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Invited Review

Adipose-derived stem cells in orthopaedic pathologies

Federico Giuseppe Usuelli[†], Riccardo D'Ambrosi[†,‡], Camilla Maccario[†,‡], Cristian Indino[†], Luigi Manzi[†], and Nicola Maffulli[§,¶,*]

[†]Foot and Ankle Unit, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy, [‡]Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Italy, [§]Department of Orthopaedics and Traumatology, Azienda Ospedaliera San Giovanni di Dio e Ruggi d'Aragona, University of Salerno, Italy, and [¶]Queen Mary University of London, Barts and the London School of Medicine and Dentistry, Centre for Sports and Exercise Medicine, Mile End Hospital, London, UK

*Correspondence address. Queen Mary University of London, Barts and the London School of Medicine and Dentistry, Centre for Sports and Exercise Medicine, Mile End Hospital, London, UK. E-mail: n.maffulli@qmul.ac.uk

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Abstract

Introduction: To examine the current literature regarding the clinical application of adipose-derived stem cells (ADSCs) for the management of orthopaedic pathologies

Sources of data: MEDLINE,SCOPUS, CINAHL and EMBASE (1950 to April 14, 2017) were searched by two independent investigators for articles published in English. Reviews, meta-analyses, expert opinions, case reports, mini case series and editorials were excluded. Furthermore, we excluded animal studies, cadaveric studies and *in vitro* studies.

Areas of agreement: ADSCs seem to produce excellent clinical results. However, the length and modalities of follow-up in the different conditions are extremely variable. Nevertheless, it appears that the use of adipose-derived stem cells is associated with subjective and objective clinical improvements and minimal complication rates.

Areas of controversy: None of the studies identified is a randomized double-blinded trial, and most of the selected studies present major limitations, and different methods, confounding the results of our review.

Growing points: It is necessary to conduct more and better studies to ascertain whether ADSCs really play a role in orthopaedic surgery with

particular attention to ADSCs harvesting method, type of administration and the conditions treated.

Areas timely for developing research: The current literature regarding the use of ADSCs for orthopaedic pathologies is limited. At present, long-term safety is the biggest challenge of ADSCs based regenerative medicine.

Level of evidence: Level IV—Study of Level I, II, III, IV

Key words: adipose-derived stem cells, regenerative medicine, stromal vascular fraction, mesenchymal stem cells, injection, PRP, scaffold

Introduction

Stromal cells can be obtained from the extracellular matrix of adipose tissue, bone marrow, synovial membranes, dental pulp, tendons, bone and periosteum trabeculae, skeletal muscle, nervous system, skin and placenta.[1–15] These cells appear to be similar in morphology and function to mesenchymal bone marrow cells, and have essentially an immunophenotype compatible with the definition of mesenchymal stem cells (MSCs), although with variation depending on the tissue of origin, method of isolation and type of culture.[9,12,16–18] These cells have been isolated and cultured, and their characteristics have been tested. The available scientific literature gives no description of the characteristics and behaviour of adipose-derived stem cells (ADSCs) in uncontrolled and not manipulated conditions.[16,19,20] The stemness characteristics, the immunomodulatory properties and the wide distribution in adults have shown MSCs as an important application for regenerative medicine and research. For these purposes, many aspects need to be considered, from the collection of adipose tissue to the method of isolation of stromal portion and subsequent culturing. These procedures impact not only on the characteristics of the cells, but also on their viability and survival after cryopreservation, and may affect the repeatability of the experimental data.[16] In general, the ideal type of MSC should be easily isolated in large amounts, should maintain the characteristics of stem cell, and should consist of minimally manipulated cells both during the phase of isolation and *in vitro*, in case of therapeutic applications.[21–23]

Classically, MSCs can be obtained from the stromal fraction of bone marrow (HBM-MSC).[2–4,24,25] HBM-MSC present several limiting features. Harvesting involves the surgical removal of the matrix portion; this is subsequently disintegrated by mechanical stress. This process allows to isolate from 0.01% to 0.001% of mononuclear cells from the harvested cells.[9,26] In addition to the low availability and the invasive method, the use of HBM-MSCs has been associated with viral infections that alter the clonogenic characteristics.[24,25,27,28] Also, HBM-MSCs harvested from aged donors show a decrease in their differentiation potential.[26,29] For these reasons, new sources of mesenchymal cells have been considered to satisfy the requirements of easy availability and stability of the cells.[3–5,22,26] Among these, the stromal fraction of adipose tissue is a reliable source of MSC. MSCs harvested from adipose tissue (ADSCs) together with the HBM-MSC are widely studied.[30] ADSCs show properties and characteristics equivalent to multipotent cells isolated from other tissues such as bone marrow.[31,32] In addition, these stromal cells are able to differentiate into several cellular lines including, adipocytes, chondrocytes, osteoblasts, hepatocytes, pancreatic cells, muscle cells and neurons like cells both *in vitro* and *in vivo*.[15,31,33–38]

The most promising aspect of ADSCs lies in their location: adipose tissue is distributed in each region of the body, and represents 10–30% of the weight of a healthy person, with an isolation yield of 5000 cells per gram of tissue. Furthermore, in surgical liposuction procedures 30 ml–6 l of lipoaspirate are

removed and would normally be discarded.[21,26] A limiting factor in the clinical application of ADSCs is the manipulation during laboratory procedures: the lipoaspirate, which contains, in addition to ADSCs, aggregates of adipocytes, collagen fibres, blood and vascular components, is processed enzymatically with collagenase A type I, and undergoes haemolysis before culturing.

