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REVIEW PAPER

Recent Clinical Trials in Adipose-derived Stem Cell Mediated Osteoarthritis Treatment

Hyeran Cho, Hyeyoung Kim, Young Guk Kim, and Kyobum Kim

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Abstract Osteoarthritis (OA), a common chronic affliction amongst the elderly and athletes, is increasing every year. Due to the limited regenerative capacities of the cartilage, surgical or physical treatments have been developed for OA. The development of surgical treatment for OA has evolved from simple joint replacement to cell-based regeneration. Especially, compared with other types of stem cells, mesenchymal stem cells (bone-marrow derived stem cell (BMSC) and adipose-derived stem cell (ASC)) are well known for their high yield, excellent differentiation capacities and easy isolation, and hence are widely applied in tissue engineering. In addition, it has been known that a capability of chondrogenic differentiation of ASCs (*i.e.*, mesenchymal stem cell population from a synovial origin) is higher than BMSCs. This review summarizes the recent clinical applications utilizing ASC and its clinical efficacy for OA treatment, estimated by applying available scoring systems. Current ASC therapy treatments in the clinical system include a direct injection of ASC supplemented with additional compounds such as platelet-rich plasma (PRP) and stromal vascular fraction (SVF) for enhancing differentiation of stem cells and the incorporation of ASC with biomaterial-based scaffolds such as fibrin and hyaluronic acid has been developed for more effective treatment than direct injection. Various scoring systems have demonstrated the clinical efficacy of ASC, and numerous results have validated the use of ASC as an OA therapy by relieving pain and improving the movement of cartilage. Furthermore, additional PRP or SVF combined with ASC have exhibited enhanced pain reduction and physiological movement of cartilage. Hence, clinical researches suggest that stem cell therapy utilizing ASC could be an effective OA treatment.

Keywords: adipose-derived stem cell, cartilage, human clinical trial, tissue regeneration

1. Introduction

Cartilage is a smooth elastic tissue present in the extracellular matrix (ECM) devoid of vessels, composed of chondrocytes, glycosaminoglycans (GAG), and hyaluronic acid (HA) [1-3]. Cartilage defect is a focal area of damage to the articular cartilage sites and osteoarthritis (OA) is one of the most typical diseases caused by cartilage defect [4-6]. OA is a common disease in elderly and athletes due to strain on the cartilage accompanying swelling, uncomfortable movement, and pain of the cartilage [7,8]. While cartilage has limited regenerative capacities, OA treatment platform have been developed adjusting surgical or physiological therapies [9,10]. Nevertheless, previous techniques temporarily improve the pain and movements but do not regenerate the damaged cartilage [11,12]. Current cartilage repair strategies developed in clinical systems that utilize autologous or allogenic cell therapies using chondrocytes have a lot of disadvantages, including donor site morbidity and fibro-cartilaginous regeneration [13,14]. Stem cell therapy is an

Hyeran Cho†, Young Guk Kim, Kyobum Kim
Division of Bioengineering, College of Life Sciences and Bioengineering, Incheon National University, Incheon 22012, Korea

Kyobum Kim* (current address)
Department of Chemical & Biochemical Engineering, Dongguk University, Seoul 04620, Korea
Tel: +82-2-2260-8597
Email: kyobum.kim@dongguk.edu

Hyeyoung Kim†
Department of Molecular Bioscience, University of Kansas, Lawrence, KS 66045, USA

†Equal Contribution.

advanced method of treating damaged cartilage because of its many advantages, *i.e.*, abundant cell source, high yield and differentiation capacities [15-18]. Researchers have developed stem cell treatment using various stem cell sources (such as bone-marrow derived stem cell (BMSC) and adipose-derived stem cell (ASC)), different cell populations, and supplementary substances such as platelet-rich plasma (PRP) or stromal vascular fraction (SVF). Especially, the ASCs have a higher yield as compared to other stem cells, and has the potential to be a therapeutic agent for damaged joint regeneration [19]. Utilizing mesenchymal stem cells (MSCs) such as BMSC and ASC have been investigated in clinical trials by varying the injected cell number and in combination with PRP or SVF [20,21]. Autologous MSCs are widely studied and are advantageous in cell transplantation because of simple isolation and abundant cell source [22]. Furthermore, the inhibitory effect of MSCs on the inflammatory reaction and regeneration of damaged cartilages has been confirmed in recent clinical trials [22,23]. Since ASC exhibits a superior ability to differentiate into a cartilage tissue as compared with BMSC [19], clinical trials for OA are actively conducted using ASC. OA is characterized by inflammatory reactions and the degradation of cartilage ECM due to molecules such as several catalytic factors and pro-inflammatory mediators [24,25]. Therefore, ASCs differentiate into cartilage cells and fill the defects of cartilage. ASCs also help to treat OA through the paracrine effect. Exosomes of ASCs are considered as mediators of the paracrine effect, and they downregulate tumor necrosis factor-α (TNF-α), IL-6, prostaglandin E2 (PGE2), and nitrogen monoxide which cause inflammatory responses. The exosomes of ASCs prevent cartilage degradation by inhibiting cyclooxygenase-2 and microsomal prostaglandin E synthase-1 (mPGES-1), which cause cartilage degradation and anti-anabolic effect [26]. Various clinical trials using ASC were developed, from direct injection with different cell numbers to implantation into defect sites in combination with additional components like PRP and SVF in HA or fibrin gel for increasing cell retention, viability, and possibility of differentiation of ASC [27-31]. Previous trials required an incubation period for expansion of ASC to obtain a homogenous cell population as well as additional surgery for patients [32,33]. However, with developing technology, it is possible to isolate the cells immediately and efficiently, shorten the duration of surgery, and reduce inconvenience to the patient [33]. Recently, 41 clinical studies conducted worldwide that apply ASC for OA treatment are published at Clinical trials.gov. ASC researches are also being actively conducted in North America and East Asia under varying conditions (Fig. 1). Most studies use not only ASC, but are in combination with PRP and SVF, and other known growth factors such as transforming growth factor (TGF)-β, insulin-like growth

