-op Pain 9 month | 9.77 | 127 |
| Pair 3 | Pre-op Symptom-Post of Symptom 9 month | 11.56 | 127 |
| Pair 4 | Pre-op AOL-Post-op AOL 9 month | 9.718 | 127 |
| Pair 5 | Pre-op QOL-Post of QOL 9 month | 14.34 | 127 |
| Pair 6 | Pre-op Sport-Post of Sport 9 month | 6.475 | 127 |

**TABLE 8** Paired *t*-test compare the difference in KOOS Score 12 months.

| | Pairs | *t* | df |
|---|---|---|---|
| Pair 1 | Pre-op AVG-Post-op AVG 12 month | 15.95 | 107 |
| Pair 2 | Pre-op Pain-Post-op Pain 12 month | 12.37 | 107 |
| Pair 3 | Pre-op Symptom-Post of Symptom 12 month | 13.2 | 107 |
| Pair 4 | Pre-op AOL-Post-op AOL 12 month | 11.68 | 107 |
| Pair 5 | Pre-op QOL-Post of QOL 12 month | 13.69 | 107 |
| Pair 6 | Pre-op Sport-Post of Sport 12 month | 6.79 | 107 |

**TABLE 8** Paired *t*-test compare the difference in KOOS Score 24 months.

| | Pairs | *t* | df |
|---|---|---|---|
| Pair 1 | Pre-op AVG-Post-op AVG 24 month | 18.67 | 59 |
| Pair 2 | Pre-op Pain-Post-op Pain 24 month | 13.79 | 59 |
| Pair 3 | Pre-op Symptom-Post of Symptom 24 month | 17.46 | 59 |
| Pair 4 | Pre-op AOL-Post-op AOL 24 month | 15.21 | 59 |
| Pair 5 | Pre-op QOL-Post of QOL 24 month | 13.84 | 59 |
| Pair 6 | Pre-op Sport-Post of Sport 24 month | 15.83 | 59 |

*Significance ($P < 0.001$).

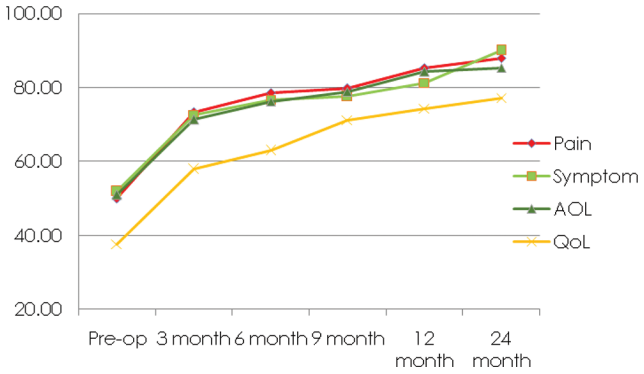


**Fig. 5** KOOS profiles pre-op and post-op up to 24 months assessment after autologous grafting of adipose tissue derived SVF done in single surgical sitting ($n = 201$). At the follow up the difference in the values are statistically significant ($P < 0.0001$) compared with the preoperative status.

ied 101 patients' 201 joints were grafted with autologous SVF in single surgical sitting with minimal follow-up of 3 months in detail, and we are able to demonstrate safety with no serious side effects reported in 24 months of follow-up and clinical improvement in a vast majority of patients. Some patient's experienced local pain and swelling at the injection site, but those symptoms were lasting shortly and were well controlled with common analgesics.

Frequency and percentage is used to describe the demographic variables of the study samples.

$P \leq 0.0001$ significance was used throughout the study. Prism GraphPad version 7.00 was used to do the statistical analysis of the study two tailed *t*-test.