Despite the promising results reported, both in regenerative and reconstructive medicine, there are still many challenges to overcome to introduce ADSCs in routine clinical practice, especially in regards to isolation, indications and safety.[39–42]

This systematic review evaluates the current application in literature of ADSCs in humans for orthopaedic ailments, and assesses the efficacy, tollerability, safety and future development *in vivo* for the management of orthopaedic conditions.

Materials and methods

MEDLINE, SCOPUS, CINAHL and EMBASE (1950 to April 14, 2017) were searched by two independent reviewers for clinical application of ADSCs in musculoskeletal medicine. This meta-analysis was conducted on the basis of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (Fig. 1).[43]

The search terms used were 'adipose stem cells' OR 'adipose mesenchymal stem cells' OR 'stromal vascular fraction' OR 'adipose-derived stem cells' AND ['orthopaedic' OR 'orthopaedic' OR 'upper limb' OR 'lower limb' OR 'joint' OR 'ankle' OR 'hip' OR 'knee' OR 'shoulder' OR 'elbow' OR 'hand' OR 'osteoarthritis' OR 'cartilage' OR 'nonunion' OR 'tendon' OR 'spine' OR 'musculoskeletal system' OR 'wrist'].

Initially, the search led to evaluate a total of 8459 articles; after initial assessment, a total of 537 articles remained. Of these, 514 were subsequently excluded based on the search criteria. Finally, 23 studies met the inclusion criteria and were included in the study.[44–66]

Inclusion and exclusion criteria

In this review, we included study of Levels I, II, III and IV regarding human subjects, with no age restriction, treated with ADSC. The assessment of level of evidence of the selected articles was performed according to 'The Oxford 2011 Levels of Evidence'.[67] Morever no follow-up limit was required as inclusion or exclusion criteria. We excluded from the study reviews, meta-analyses, expert opinions, case reports, case series with less than five patients, animal studies, *in vitro* studies and editorials. Two independent reviewers analysed and evaluated all the information available from the articles. In cases of disagreement between the two reviewers, a third senior reviewer was asked to evaluate and analyse the articles.

Results

The details of the 23 articles included in the present systematic review are reported in Table 1.[44–66] Of the 23 studies selected, 11 (47.82%) were Grade IV of level of evidence,[44–48,55,56,58,61–63] eight (34.78%) Grade III,[49–54,57,64] three (13.05%) Grade II[59,60,65] and one (4.35%) Grade I.[66] Seven articles (30.43%) described the results of treatment of isolated chondral or osteochondral lesions (of which five of the knee and two of the ankle),[47–49,51,52,54,59] two articles (8.69%) dealt with tendinopathy (one lateral epicondilopathy and one Achilles tendinopathy),[61,66] in three (13.05%) articles ADSCs were used for the treatment of deformity (one varus knee and two varus ankles),[50,53,60] in nine studies (39.13%) stem cells were used for OA (eight knee OA and one for OA in different joints)[45,46,55–58,62,64,65], one article (4.35%) dealt with bone defect,[44] while one (4.35%) article evaluated the safety related to the treatment of joint disease.[63] Altogether, 1746 procedures were considered; all investigations were published between 2012 and 2017. The total number of complications identified in the present review is 254/1746 (14.55%), most of which were minor, such as joint pain or other complications not directly related to the treatment. Four studies (17.39%) used ADSCs in combination with PRP.[56,60,63,65] For what concerns clinical applications, six studies (26.0%) used a direct intra- or peri-articular or intra- or peri-tendinous; 15 studies (65.3%) used ADSCs during surgical procedure, while in the remaining two (8.7%) injections were performed on the same day of the arthroscopy after stem cell preparation (3–4 h).