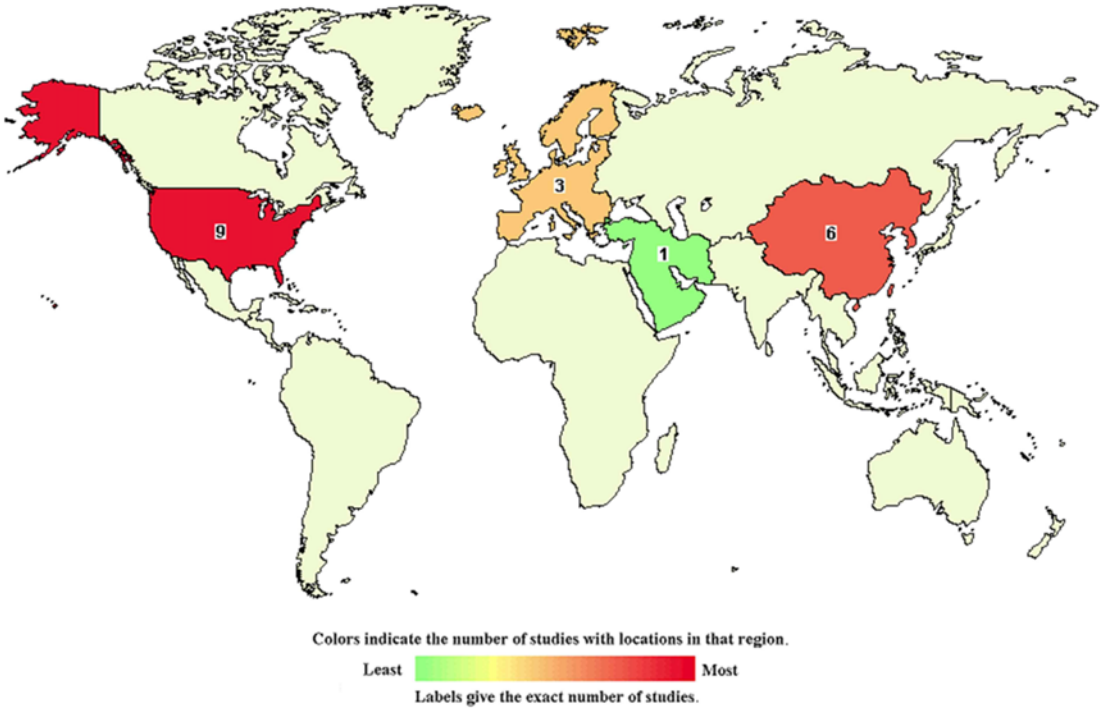

Fig. 1. Status of ASC clinical trials by continents (Keyword; Adipose-derived stem cell, Osteoarthritis). Data from www.clinicaltrials.gov (retrieved Jan 31, 2019).

factor 1, and fibroblast growth factors. Cell-growth factor-containing fractions have exhibited enhanced chondrogenesis of stem cells in *in vitro* and *in vivo* experiments [34,35]. It is thus expected that these fractions will be clinically more efficacious, and have therefore been applied by many researchers. This article summarizes an overview of recent tissue engineering approaches in clinical trials that report the improvement of damaged cartilage, especially in OA, by utilizing autologous or allogenic ASC by injection or implantation at the affected sites.

2. Clinical Evaluation of Cartilage Regeneration Using Various Scoring Systems

To evaluate cartilage regeneration and functional joint improvement for OA, a series of scoring systems have been developed to specifically analyze pain level, physical activities, and morphology/quality of regenerated cartilage tissues at the damaged joint sites. Currently available scoring systems to evaluate progress and restoration of OA are summarized in Table 1. Visual analogue scale (VAS) is a unidimensional evaluation of pain intensity, and is self-completed by the respondent [36,37]. This scale provides intensity of postoperative pain from the patient's perspective. In terms of score interpretation, a higher VAS score indicates greater pain intensity. This method takes less than a minute to complete, and easily exhibits small changes and improvements of pain at each given time point.

The American Orthopedic Foot & Ankle Society (AOFAS) score is a standardized measurement of foot/ankle functionality to evaluate pain (assessed by patient), alignment (assessed by physicians), and function (assessed by both patients and physicians). Its subjective scales have demonstrated reliability and validity [38], whereas the objective scales are not predictable for their reliability and validity [39]. Nevertheless, it is one of the most widely-used instruments in clinical studies, and provides the best comparison between various studies [40].

The International Cartilage Repair Society (ICRS) grade is a validated scoring system that classifies the grade from 0 to 4 in degree of defect repair, integration to border zone, and macroscopic appearance of tissue morphology [41]. This evaluation for the status of OA sites is primarily based on hierarchical zonal morphologies and integration.

The Kellgren-Lawrence (K-L) grade was designed to assess the severity of knee cartilage, and is evaluated solely by plain radiograph images (Fig. 2) [42]. K-L grade helps the physicians to make clinical decisions to define useful surgical management. This system scores from 0 to 4, based on the comparison between radiographic images of patients and standard images. Since K-L grade requires

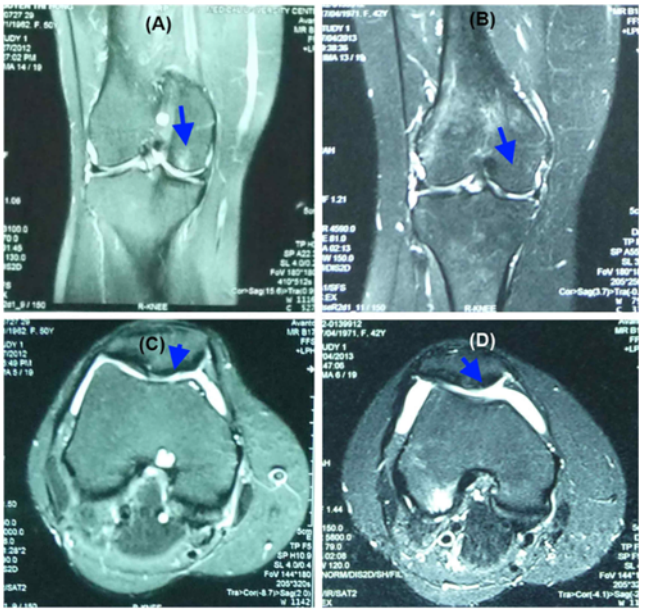

Fig. 2. Representative cartilage region to analyze regenerated cartilages using MRI images. Reproduced with copyright permission from [42].

radiographic imaging, evaluations are performed with limited other investigations [43,44].

The Knee injury and Osteoarthritis Outcome Score (KOOS) system evaluates consequences during the OA period, by examining patient responses for symptoms, stiffness, pain, function of knee, and quality of life. Furthermore, the KOOS score is useful during examinations involving short-term and long-term monitoring of the patient [45-47]. Similarly, the Western Ontario and McMaster Universities osteoarthritis index (WOMAC) was developed to evaluate pain, stiffness, and physical function of the cartilage [48]. Various studies confirm the validity and reliability of the WOMAC index for OA patients, based on the correlation with other pain measurement outcomes [49-51].

The International Knee Documentation Committee (IKDC) subjective evaluation system is one of the most reliable outcome reporting tools that evaluates the improvement of symptoms, knee function, and sports activities [52,53]. This patient-oriented questionnaire is a reliable method for people afflicted with diverse knee disorders (*i.e.*, knee-specific measurement), although this is not a disease-specific evaluation [52,54].