## RESULTS

All the patients examined in this study were of various age groups ranging from 29 to 84 years. Also, all the grades of OA patients were involved to study the effect of adipose-derived SVF intra articular grafting. No adverse events related to the intra-articular grafting were reported, including no acute pain areas, no inflammation at site and no infection has been reported. Few cases were reported of patellar tendonitis at third week that resolved spontaneously. No infections or interventions related to the lipoaspiration sites were recorded expect bruising and itching at site for few days. The result showed the significant improvement in the joint functions after 24 months. The KOOS score changed significantly from pre-op average to post-op average after 24 months. Overall, all the patients reported reduction in the pain, can move normally and carry out their routine living activities. Moreover, all the patients showed no side effects or complication to autologous grafting of adipose-derived SVF in single surgical sitting.

## DISCUSSION

Knee OA is a common chronic orthopaedic disease that significantly reduces the patient's QOL. This clinical study showed that SVF grafting brought good results for patients

with OA patients. We have seen significant improvement in pain & symptom score as early as it shows strong anti-inflammatory and analgesic effect of SVF derived from autologous adipose tissue obtained by lipoaspiration. The anti-inflammatory and pain reduction effects are also contributed by soluble factors secreted from the SVF. Pericytes secrete many important soluble factors, such as HGF, VEGF, NGF, EGF, FGF, and TGF[27–29]. Adipose-derived SVF yields a heterogeneous population of cells including pericytes – progenitor cells with multipotent differentiation potential. SVF cells transcribe many genes that are implicated inflammation, angiogenesis and tissue repair[30]. It is suggested that adipose-derived SVF can have antifibrotic properties by the reduction of local infiltration of inflammatory cells into tissue by the secretion of antifibrotic factors such as interferon-γ and matrix metalloproteinases[30], and by the decrease of pro-fibrotic factors such as transforming growth factor-β[31]. Advantages of SVF include (1) ease of obtaining cells from lipoaspirates, (2) larger pool of adipose-derived pericytes compared with the pool of bone marrow-derived pericytes cells (BM-MC) and (3) stronger angiogenic and regenerative potential of adipose- derived pericytes compared with bone marrow derived pericytes[32].

In this study, all patients showed improved joint function after 12 and 24 months. Moreover, there were no side effects or complications related to microorganism infection, graft rejection, or tumorigenesis. These results provide a new opportunity for OA treatment[22]. The patients will be further monitored and longer follow-up data will help to answer question about durability and long-term safety of adipose-derived SVF. Although in a clinical study, with autologous grafting of SVF in a single surgical sitting almost all patients showed significant improvement in all clinical outcomes at the final follow-up examination. All clinical results significantly improved at 24 months follow-up compared to initial examination before the treatment ($P < 0.0001$) (Table 5–9, Fig. 5). Moreover, one patient underwent total knee arthroplasty during this 24-month period. Another limitation of our study is no randomization and no placebo control. There were two reasons for designing that case control study: 1) ethical aspect and 2) economical aspect. We believe it would be rather unethical to ask placebo group of patients to undergo lipoaspiration and placebo administration to the joint with OA. Since this study was designed as autologous grafting of adipose-derived SVF in a single surgical sitting, there is strong previously documented clinical evidence of safety of autologous non-manipulated or minimally manipulated cell therapies[33]. On the other hand, this study is well designed and strong evidence for minimal risks based on previous studies exists, can lead to a cost-effective, safe, ethical and objective evaluation of a novel treatment.

## CONCLUSION

To summarize, autologous grafting of adipose-derived SVF done in a single surgical sitting is a safe and efficient method for treating OA. The efficiency of grafting clearly improved after 6, 9, 12 and 24 months (Table 5–9, Fig. 5). None of the patient has got adverse or side effect of SVF grafting. Overall, 94% of patients were satisfied with autologous grafting done in single surgical sitting which proves efficacy of grafting. Pain was sturdily reduced after procedure and the QOL was significantly improved. This study suggests that autologous grafting of adipose-derived SVF done in a single surgical sitting is a promising minimally invasive procedure for OA patients.

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