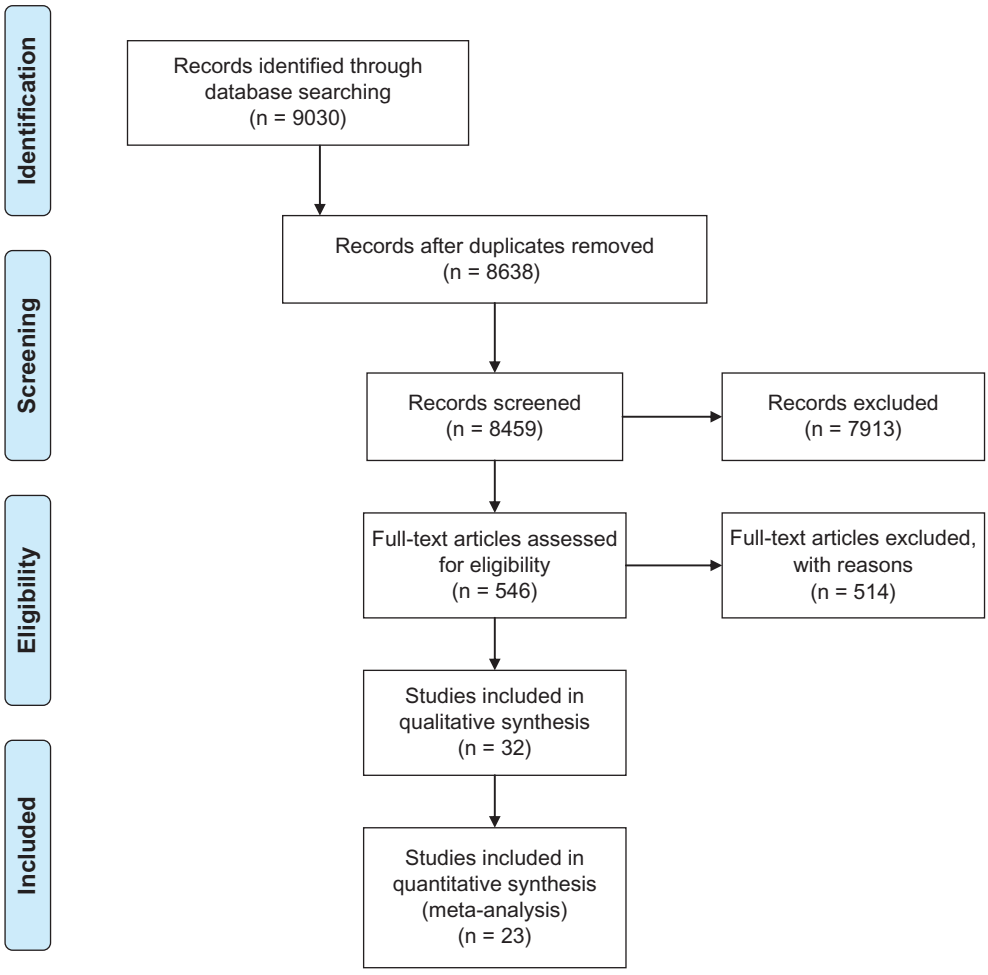

Fig. 1 PRISMA flowchart. Moher D, *et al.*[43]

Osteochondral lesions

Kim *et al.* in 2015 treated 54 patients (56 knees) with symptomatic knee OA using ADSCs, harvested from the patient's buttock.[49] Patients were divided into two groups:

- ADSCs implantation without a scaffold (Group 1 – 39 knees)
- ADSCs loaded in fibrin glue scaffold (Group 2 – 17 knees)

Both groups showed significant clinical improvement (IKDC and Tegner score). Nine lesions in Group I (23%) and 12 (58%) in Group 2 reached a grade of I or II using the ICRS classification. In Group I, being overweight and having a larger size of the lesion were significant negative predictive factors.

The same investigators compared the results of two groups of patients undergoing different treatment with ADSCs harvested from the patient's buttock:

- ADSCs injection in association with PRP (injection group; $n = 20$)
- ADSCs implantation on a fibrin glue scaffold (implantation group; $n = 20$)[51]

Second-look arthroscopy, performed more than 1 year after the index treatment, showed significant improvement in IKDC and Tegner activity. Moreover, at final follow-up (28.6 months post-operatively), IKDC and Tegner activity scores

Table 1 Controlled clinical studies investigating the use of adipose-derived stem cells in orthopaedic conditions

Article	Type of study/ level of evidence	Pathology	Cell type and source	Injection/implantation	Study design	Number of patients/ mean age	Follow-up	Results	Complications
Elbow									
Lee *et al.* 2015, Stem Cell[61]	Case series, Level IV	Lateral epicondylosis	Allo-ADSCs Human subcutaneous fat tissue from healthy donors	One injection under US guidance	Allo-ADSCs (10^6 or 10^7 cells in 1 ml)	12 patients/51.8 years	52 weeks	VAS scores progressively decreased and elbow performance scores improved. Tendon defects significantly decreased	Six mild swelling Two joint effusion One delayed elbow pain
Usuelli *et al.* 2017, KSSTA[66]	Randomized controlled clinical trial, Level 1	Non-insertional Achilles tendinopathy	ADSCs manually lipoaspirated from the patient's abdominal subcutaneous tissue. Two very thin patients required to have adipose tissue harvested from the internal side of the thigh	ADSCs US-guided injection into the lesion location, intratendon and in the peritendon area	PRP or ADSCs single US-guided injection	44 patients (18–55 years) – 56 tendons: 23 patients (28 tendons) PRP injection 21 patients (28 tendons) ADSCs injection	6 months	Both treatments allowed for a significant improvement VAS, AOFAS and VISA-A scored significantly better at 15 and 30 days in the ADSCs in comparison to PRP group. At the following time points the scores were not significantly different between the two groups	Neither serious side effects nor adverse events were observed during the follow-up period. Five patients of the ADSCs groups also complained for haematoma and cutaneous discomfort at the adipose tissue harvest site for about a week after the procedure
Chondral and osteochondral lesion									
Kim *et al.* 2015, Am J Sports Med[49]	Cohort study, Level III	Isolated full-thickness articular	ADSCs harvested from the patient's buttock	ADSCs implantation without a scaffold vs ADSCs	Arthroscopic debridement and ADSCs with	54 patients (56 knees)/57.5 years:	28.6 months	IKDC score and Tegner activity scale in each	None