The Lysholm knee scoring system is designed to assess functional instability of the knee, especially for anterior cruciate ligament injuries and knee chondral defects [47,55]. Lysholm scores evaluate condition-specific outcomes including limp, locking, pain, stair-climbing, use of supports, instability, swelling, and squatting [55]. However,

Table 1. Scoring systems for assessing knee function and cartilage regeneration

Ref.	Scoring system	Purpose	Properties	Outcomes
[36,37]	VAS	Evaluate pain of patients	- Hard to compare a scale of pain with other groups - Effectively exhibited changes within individuals	- No pain (0) – unbearable pain (10) at 10 cm line scale - Distance on the 10 cm line determined the score
[38-40]	AOFAS	Evaluate pain, function, and alignment of foot and ankle	- Consist of three parts; pain, function, alignment of foot and ankle - Surveys are included subjective and objective questions	- Total 100 points composed of pain (40 points), function (50 points), and alignment (10 points) - High score indicates healthy states
[41]	ICRS grade	- Patient: Evaluate symptoms, activity level, and function at injury - Surgeons: Evaluate the status of defects sites	- Consists of two parts 1) Patient: contained localization, onset of symptoms, physical function at injury, activity level 2) Surgeons assess degree of defect repair, integration to border zone, and macroscopic appearance	- Grade 0; normal - Grade 1; almost normal but have superficial cracks and fissures - Grade 2; lesion extend down to no more than 50% of cartilage depth - Grade 3; lesions extend down to calcified layer or over 50% of cartilage depth - Grade 4; lesions extend down to subchondral bone plate or trabecular bone.
[42-44]	K-L grade	Assess the severity of knee	- Assess by using a plain radiograph - Survey work performed with limited number of films due to hazard of radiation - Radiograph images should be assessed by the same observer or two observers - Scoring should be performed following standard films	- Grade 0; normal – absence of radiographic image changes of osteo-arthrosis - Grade 1; exhibited possibility of osteophytic lipping and narrowing of joint space - Grade 2; exhibited osteophytes and narrowing of joint space - Grade 3; exhibited multiple osteophytes, clear narrowing of joints space, and possibility of deformation of bone - Grade 4; large osteophytes, clear narrowing of joint space, critical sclerosis, and clear deformation of bone
[45-51]	KOOS	Evaluate symptoms, stiffness, pain, function of knee, and quality of life	- Only evaluated by patients - Each question belongs to one subscale; Pain, Symptoms, Function in daily living, sport and recreation, Quality of life	- Each questions were assigned by the scores following instructions; None (0), Mild (1), Moderate (2), Severe (3), Extreme (5) - Each average subscale score is converted by formulae - High score indicates normal state
[49-51]	WOMAC	Evaluate pain, stiffness, and physical function	- Revealed weakness in stiffness subscale with low test-retest reliability - Could not detect change of physical function	- Assessed by 24 questions about pain, stiffness, and physical function - High score indicates worse patients
[52-54]	IKDC grade	Evaluate improvement of knee symptoms, function, and activities	- Detect improvement of symptom, function, and sports activities - Subjective scale	- Each questions were assigned by the score from 0 (*i.e.*, constant or worst pain) to 10 (*i.e.*, never or no pain) - IKDC Score is determined by calculating that (sum of score in each part) / (maximum possible score in each parts) * 100
[44,56]	TAS	Evaluate level of activity based on work and sports	- Represent specific activity using numerical scale - Requires opinions to the patients about possible activity	- Patients select the level of activity that they can perform - Compared present and past injury level
[44,47, 55,56]	Lysholm knee scoring scale	Evaluate functional instability of knee	- Emphasized the evaluation of instability of cartilage - Requires opinions of patients about symptoms, own functional view, and instability	- Each statements described in section have scores - High score indicates normal state

this method exhibits limitations of weak measurements in daily activities and sports, and suboptimal performance in consistency with varying knee conditions. Hence, the Tegner activity scale (TAS) was developed to evaluate levels of functional activities as a complement to the Lysholm score. This clinician-administered tool describes 11 levels of activities (from level 0 of "Sick leave or disability pension because of knee problems" to level 10 of "Competitive sports-soccer, football, rugby (national elite)"); both Lysholm and TAS scores are commonly used together [44,56].

3. Currently Completed Clinical Approaches in ASC Administration for Cartilage Repair

3.1. Safety and stability of autologous compartments

Recent clinical trials involving administration of ASC applying various strategies are summarized in Table 2. The aim for early pilot reports is to evaluate the safety of autologous compounds such as SVF or PRP for knee OA, and potential improvement of knee pain [57]. In this trial, the SVF suspension was injected to 8 knees from 6 patients (5 female and 1 male, 59 years of mean age). The initial status of each patient was classified as K-L grades I to III, and VAS score of 4 or greater. Adipose tissue was harvested from the abdomen, flanks, and/or lateral thighs, having average 173.5 mL of lipo-aspirate harvest, with an average viable SVF cell count of 14.1 million. After aspirating 5 mL fluid from the intra-articular space, 3 mL of SVF suspension in Lactated Ringer's was injected. Pain and mobility assessment were conducted by the WOMAC Index and VAS scoring, preoperatively and postoperatively at 3 months and 1 year. The clinical outcomes report a decreased WOMAC score from a preoperative mean 32.9, to a postoperative mean 10.8 at 3 months, and 9.4 at 1 year. The VAS score also decreased from a preoperative mean of 5.9 to a postoperative mean of 1.8 at 3 months and 2.1 at 1 year, representing an improved outcome. Orthopedic evaluation exhibited increased, but not significant, range of motion (ROM), and the timed up-and go (TUG) testing reduced by 48% from an initial value of 5.4 sec to 2.6 sec postoperatively at 3 months. No significant changes were observed in the MRI images between 0 and 3 months due to the short period of follow-up. Compared with standard-of care treatments (*i.e.*, visco-supplementation and corticoid steroid injections) and other previous studies [58-62], the procedures in this study are advantageous as a potential OA therapy due to consequent pain reduction, longer maintenance time for 1 year, and positive clinical outcomes.

Based on the anti-inflammatory properties of cells in SVF, a hypothetical mechanism of how adipose-derived SVF reduces OA knee pain has been proposed. SVF exhibits a capability of immunosuppression and could release anti-inflammatory molecules such as IL-1, IL-10, receptor antagonist (IL-1ra), indoleamine 2,3-dioxygenase, TGF-β, and PGE2 [63]. To overcome the few obstacles, following have been suggested for improving clinical results: (1) using a standard MRI for detecting trivial changes postoperatively; (2) studies on the OA evaluation system need to contain multiple questions for clear investigations; (3) sophisticated injection of SVF into the intra-articular site; (4) increasing the number of experimental groups and establishing a control group. In conclusion, the treatment of OA by administering adipose-derived SVF on the OA knee has potential future clinical application due to its safety (the reduction of pain) and feasibility (being potential therapy).

In addition to the incorporation of autologous SVF and PRP to facilitate ASC integration, autologous microfragmented fat tissue can alternatively be utilized for OA treatment [64]. In 2017, Hudetz *et al.* reported that transplantation of microfragmented adipose tissue into 32 knees of 17 patients improved the deposition of cartilage-specific ECM components. Increasing proteoglycan contents on lateral tibial condyle and both patellar facets were observed at 12 months in the delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) images. VAS outcomes of patients exhibited significantly reduced pain score regardless of movement or rest, and without significant changes in inflammation markers including c-reactive protein and immunoglobulin G glycome level in the both local (synovial fluid) and systemic (blood plasma) systems. Therefore, intra-articular administration of autologous microfragmented fat tissue facilitated the cartilage ECM deposition and chondrocyte proliferation via trophic and immunomodulatory mechanisms. Consequently, this approach could also influence biochemical changes in the cartilage and architectural restoration without any adverse events, including chondrotoxicity.

One proof-of-concept phase I/II clinical trial evaluated the safety and efficacy of intra-articular injection of autologous ASCs in patients with knee OA [65], including a 2-year follow-up study that verified the long-term efficacy of injected ASCs to the OA sites [66]. Abdominal subcutaneous fat was collected from participants (n=18) by liposuction, and isolated ASCs prepared in saline were injected with varying cell populations containing 1 (low), 5 (mid), and 10 (high) $\times 10^7$ cells. The high dose group showed significant decrease in the WOMAC score (70%) at the 1-year follow-up, when compared with baseline score. In addition, the size of cartilage defect decreased most efficiently in this group, indicating the regeneration of thick and hyaline-like cartilages. This resulted in enhanced

Table 2. Summary of OA treatment by using ASC in clinical systems

Ref.	Group	Administration	Scoring outcome	Radiograph and physiological therapy outcome
[57]	6 Knee OA patients Patient 1 : 4.1×10^7 cells (Left side) Patient 2 : 7.0×10^6 cells (Both side) Patient 3 : 1.71×10^7 cells (Left side) Patient 4 : 1.76×10^7 cells (Left side) Patient 5 : 7.6×10^6 cells (Both side) Patient 6 : 7.9×10^6 cells (Right side)	Intra-articular injection of SVF suspension	1. WOMAC score decreased until 1 year 2. VAS scores were decreased until 1 year	1. ROM increased but was not statically significant 2. TUG results at 3 months were reduced compared with before treatment 3. No significant changes in MRI images
[64]	17 Knee OA patients (not mentioned amount of ASC)	Intra-articular injection of micro-fragmented adipose tissue	The VAS score at the last follow-up was decreased compared with initial states regardless of movements or rest	dGEMRIC images exhibited that increased GAG content in regions of interest compared with initial GAG contents.
[65,66]	18 Knee OA patients Low-dose: 1.0×10^7 cells Mid-dose: 5.0×10^7 cells High-dose: 1.0×10^8 cells	Direct ASCs injection	1. High-dose group exhibited reduced WOMAC score and VAS score at 1 year 2. The knee score of KSS increased in low-dose group and high-dose group at 1 years 3. The function score of KSS increased in low-dose group until 1 year 4. No significant improvement in all groups at 2 years compared with 1 year	1. High-dose group at 2 years exhibited decreased defect size in medial femoral regions 2. Mid-dose group exhibited disappeared most of regenerated cartilage tissue at 2 years 3. No significant changes in lose-dose group were observed until 2 years
[69]	18 Knee OA patients (2×10^6 cells 10×10^6 cells 50×10^6 cells)	Intra-articular injection of ASC suspensions	1. WOMAC outcomes in low-dose group exhibited significantly decreased at 6 months and the other groups were not exhibited significant difference. 2. VAS scores in low-dose group at 6 months were only significantly decreased about 41.2 compared to baseline 3. KOOS index in low-dose group at 6 months were significantly enhanced compared to baseline	1. Overall dGEMRIC images exhibited increased dGEMRIC index but there was no significant differences regardless of injected cell number 2. MRI images exhibited decreased cartilage regions and there was no significant difference in all groups
[70]	25 Knee OA patients in each groups (Control group: PRP without ASC Study group: PRP with ASC) (1.89×10^6 cells)	Injection of PRP solution containing ASC	1. In the control group only 19.4 point increased in Lysholm score, 0.8 points increased in VAS score, and decreased 1.7 points in VAS 2. In the study group, the clinical result was improved in the Lysholm score (increased 26.9 points), TAS (increased 1.3 points), and VAS (decreased 2.2 points) 3. The older patients (> 55 years) exhibited increased Lysholm score and decreased TAS 1.7 point in VAS, indicating that injection of MSC might be effective on the younger patients	ICRS grade results exhibited that the patients who have ICRS grade 3 enhanced VAS outcomes patients than those ICRS grade 4
[71]	30 Knee OA patients (4.2×10^7 cells)	Intra articular injection of PRP, $CaCl_2$	1. Lysholm scores were increased 2. Decreased VAS score 3. Improved five scales of KOOS at 2 years	25 patients exhibited improved or maintained cartilage status assessed by K-L grade at 2-year follow-up
[30]	Group 1 - not underwent 2nd arthroscopic surgery (23 knees) Group 2 - underwent 2nd arthroscopic surgery (37 knees) (3.8×10^6 cells)	Injection of ASC suspension	1. TAS and IKDC score were increased after ASC implantation in all group 2. Overweight patient and patients who have size of lesions > 5.4 cm^2 in group 2 had significant worse outcome according to IKDC, TAS, and ICRS	Arthroscopic surgery images exhibited regenerated cartilage surfaces

Table 2. Continued

Ref.	Group	Administration	Scoring outcome	Radiograph and physiological therapy outcome
[72,73]	49 Ankle OA patients, 23 ankles: without ASC 26 ankles: with ASC (4.1×10^6 cells)	Injection of ASC after arthroscopic marrow-stimulation procedure	1. The VAS scores were significantly decreased in group 1 and 2 at final follow-up 2. The AOFAS scores were significantly increased in group 1 and group 2 at final day 3. There were no significant differences in the mean VAS and AOFAS scores between group 1 and 2 at final follow-up	1. There was no ICRS grade 1 repair in group 1 at final follow-up 2. 26.1% of patients were evaluated as ICRS grade 2 in group 1 at final follow-up 3. In group 2, ICRS grade 1 was 3.8% and grade 2 was 42.3% at final follow-up 4. ICRS grades at final follow-up between group 1 and 2 had a significant difference
[74]	Group A: treated with arthroscopic marrow stimulation (37 ankles) Group B: MSC injection, followed by arthroscopic marrow stimulation (31 ankles) (3.9×10^6 cells)	Injection of ASC suspension	1. The VAS score and AOFAS score of each group were significantly improved at final follow-up 2. The Roles and Maudsley score in group B exhibited greater improvement compared with group A 3. The TAS score exhibited a significant difference between groups with higher level in group B at the final follow-up	1. The patients who did not undergo ASC injections exhibited i) fibrotic tissue regeneration in the lesion observed by second-look arthroscopic surgery and ii) cartilage defects in the lesions observed by MRI 2. The patients who underwent ASC injections exhibited filled cartilage defects observed by second-look arthroscopy and MRI
[79]	49 Knee OA patients (4.3×10^6 cells)	Implantation of fibrin gel containing ASC	1. Lesion size assessed by IKDC score was increased at final follow-up 2. Significant enhancement of activity level assessed by TAS was observed under 60 years.	-
[28]	20 Knee OA patients (4.4×10^6 cells)	Arthroscopic implantation fibrin gel containing ASC	1. The IKDC scores were significantly increased at the final follow-up 2. The TAS results exhibited abundant patients in the 0 and 1 grade at final follow-up	MOAKS scores exhibited enhanced grade in the size of cartilage loss area and percentage of full-thickness cartilage loss
[27]	56 Knee OA patients Group1: SVF (3.9×10^6 cells) Group2: Fibrin gel contained SVF	Injection of SVF or fibrin gel	1. Group 1: the patients who have lesions larger than 5.7 cm^2 exhibited significantly worse outcomes in IKDC score and TAS 2. Group 2: IKDC scores and TAS results exhibited no significant differences in group 2 organizing lesion size, age, location, *etc.*	1. 23% of lesions had achieved a normal or near-normal state (ICRS grade 1, 2) in group 1 2. 58% patients in group 2 were assessed as 1 or 2 grade by using ICRS
[82]	2 Patients: Hip 2 Patients: Knee (not mentioned amount of ASC)	PRP, HA, $CaCl_2$	1. The first and second patients exhibited improved VAS score and range of motion 2. The third patients exhibited decreased VAS score and increased flexion of knee and range of motion 3. The forth patients exhibited decreased VAS score and improved flexion of the knee	1. The first patients exhibited significant filling of bone defect in MRI 2. MRI image of second patients indicated significant filling of bone defects with a possibility of bone matrix formation 3. The third and forth patients exhibited significant increase in the thickness of meniscus cartilages
[83]	91 OA patients (knees, hips, low backs, ankles) (not mentioned amount of ASC)	Injection of SVF	VAS scores were significantly decreased until 3 months after SVF injection	No evidence of tumor formation in 27 joints that were analyzed by MRI
[84]	3 Knee OA patients (not mentioned amount of ASC)	Implantation of HA containing PRP	The VAS score of patients 1, 2, 3 were significantly decreased between pretreatment and 16 weeks after treatment	1. The ROM of patient 1, 2, and 3 were significantly improved at last follow-up 2. MRI images exhibited increased cartilage-like tissue on the medial side of the knee