Continued

Table 1 *Continued*

Article	Type of study/ level of evidence	Pathology	Cell type and source	Injection/implantation	Study design	Number of patients/ mean age	Follow-up	Results	Complications
		cartilage lesion in OA knees (Kellgren–Lawrence Grades 1–2)	through tumescent liposuction	implantation with a scaffold (fibrin glue)	scaffold vs without scaffold (mean of 3.9×10^6 stem cells)	37 patients (39 knees) were with ADSCs implantation without a scaffold (Group 1) 17 patients (17 knees) implantation of ADSCs loaded in fibrin glue as a scaffold (Group 2)		group significantly improved. Nine of the 39 lesions (23%) in Group 1 and 12 of the 17 lesions (58%) in Group 2 achieved a grade of I or II	
Kim *et al.* 2015, Am J Sports Med[51]	Cohort study, Level III	Isolated, full-thickness articular cartilage lesion (Kellgren–Lawrence Grade 1–2)	ADSCs harvested from the patient's buttock through tumescent liposuction	ADSCs injection with PRP vs ADSCs implantation with a fibrin glue scaffold	Arthroscopic debridement and injection (4.07×10^7 stem cells) vs implantation (3.96×10^6 stem cells)	40 patients/59.25 years: 20 injection 20 implantation	12.6 months second-look arthroscopy 28.5 months for injection group 28.8 months for implantation group	IKDC and Tegner activity scores significantly improved in both groups at the time of second-look arthroscopic surgery At final follow-up, the mean IKDC and Tegner activity scores in the implantation group had improved further	None
Kim *et al.* 2015, Am J Sports Med[47]	Case series, Level IV	Cartilage lesion	ADSCs harvested from the patient's buttock through tumescent liposuction	ADSCs implantation in a fibrin glue scaffold	Arthroscopic debridement + implantation (4.3×10^6 stem cells)	49 patients (55 knees)/58.1 years	26.7 months	The mean pre- and post-operative IKDC and Tegner activity scores significantly	None

								improved. Twenty-four patients reported the surgery as excellent (43.6%), 17 as good (30.9%), 11 as fair (20.0%), and 3 as poor (5.5%)	
Kim *et al.* 2016, Osteoarthritis and Cartilage[48]	Case series, Level IV	Isolated articular cartilage lesion in OA knees (Kellgren–Lawrence Grades 1 and 2)	ADSCs harvested from the patient's buttock through tumescent liposuction	ADSCs implantation in a fibrin glue scaffold	Arthroscopic debridement + implantation (4.4×10^6 stem cells)	20 patients (24 knees)/57.9 years	27.9 months	The clinical outcomes significantly improved both for IKDC and Tegner activity scale. The cartilage lesion grades at follow-up MRI were significantly better than the preoperative values	None
Koh *et al.* 2016, Arthroscopy[59]	Prospective comparative study, Level II.	Single ICRS Grade III/IV symptomatic cartilage defect (>3 cm^2) on the femoral condyle	ADSCs harvested from subcutaneous adipose tissue	ADSCs implantation with fibrin scaffold	ADSCs with fibrin glue implantation and MFX vs MFX alone (4.97×10^6 stem cells)	80 patients/37.5 years: 40 patients MFX and ADSCs (Group 1) 40 patients MFX treatment alone (Group 2)	MRI 24.3 months Clinical 27.4 months	Group 1 had complete cartilage coverage. Significantly better signal intensity was observed in Group 1, compared with Group 2. KOOS pain and symptom subscores were significantly	None

Continued

Table 1 *Continued*

Article	Type of study/ level of evidence	Pathology	Cell type and source	Injection/implantation	Study design	Number of patients/ mean age	Follow-up	Results	Complications
								greater in Group 1 than in Group 2	
Kim *et al.* 2013, Am J Sports Med[54]	Cohort study, Level III	OLTs	ADSCs derived from buttock fat pad	ADSCs injection along with arthroscopic marrow stimulation	Arthroscopic marrow stimulation alone vs ADSCs (3.9×10^6 stem cells) + marrow stimulation	65 patients/56.8 years: 35 marrow stimulation alone (Group A) 30 ADSCs + marrow stimulation (Group B)	21.8 months	VAS and AOFAS improved significantly in both groups. VAS and AOFAS significantly greater in Group B.The Roles and Maudsley score showed significantly greater improvement in Group B than in Group A. The Tegner activity scale score was significantly improved in Group B (but not in Group A)	None
Kim *et al.* 2014, Am J Sports Med[52]	Cohort study, Level III	OLTs	ADSCs harvested from the patient's buttock	ADSCs injection with arthroscopic marrow stimulation	Arthroscopic marrow stimulation vs ADSCs (3.94×10^6 stem cells) + marrow stimulation	50 patients/46.1 years: 26 arthroscopic marrow stimulation alone (conventional group) 24 arthroscopic marrow stimulation with	21.9 months	All clinical outcomes, including the VAS, AOFAS, and Tegner scores, improved significantly in the ADSCs group compared with the conventional group.	None