ICRS grade in the medial femoral and tibial condyle, as observed in the second-look arthroscopy. Moreover, histological evaluation of biopsy using Safranin O, type I collagen and type II collagen staining showed marked improvement in the medial and femoral condyle defects due to newly regenerated articular cartilage tissues. However, no significant differences were observed in the lower dose groups at the 2 years follow-up. Knee Society Clinical Rating System (KSS) score in the high-dose group exhibited a significantly increased score (78.6%) at the 1-year follow-up as compared to the baseline, while but a low-dose group reveled 117.9% increase at the 1-year follow-up. A similar pattern was presented for the KOOS score, except for sports and quality of life subscales. Structural outcomes by K-L grade exhibited medial compartment regeneration at 6 months in the high-dose group. MRI evaluations indicated regenerated articular cartilages in the medial femoral and tibial condyles, and decreased defect size and increased cartilage volume in the medial femoral, in high-dose group up to 2 years, with no significant difference in the low dose groups. Overall, these results reveal that administration of high-dose ASC results in regenerated cartilage regions, decreased pain, and enhanced movement in the elderly. This indicates that cell therapy using ASC improves the OA over 2 years, and number of cells injected should be considered for enhancing the efficacy.

3.2. Controlling parameters for ASC-mediated OA treatments

Although the number of injected ASCs is perhaps an important regulatory parameter for OA treatment, clear evidence for ASC dose is still under investigation, especially at the clinical level. For example, Orozco *et al.* reported that 4.0×10^7 BMSCs improved the cartilage morphology and quality in 11 of the 12 patients after 1 year of treatment [67]. Sekiya *et al.* suggested that injecting 4.7×10^7 synovial MSCs in 10 patients enhanced their outcomes in terms of MRI score, qualitative histology, and Lysholm score [68]. However, Pers *et al.* reported no dose-dependent clinical outcome observed in the Phase I trial of ASC administration (Fig. 3) [69]. In this trial, all participants were diagnosed at 3 to 4 grade of K-L scale and were divided into 3 groups being administered different doses of ASC (2×10^6, 10×10^6, and 50×10^6 cells). Clinical outcomes exhibited significantly improved pain, function, and stiffness of the

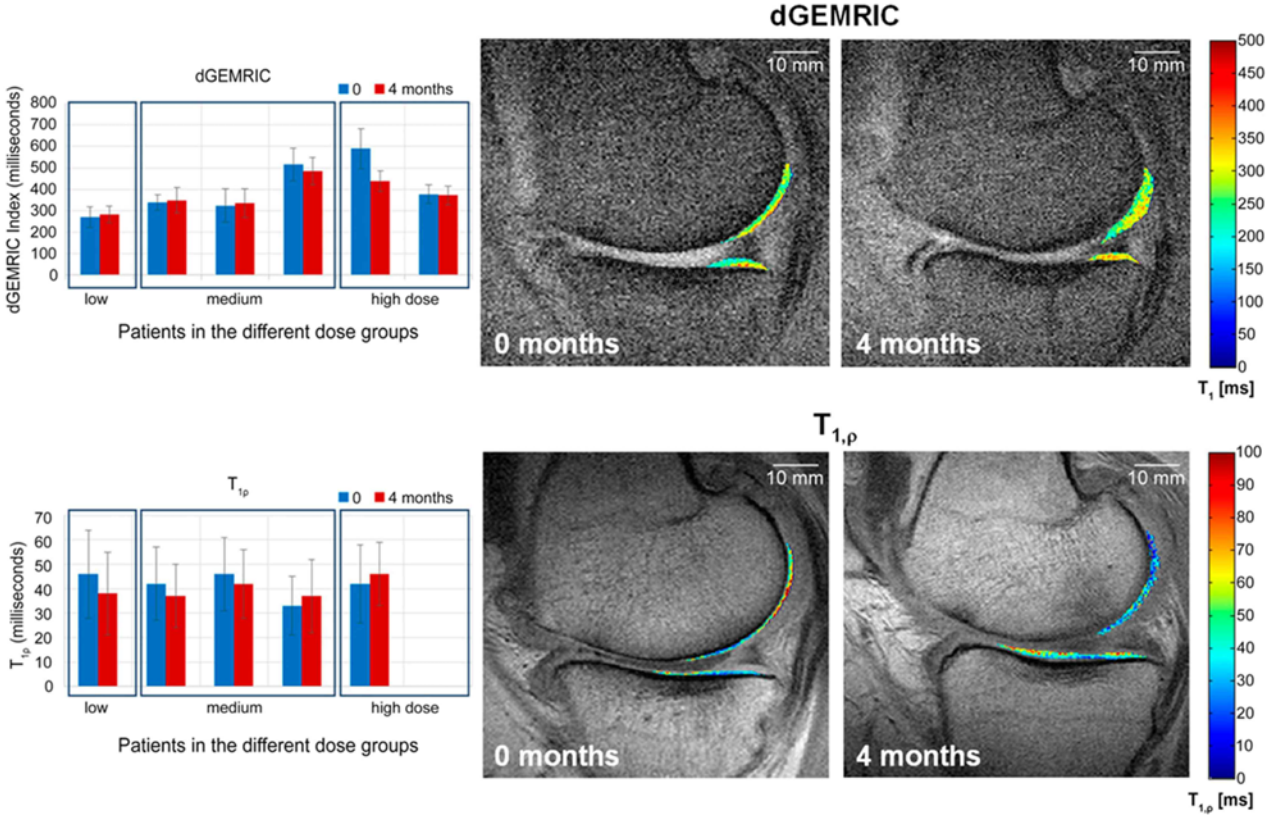

Fig. 3. dGEMRIC and MRI images of patients. Left images exhibited dGEMRIC and T_{1rho} values of different dose group at before and 4 months after the treatment. MRI images exhibited cartilage changes in low-dose group observed by dGEMRIC and T_{1rho}. Increased dGEMRIC and decreased T_{1rho} described improved cartilage regeneration. Reproduced with copyright permission from [69].

OA knee as compared to pre-treatment states in only the 2×10^6 dose group. A clear correlation between ASC numbers and clinical outcomes for OA treatment was not observed due to the small sample size (6 patients in each dose group). Nevertheless, the highest pain level at baseline in pre-treatment states (*i.e.*, inflammatory milieu) of patients in the low-dose group might prime the injected ASCs to exert their immune-modulatory functions and subsequently improve the patient responses. Another clinical trial reported using infrapatellar fat pad derived MSCs, and proposed that 1.2 - 2.3 $\times 10^6$ stem cells in 3 mL of PRP carrier is optimal for clinical chondrogenesis in knee OA treatment [70].

In general, clinical outcomes of ASC administration for the treatment of knee OA could be evaluated using arthroscopic inspection [30,71]. Koh *et al.* performed a series of clinical trials to investigate the effect of intra-articular injection of ASCs using the Lysholm score, KOOS, VAS score, radiographic evaluation, and second-look arthroscopic findings. One of their early trial included 30 elderly patients (5 men and 25 women, mean age 70.3 (range 65-80) years) who had failed nonsurgical treatment and were diagnosed as idiopathic or secondary knee OA (K-L grade 2 or 3 OA in multiple compartments). ASCs were prepared from the subcutaneous fat tissue extracted from the patients' buttock, and a mean of 4.0 $\times 10^6$ cells (*i.e.*, 9.7% of 4.2 $\times 10^7$ SVF cells) in 3.0 mL of PRP were implanted with arthroscopic lavage. After surgery, the patients underwent clinical assessments conducted at the 3-month, 12-month, and 2-year follow up visits. Clinical outcomes indicated that the ASC injection was effective in cartilage healing, alleviating pain, and enhancing functions in the elderly patients with knee OA, showing significantly increasing mean Lysholm and VAS scores, from the preoperative to the 2-year follow-up. The median KOOS was also improved in all five categories including pain, symptoms, activities of daily living, sports/recreation, and quality of life (Fig. 4). A statistically significant association between patient's age and mean improvement from baseline was observed in all KOOS subscales only at the 2-year follow-up. On second-look arthroscopy, 14 of the 16 elderly patients (87.5%) experienced improved or maintained cartilage status at least 2 years postoperatively, and only 5 patients demonstrated worsening of the K-L grade. A subsequent study also investigated the clinical efficacy of ASC injection on knee OA treatment and the downstream factors that influence the results after MSC [30]. This study enrolled 56 patients (60 knees) diagnosed with K-L grade 1-2 and presenting with symptoms of knee joint pain and/or functional limitations. Joint function and sports activities of the patients were evaluated both pre- and post-operatively by IKDC score and TAS. Patients were classified into two groups, depending whether they had undergone a previous second-arthroscopic procedure. Following removing all the unstable and damaged cartilages from the lesion, the prepared ASC suspension (isolated from the patients' buttocks 1 day prior to arthroscopic surgery) containing a mean of 3.8 $\times 10^6$ cells/mL was filled into the cartilage lesion under arthroscopic guidance. The clinical outcomes revealed improved mean IKDC score (from 38.0 to 61.0) and mean TAS score (from 2.5 to 3.6) at the final follow-up (mean 26.5 months). Interestingly, when associations between patient characteristics and outcomes were considered, statistical significance was found between patient weight and clinical and arthroscopic outcomes. Overweight patients (BMI $\geq$ 27.5 kg/m^2) and patients with

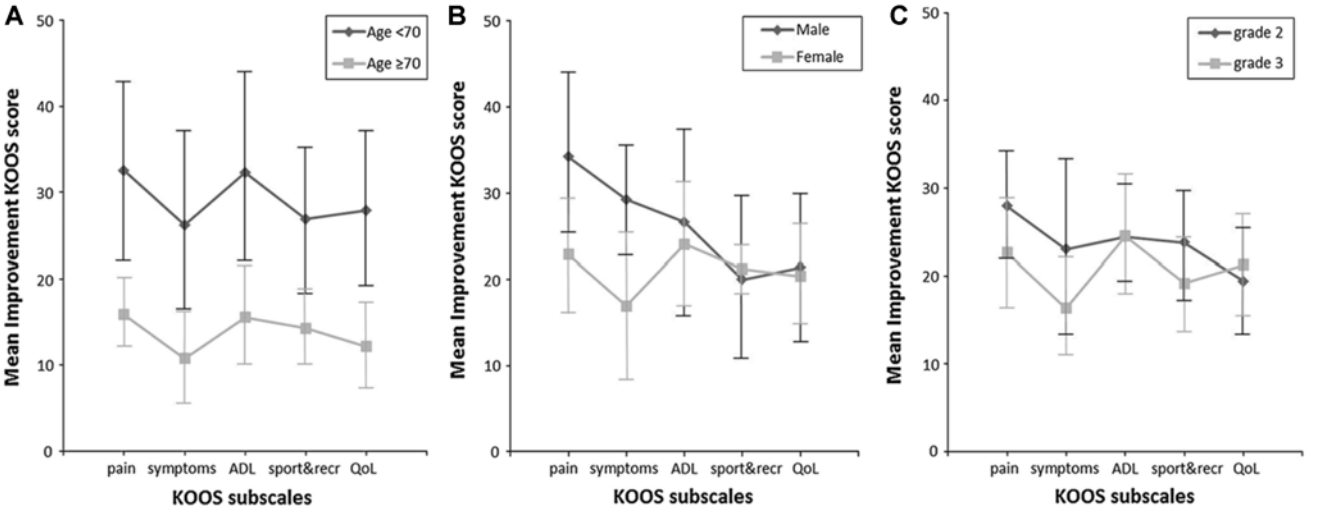

Fig. 4. Analyzed data of ASC treated patients using KOOS at 2-year follow-up by grouping A. age, B. gender, and C. K-L grade. QoL: Quality of life. Reproduced with copyright permission from [71].

size of lesions > 5.4 cm^2 had significantly worse outcomes with respect to the IKDC score, TAS score, and ICRS grade, indicating that both factors affect the clinical outcomes in ASC implantations. In the second-look arthroscopic findings, 2 of the 37 lesions (5%) were grade I (normal), 7 (19%) grade II (near normal), 20 (54%) grade III (abnormal), and 8 (22%) grade IV (severely abnormal), indicating only 24% of lesions had achieved a normal or near-normal state (ICRS grade I or II) at the second arthroscopic procedure. Taken together, these results indicate that compared to conventional treatment such as microfracture surgery in OA knees, ASC implantation exhibits improved clinical outcomes, thereby implying it to be a potential efficacious therapy for OA patients.