					an injection of ADSCs (ADSCs group)			Significant difference in the mean MOCART score between the conventional and ADSCs groups	
Deformity									
Koh *et al.* 2014 Arthroscopy[60]	Prospective comparative study, Level II.	Varus knee deformity	ADSCs harvested from both buttocks	One injection	Arthroscopy and injection of ADSCs + PRP. Subsequently HTO was performed	44 patients /53.2 years: 23 PRP (2 units of 3 ml) alone 21 PRP (2 units of 3 ml) + ADSCs (4.11×10^6 stem cells)	24 months	ADSCs-PRP group showed significantly greater improvements in the KOOS subscales for pain and symptoms. The ADSCs-PRP group showed a significantly greater improvement in the VAS. Partial or even fibrocartilage coverage was achieved in 50% of the ADSCs-PRP group and in only 10% of the patients in the PRP-only group	None
Kim *et al.* 2016, Arthroscopy[50]	Retrospective comparative study, Level III	Varus ankle osteoarthritis	ADSCs harvested from the patient's buttock	ADSCs injection along with arthroscopic marrow stimulation	Arthroscopic marrow stimulation vs ADSCs (4.1×10^6 stem cells) + marrow stimulation	49 patients/53.9 years: 23 ankles underwent marrow stimulation alone (Group 1),	27.6 months 12.5 second-look arthroscopies	The mean VAS and AOFAS score improved significantly for both groups. The VAS and	None

Continued

Table 1 *Continued*

Article	Type of study/ level of evidence	Pathology	Cell type and source	Injection/implantation	Study design	Number of patients/ mean age	Follow-up	Results	Complications
						and 26 underwent marrow stimulation with ADSCs injection (Group 2).		AOFAS scores were significantly better in Group 2. Significant differences in ICRS grades between the groups	
Kim *et al.* 2016, J Exp Orthop[53]	Retrospective comparative study, Level III	Varus ankle OA	ADSCs harvested from the patient's buttock	ADSCs injection	Arthroscopic marrow stimulation and SMO alone vs arthroscopic marrow stimulation and SMO + ADSCs (4.0×10^6 stem cells)	62 patients (64 ankles)/51.8 years: 31 patients /33 ankles marrow stimulation alone (Group I) 31 patients/31 ankles marrow stimulation with ADSCs injection (Group II)	12.8 months	The mean VAS and AOFAS score 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	None
Osteoarthritis									
Fodor *et al.* 2016, Aesthet Surg J[45]	Case series, Level IV	OA knee	ADSCs obtained through enzymatic disaggregation of lipoaspirate from the	One intra-articular injection	ADSCs injection (14.1 millions)	Six patients (8 knees)/59 years	12 months	Significant improvement in WOMAC and VAS scores at 3 months and, maintained at 1	None

			abdomend, flanks or lateral tights					year. ROM and TUG both improved from preoperative to 3 months. MRI showed no detectable structural differences	
Pers *et al.* 2016, Stem Cells Transl Med[64]	Cohort study, Level III	OA knee	Autologous ADSCs	One intra-articular injection	Low dose (2×10^6 cells) vs medium dose (10×10^6 cells) vs high dose (50×10^6 cells)	18: 6 low dose 6 medium dose 6 high dose 64.6 years	6 months	Patients treated with low-dose ADSCs experienced significant improvements in pain levels and function compared with baseline	Four transient knee joint pain and swelling after local injection
Jo *et al.* 2014, Stem Cells[46]	Case series, Level IV	OA knee of Grade 2 or more according to Kellgren–Lawrence	ADSCs from abdominal subcutaneous fat	Arthroscopic examination + injection	Phase I: Low dose (1.0×10^7 cells) Mid dose (5×10^7 cells) High dose (1.0×10^8 cells) Phase II: nine patients, High dose	18 patients/62 years: 9 in Phase I 9 in Phase II	6 months	The WOMAC score improved in the high-dose group. The size of cartilage defect decreased while the volume of cartilage increased in the high-dose group. Histology demonstrated thick, hyaline-like cartilage regeneration	Nine minor complications non-treatment related one urinary stone two bilateral knee pain
Koh *et al.* 2012, The Knee[57]	Therapeutic case–control study; Level III.	Knee OA	ADSCs derived from the infrapatellar fat pad	Injection of ADSCs + PRP with arthroscopic debridement	Firtst time: Arthroscopic treatment Injection of the stem cells the same day of arthroscopy	25 patients/54.2 years	16.4 months	The mean Lysholm, Tegner activity scale, and VAS scores of patients in the study group improved	One marked pain with swelling after the injection

Continued

Table 1 *Continued*

Article	Type of study/ level of evidence	Pathology	Cell type and source	Injection/implantation	Study design	Number of patients/ mean age	Follow-up	Results	Complications
					after stem cells preparation (3–4 h) 1.89×10^6 stem cells + 3.0 ml of PRP PRP was administered every 7 days as the second and third rounds of treatment.			significantly by the last follow-up visit	
Koh *et al.* 2013, Arthroscopy[58]	Therapeutic case series, Level IV	Kellgren–Lawrence Grade 3 OA in multiple compartments or Grade 4 OA in only one compartment,	ADSCs harvested from infrapatellar pad	One injection of ADSCs + PRP	Arthroscopic treatment + fat harvesting Injection of the stem cells the same day of arthroscopy after stem cells preparation (3–4 h) injection (1.8×10^6 stem cells + 3.0 ml of PRP)	18 patients/54.6 years	24.3 months	WOMAC and VAS decreased significantly while Lysholm scores also improved significantly. MRI score had significantly improved	One marked pain with swelling after the injection
Koh *et al.* 2014, Am J Sports Med[55]	Case series, Level IV	Knee OA	ADSCs harvested from patient's buttocks	ADSCs implantation	Arthroscopic ADSCs implantation (3.8×10^6 stem cells)	37 patients/52.6 years	26.5 months	IKDC and Tegner activity scale scores were significantly improved 94% patients reported good to excellent satisfaction	None
Koh *et al.* 2015, KSSTA[56]	Therapeutic case series study, Level IV	Knee OA	ADSCs harvested from subcutaneous tisse	One injection	ADSCs (4×10^6 stem cells) + PRP (3.0 ml) combined with arthroscopic lavage	30 patients/70.3 years	24 months	Almost all patients showed significant improvement in all clinical outcomes. All	Three slight knee pain

								clinical results significantly improved at 2-year follow-up compared to 12-month follow-up. On second-look arthroscopy, improved or maintained cartilage status at least 2 years was noted postoperatively	
Nguyen *et al.* 2016, Stem Cell Transl Med[65]	Prospective comparative study, Level II.	Knee OA (Kellgren–Lawrence Grade II–III)	Autologous ADSCs harvested from the abdomen	Arthroscopic microfracture and ADSCs Injection	Isolated arthroscopic microfracture vs arthroscopic microfracture + ADSCs (10^7 ADSCs cells/ml) suspended in PRP	30 patients: 15 patients placebo group (58,2 years) 15 patients treatment group (58.6 years)	18 months	All treatment group patients had significantly reduced pain and WOMAC scores, and increased Lysholm and VAS scores compared with the placebo group. Outerbridge classification, measured with MRI, showed non-differences between the two group, but a different trend was observed: infact Outerbridge score scores	None

Continued

Table 1 *Continued*

Article	Type of study/ level of evidence	Pathology	Cell type and source	Injection/implantation	Study design	Number of patients/ mean age	Follow-up	Results	Complications
								increased in the placebo group over time but decreased in the treatment group	
Michalek *et al.* 2015 Cell Transplant[62]	Case series, Level IV	Grade 2–4 degenerative OA	ADSCs obtained with liposuction	One intra or peri-articular injection	ADSCs (25×10^6 ADSCs cells)	1114 patients/62 years	17.2 months	Most patients gradually improved 3–12 months after the treatment. At least 75% Score improvement was noticed in 63% of patients and at least 50% Score improvement was documented in 91% of patients 12 months after ADSCs therapy	47 local pain < 24 h 38 local pain > 24 h 58 local swelling < 72 h 12 local swelling > 72 h 9 fever <38°C < 24 h 4 fever >38°C > 24 h 5 reactive synovitis 3 headache 2 deep venous thrombosis 1 infectious synovitis
Bone defects									
Dufrane *et al.* 2015 Medicine (Baltimore)[44]	Case series, Level IV	Three bone tumours (two osteosarcomas, one Ewing sarcoma) three nonunions due to congenital pseudoarthrosis	Subcutaneous biopsy	3D Graft from cortical bones of selected human donors	3D graft implantation (16 million of ADSCs)	Six patients/9.6 years	11–47 months	The final osteogenic product was stable, did notrupture with forceps manipulation, did not induce donor-site morbidity, and was easily implanted directly into the bone defect	One allograft removal because of intercalary allograft infection more than 10 months post-transplantation one material removal due to sepsis following screw and plate

										infection by *Staphylococcus aureus* at 10 months post-transplantation one surgical revision was performed at 9 months, due to incomplete or inefficient bone consolidation
Safety										
Pak *et al.* 2013, BMC Musculoskelet Disord[63]	Case series, Level IV	Joint disease	ADSCs Abdomen	One intra-artiuclar injection	ADSCs + PRP (2 ml buffy coat PRP + 10 ml of ADSCs)	91 patients (100 joints)/ 51.2 years	26.62 months	VAS improvement. MRI failed to demonstrate any tumour formation at the implant sites	37 swelling/effusion	

ADSCs = adipose-derived stem cells; US = ultrasound; VAS = visual analogue score; OA = osteoarthritis; WOMAC = Western Ontario & McMaster Universities Arthritis Index; ROM = range of motion; TUG = time up and go; MRI = magnetic resonance imaging; PRP = platelet rich plasma; KOOS = knee injury and osteoarthritis outcome score; ICRS = International Cartilage Repair Society; HTO = high tibial osteotomy; SMO = supramalleolar osteotomy; AOFAS = American Orthopaedic Foot and Ankle Society Score; MOCART = magnetic resonance observation of cartilage repair tissue; IKDC = International Knee Documentation Committee; MFX = microfracture.

showed a further improvement in the implantation group, with a higher IKDC in the implantation group. The ICRS grades were significantly higher in the implantation group.