Another clinical trial investigated how ASC injection improves the cartilage regenerative efficacy of patients who underwent marrow stimulation with lateral sliding calcaneal osteotomy for varus ankle OA [72]. Apart from mechanical improvement of the lateral sliding calcaneal osteotomy via altering weight-bearing axis and suitable mechanical environment for halting degenerative changes in the articular cartilage, cartilage regeneration of medial osteoarthritic lesions is inevitable for the fundamental recovery of joint cartilage sites. A previous study reported that patients with osteochondral legions of the talus show superior clinical outcomes by combined administration of MSC and marrow stimulation, as compared to marrow stimulation alone [73,74]. The arthroscopic marrow-stimulation procedure for arthritic lesions was performed in a standardized manner in patients with Outerbridge classification grade 4: (1) debridement of all unstable and damaged cartilage in the lesion, (2) microfracture or abrasion arthroplasty, and (3) injection of ASCs (4.5×10^7 SVF cells, containing a mean of 4.1×10^6 stem cells) into the lesion site after microfracture. All patients were clinically and radiologically evaluated preoperatively and during follow-up using VAS score and AOFAS ankle-hindfoot score for clinical evaluations, and weight-bearing antero-posterior and later radiographs for radiologic evaluations. In an effort to evaluate cartilage regeneration, second-look arthroscopy was conducted with ICRS grade, following removal of the screws after radiologic and clinical confirmation of the osteotomy site. The clinical results showing improved VAS and AOFAS scores as well as enhanced ICRS grade with a significant correlation (Kruskal-Wallis test), demonstrates the viability of dual treatment using arthroscopic marrow stimulation and ASC injection after lateral sliding calcaneal osteotomy with varus ankle OA, as compared with marrow stimulation alone. Therefore, it was speculated that marrow-stimulation treatment recruits the progenitor cell population in the bone marrow, leads to coverage of the lesion with fibrous cartilage, and additional injection of ASCs effectively facilitates cartilage regeneration MSCs as a supplementary strategy [75]. Additionally, an important mechanism of ASCs for osteoarthritis therapy is paracrine signaling mediated stimulation of endogenous cell proliferation, prevention of apoptosis, improvement of blood flow in defected or diseased joints, and subsequent initiation of repair procedure [42,76-78]. Moreover, easy preparation and handling, adhesive selection of ASCs onto plastics from heterogenous crude stromal fraction, and prolonged differentiation potential as compared to the bone marrow derived progenitor cells are advantages of the ASC-based cell therapy.

Furthermore, Kim *et al.* investigated the intrinsic factors associated with clinical outcomes in the ASC injection for knee OA treatment [79]. In this study, 49 patients were classified into subgroups depending on age (< 50, 50-59, and ≥ 60 years), sex, side of involvement (right and left), BMI (< 20.0, 20.0-24.9, 25-29.9, and ≥ 30.0 kg/m^2), lesion size (< 3.0, 3.0-5.9, 6.0-8.9, and ≥ 9.0 cm^2), and lesion location (medial femoral condyle, lateral femoral condyle, and trochlea). Autologously obtained ASCs (an average of 4.3×10^6 cells) were mixed with fibrin glue as a scaffold, and implanted into the defected lesion under arthroscopic guidance. For clinical evaluation during the 26.7 months of the mean follow-up period, all participants were evaluated by following the OA scoring system, IKDC and TAS to detect improvement or deterioration in symptoms, function, and sports activities due to knee impairment. The clinical results indicated that ASC implantation resulted in an increase in mean scores of both IKDC and TAS, from 37.7 and 2.2 to 67.3 and 3.8, respectively. There were significant differences at final follow-up with respect to the age and lesion size groups; however, genders, side of involvement, or BMI groups showed no difference. Hence, after multivariate analyses, it was concluded that (1) two major factors that influence the clinical outcomes of ASC administration are age of the patient and lesion size at the OA sites, and (2) the age of 60 years and a lesion size of 6.0 cm^2 were considered as a cutoff value for resulting recommended outcomes.

While most clinical trials have investigated the safety and efficacy of localized injection of ASCs in knee defect sites, a possibility of systemic transplantation of MSC through intravenous (IV) injection has recently been examined [80,81]. This phase I study with 9 rheumatoid arthritis patients evaluated the safety and tolerability of umbilical blood-derived MSCs with the intravenous infusion. After four injections of 2.5 to 10×10^7 MSCs, vital signs of patients demonstrated no negative safety issues in all groups up to 4 weeks. Interestingly, a test for serum cytokine level reveals reduced expression of IL-1β,

IL-6, IL-8, and TNF-α after 24 h of MSC IV injection in the high-dose (10×10^7 cells) group. In addition to the general route of stem cell administration into the local knee defects, this clinical study demonstrates that systemic administration could also be a feasible option, especially for immune-suppress treatment.

4. Biomaterials as a Scaffold for Enhanced ASC Localization

Based on previous studies, recent clinical ASC administrations have often been combined with utilizing biomaterial matrix as a scaffold for localization of transplanted progenitor cell populations at the specific defect sites [27,28]. A direct intra-articular injection of ASCs in suspension form might result in dissipation of the delivered cell population, limited cell retention and survival, and subsequently low levels of cellular engraftment at the damaged sites. Although a need of scaffold materials in a clinical setting is still under investigation for optimizing outcomes [29], a series of trials have recently investigated the clinical efficacy of tissue engineered materials-based ASC implantation for OA treatment.

Fibrin is the most frequently used biomaterial for ASC transplantation [31]. Due to its high biodegradability, biocompatibility, injectability, and ease of handling, recent approaches have evaluated its superior clinical outcomes on ASC implantation. For instance, in a previously discussed article, Kim *et al.* reported that a direct ASC implantation into the large size of the cartilage lesion (≥ 5.4 cm^2) in knee OA defect results in poor clinical outcomes [30], hence, a subsequent clinical trial by the same research group evaluated the efficacy of clinical and second-look arthroscopic outcomes of MSC implantation using fibrin glue as a scaffold [27]. In this study, the second-look arthroscopies were conducted among OA patients with K-L grades 1-2 at a mean of 12.3 months postoperatively. Combining lyophilized human plasma fibrinogen (71.5 - 126.5 mg/mL) in 1 mL of aprotinin solution, thrombin (4.9 - 11.1 mg/mL) dissolved in calcium chloride solution (13.9 - 15.6 mg/mL), and a mean of 4.2×10^7 SVF cells containing an average 3.9×10^6 ASCs instantaneously form a fibrin gel mixture. Along with significantly enhanced clinical outcomes at 28.6 months of final follow up with increased IKDC and TAS scores in both groups, with or without fibrin materials, the prognostic factors (*i.e.*, body mass index (BMI) and size of cartilage lesions) in ASC implantation were also investigated. Patients with high BMI (> 27.5 kg/m^2) had significantly worse clinical outcomes than those with a lower BMI (< 27.5 kg/m^2) in the group administered without fibrin assist. However, this was not found in the high BMI patient group administered ASC implantation with a fibrin scaffold. Moreover, 58% of the lesions had achieved a normal or near-normal state (ICRS grade I or II) in patients with fibrin assisted ASC implantation, as compared to 23% in the group without fibrin. Also, strong correlations between size and ICRS overall repair grades were identified in both groups. These results support the theory that lesion size might be an important prognostic factor, and fibrin glue could be an effective scaffold in ASC implantation in OA knees, if some limitations (sample size, follow-up period, the optimized method for fibrin-cell mixture, and number of ASCs) are improved. In addition, analyzing MRI images also verified the improved efficacy of ASC implantation with fibrin gel [28]. Totally, 24 patients presenting with knee joint pain, limited movement, and experiences of nonsurgical treatments within 3 months, underwent ASC implantation (notably, regardless of cell number) with fibrin glue. MRI evaluations as per the MRI Osteoarthritis Knee Score (MOAKS) system evaluated the cartilage lesions before and after surgery for size and depth of the damaged cartilage. In addition to increased IKDC and TAS score, MRI outcomes exhibited that grade of the size of cartilage loss area was enhanced, and percentage of full-thickness cartilage loss grade was improved. Hence, this clinical report also concluded that ASC implantation embedded in fibrin glue to the damaged cartilage sites could treat cartilage defect and full-thickness cartilage loss region.