Kim *et al.* investigated patients with symptomatic knee OA who underwent implantation of ADSCs harvested from the buttocks.[47] 49 patients (55 knees) were evaluated retrospectively, with significant clinical improvements (IKDC and Tegner activity scores). Almost half of the patients (43.6%) was fully satisfied with the treatment, 50.9% of the patients reported their satisfaction as good or fair, while 5.5% remained unsatisfied. No significant differences were found between clinical outcomes and demographic datas.

Kim *et al.* reported the clinical and imaging outcomes of ADSCs implantation, harvested from the buttocks, in 20 patients (24 knees) with knee OA.[48] Two years after arthroscopic ADSCs implantation, all clinical outcomes improved significantly; at MRI, the cartilage lesions appeared significantly improved compared to the preoperative status.

Koh *et al.* compared the clinical and imaging efficacy of ADSCs, collected from subcutaneous adipose tissue from the patient's buttock in two group of patients with symptomatic chondral lesion of the knee (ICRS Grade III or IV):

- ADSCs with fibrin glue and microfracture (MFX) treatment (Group 1, $n = 40$)
- MFX treatment alone (Group 2, $n = 40$)[59]

At final follw up, 26 patients (65%) in Group 1 showed a complete cartilage coverage of the lesion, while in Group 2 this happened in 18 patients (45%). The MOCART score was significantly higher in Group 1 compared to Group 2 patients. Clinical follow-up at 27.4 months showed higher KOOS in Group 1 than in Group 2. Second-look arthroscopy, performed in 57 knees (30 in Group 1 and 27 in Group 2), highlighted good repair tissue quality with no differences between the two groups.

Kim *et al.* treated arthroscopically 65 elderly patients (>50 years) for ostechondral lesion of the talus (OLT), dividing them into two groups:

- isolated marrow stimulation (Group A – 37 ankles) and
- marrow stimulation in association with ADSC harvested from the buttock (Group B – 31 ankles)[54]

At final follow-up, patients in Group B showed greater clinical improvements compared to those in Group A, especially in lesions greater than 109 mm^2.

The same group compared imaging results in 49 patients (50 ankles) who underwent arthroscopic treatment for symptomatic OLTs.[52] Patients were divided in two groups:

- marrow stimulation alone (conventional group – 26 ankles),
- marrow stimulation in association with ADSCs injection, harvested 1 day before arthroscopic surgery from the patient's buttock (ADSCs group – 24 ankles).

At final follow-up, the ADSCs group showed higher clinical (American Orthopeadic Foot and Ankle Score (AOFAS), and Tegner scores) and imaging (MOCART) scores, with a significant association between MOCART and clinical scores in both groups. Age, lesion size and the presence of subchondral cysts were correlated with a lower MOCART score in the conventional group but not in the ADSCs group.

Tendinopathy

In 2015, Lee *et al.* reported the clinical outcomes and complications in 12 patients treated with allogenic ADSCs, isolated from lipoaspirates of human subcutaneous adipose tissue obtained from healthy donors in treating lateral epicondylopathy.[61] Patients were randomized into two groups (six patients per group), and were administered, respectively, 10^6 or 10^7 cells in 1 ml. No complications were reported throughout the entire follow-up of 52 weeks; clinically, the visual analogue score for pain (VAS) reduced significantly from 66.85 mm to 14.8 mm, while elbow performance scores improved reaching a value of 90.6 at final follow-up.

Usuelli *et al.* compared compared the clinical efficacy of PRP and ADSCs injection for the treatment of non-insertional Achilles tendinopathy in 44 patients (56 tendons):

– 23 patients (28 tendons) treated with single US-guided PRP injection;
– 21 patients (28 tendons) trated with single US-guided ADSCs injection lipoaspirated from the patient's abdominal subcutaneous tissue. Patients were re-evaluated at 15, 30, 60, 120 and 180 days from the index treatment, using the VAS pain scale, the VISA-A, the AOFAS Ankle-Hindfoot Score, and the SF-36 form. Imaging assessment included US and MRI. The ADSCs group showed higher VAS, AOFAS and VISA-A respectively at 15 and 30 days from the treatment, with no differences between the two groups at the final follow-up. Neither serious side effects nor adverse events were observed during the follow-up period. Five patients in the ADSCs groups also reported haematoma and cutaneous discomfort at the adipose tissue harvest site for ~1 week after the procedure.[66]

Deformity

Koh *et al.* treated 44 patients who underwent an open wedge high tibial osteotomy (HTO) for varus deformity, with or without ADSCs harvested from both buttocks:

– 23 patients received only PRP injection, and
– 21 underwent ADSCs therapy plus PRP injection.[60]

All patients underwent a second-look arthroscopy at the moment of removal of the fixation devices, revealing fibrocartilage coverage of the lesion in 50% of the ADSCs-PRP group patients, and only 10% in the other group. The ADSCs-PRP group reported significant greater improvements in all clinical scores compared to the PRP-only group, except for the mean Lysholm score. Imaging parameters (femorotibial angles and weight-bearing lines) did not show any pre- or post-operative differences between the two groups.