Further studies were undertaken to investigate if HA could support the ASC-associated physiological performance at the OA sites. Park reported the outcomes of clinical trials in 4 individuals with osteonecrosis of hips (the first two cases) and OA of knees (the latter two cases) [82]. For investigating the regenerative efficacy, autologous ASCs along with PRP, HA, calcium chloride ($CaCl_2$), and dexamethasone were percutaneously implanted. Experimental procedures included (1) initial patient classification according to degree of VAS pain score and MRI results, (2) preparation of autologous ASCs from each patient, injection of ASCs into the affected area, and subsequent injection of PRP/$CaCl_2$, and (3) clinical assessment by MRI scan, VAS, and functional rating index scoring system during follow up periods. In terms of knee joint treatment, the third case concerned a 70-year-old Korean woman with OA having over 5 years history of right knee pain and VAS score 7 at rest. An 8.5 cm^3 mixture of ASCs, PRP, dexamethasone, and HA was injected into the medial and lateral sides of the knee, and she was also provided additional PRP and dexamethasone injections over the next four weeks. During the follow-up at the 7th week and 12th week of ASC injection, the VAS score indicated improvement

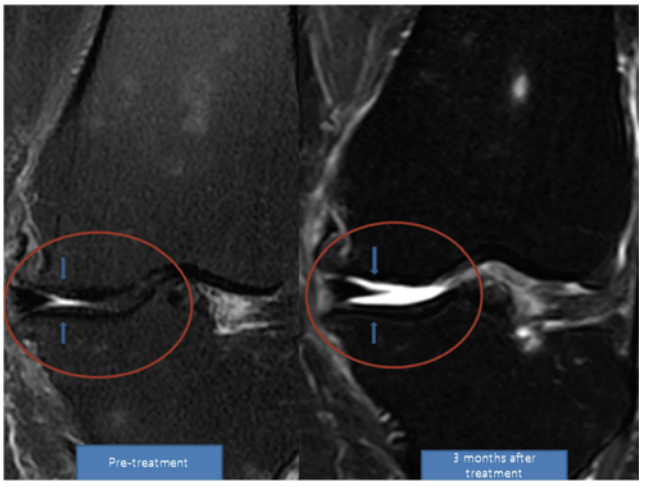

Fig. 5. Sagittal T2 view images of the knee observed by MRI. MRI images exhibited increased thickness of medial meniscus cartilage. Reproduced with copyright permission from [82].

of pain to more than 80% and 90%, respectively. Flexion of knee and range of motion were also improved, along with significant increase in the thickness of meniscus cartilage on the medial side of the right knee, as observed in the post-treatment MRI images. The final case was a 79-year-old Korean woman with bilateral knee pain (VAS score 8), mild joint swelling, deformity of the knee, a decreased range of motion, and tenderness with flexion due to OA. After the same ASC treatment, the pain improved over 50% and 90% indicating improvement of flexion of the knee, in the follow-up from the 4th week to 12th week, respectively. A significant increase in the height of her meniscus cartilage on the anterior medial side of the left knee is presented in Fig. 5. This series of clinical case reports presents clear MRI evidence of apparent bone regeneration in osteonecrosis of femoral heads and meniscus cartilage regeneration in OA of human knees. Combinatorial administration of ASCs, PRP, HA, and $CaCl_2$ therefore offers a possibility of successful human clinical outcomes for bone and cartilage regeneration.

Another study investigated the safety standards regarding human orthopedic applications using ASCs in the form of SVF with PRP by implanting into articular joints [83]. The retrospective study was performed for 91 patients with 100 articular joints including knees, hips, lower back, and ankles. Adipose tissue was harvested from the patients' lower abdomen area, and 10 mL of ASC-containing SVF was obtained. The prepared ASCs was mixed with 2 mL PRP derived from autologous blood. The final mixture was prepared with 0.5% (w/v) HA and supplemented with $CaCl_2$, to be used as the scaffold and for activation of platelets, respectively. This ASC mixture was injected into the specific joint under ultrasound guidance, and patients were administrated additional autologous PRP each week over a one-month period. After ASC/PRP implantation, the VAS score gradually decreased from 26.62 at pre-treatment to 4.43 at the end of the 3 months follow-up. A similar administration approach using SVF, PRP, and additional HA has recently been conducted for 3 patients [84]. At the start and end point, the VAS, functional rating index (FRI), range of motion (ROM), and MRI were performed and compared. In all 3 patients (60 to 87 years old, over 7 VAS score before treatment), they reported (1) pain relief (*i.e.*, lower VAS scores), (2) formation of increased cartilage-like thickness tissue by MRI evaluation after 16 weeks, and (3) enhanced ROM. This report therefore suggests that ASC injection with autologous PRP improves the FRI, VAS, and ROM with morphogenic changes in MRI, over a longer duration. It could also be speculated that (1) a variety of native growth factors in PRP stimulate the chondrogenic differentiation of ASC to mature chondrocytes, and (2) HA functions as a scaffold for cellular adhesion and localization. Therefore, these series of clinical trials demonstrate the functional advantages of utilizing autologous PRP as native biological stimuli, HA for supporting scaffold biomaterials, and $CaCl_2$ as biochemical activator for an enhanced ASC administration strategy.

5. Summary

Several clinical trials have been carried out for the regeneration of cartilage tissue by utilizing various stem cells and components. Previous *in vitro* and *in vivo* studies suggest that ASC exhibits potential advantages over other stem cells for advanced treatment of OA. Hence, ASCs have been injected or implanted at the damaged cartilage sites. Clinical trials were performed by changing (1) ASC population, (2) method of administration (*i.e.* direct injection or implantation suspended in hydrogel), (3) combination with PRP or SVF, and (4) surgical procedure. The patients were carefully monitored by radiography and scoring systems for any physiological changes over 6 months after treatment. Although patients who underwent ASC treatment showed no significantly enhanced recovery between the 1-year and 2-year follow-ups, clinical ASC therapy outcomes exhibited (1) decreased pain, (2) improved movement of joint, and (3) decreased size of defect sites in the radiograph outcome. Taken together, ASC appears to be potential therapeutic agents which could be utilized as promising OA regeneration candidates. Furthermore, development of scaffolds and the combination of additional compounds would help overcome the current clinical limitations.

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