Kim *et al.* assessed 49 patients with ankle osteoarthritis and varus deformity treated with arthroscopic marrow stimulation and lateral sliding calcaneal osteotomy: 23 ankles underwent marrow stimulation alone (Group 1), and 26 underwent marrow stimulation in association with ADSCs injection derived from the fat pad harvested from their buttock (Group 2).[50] Second-look arthroscopy more than 1 year after surgery evidenced a higher ICRS score in Group 2 patients. At the latest follow-up, clinical outcomes (VAS, AOFAS) had improved significantly in all patients, and were significantly higher in Group 2 patients.

Recently, Kim *et al.* reported clinical and imaging results in two different groups of patients who had undergone supramalleolar osteotomy and second-look arthroscopy:

– bone marrow stimulation alone (Group I – 33 patients).
– bone marrow stimulation plus ADSCs injection (harvested from both buttocks) (Group II – 31 patients).[53]

Both groups reported significant improvement in VAS and AOFAS scores; moreover, Group II showed a significant higher AOFAS and lower VAS at the final follow-up. Second-look arthroscopy revealed a better ICRS grade in Group II.

Osteoarthritis

Fodor *et al.* treated six patients with symptomatic osteoarthritis (OA) of the knee (Grade I–III of the Kellgren-Lawrance scale) using a direct injection of autologous ADSCs harvested from the abdomen, flanks, and/or lateral thigh.[45] No adverse events such as infections or pain were recorded. At 3 months, all patients reported clinical improvements maintained up to 1 year. All patients resumed normal activities of daily living with decreased knee pain.

Recently, Pers *et al.* assessed the effect of a an intra-articular injection of ADSCs, obtained by liposuction under local anaesthesia in 18 patients with knee OA.[64] Three patient cohorts, each consisting of six patients, were treated with infiltration at increasing cell amounts:

– low dose (2×10^6 cells),
– medium dose (10×10^6 cells), and
– high dose (50×10^6 cells).

Six months after the injection, no adverse events were reported. Only four patients reported transient

knee discomfort immediately after the injection. Of the three cohorts, the low-dose group reported significant improvements in pain relief.

Jo *et al.* evaluated clinical outcomes of intra-articular injection of autologous ADSCs, harvested from the abdominal subcutaneous fat by liposuction, for knee OA in 18 patients.[46]

The study protocol consisted of two phases:

- Phase I: three cohorts of three patients each with increasing dose (low, mid and high).
- Phase II: one cohort of nine patients all treated with high dose.

No complications were reported. In the high-dose group, the WOMAC score showed significant improvement 6 months after treatment; moreover, arthroscopy revealed a reduction of the size of the lesion with an increase of the cartilage, confirmed at histology to have the appearance of regenerated hyaline cartilage.

Koh *et al.* assessed the efficacy of ADSCs injection harvested from the infrapatellar fat pad for the treatment of 25 knee OA.[57] Each patients underwent arthroscopic debridement in association with ADSCs injection. At the end of the observation period, all patients showed significant clinical improvements (Lysholm, Tegner and VAS) with no adverse effects.

The same group evaluated the outcomes of 18 patients who underwent intra-articular injections of autologous ADSCs, harvested from the inner aspect of the infrapatellar fat pad, for symptomatic knee OA.[58] ADSCs in association with 3.0 ml of PRP were injected directly into the joints. WOMAC, VAS and Lysholm scores improved significantly at the final follow-up. MRI showed an improvement in the cartilage whole-organ MRI score.

Koh *et al.* investigated the effect of ADSCs, harvested 1 day before arthroscopy, from the patients' buttocks through tumescent liposuction.[55] Moreover, the authors investigated the predictive factors in outcome of treatment with ADSCs. After ADSCs implantation for cartilage defect, 37 patients underwent second-look arthroscopy, performed at a mean follow-up of 26.5 months. According to the ICRS classification, two (5%) lesions were classified as Grade I, seven (19%) as Grade II, 20 (54%) as Grade III, and eight (22%) as Grade IV. Almost all patients (94%) were satisfied with the operation.

Koh *et al.* treated 30 elderly patients with arthroscopic debridement and ADSCs injection, harvested from both buttocks, for symptomatic knee OA.[56] Clinical parameters improved significantly in almost all patients, with five patients older than 65 showing worsening in Kellgren-Lawrance scale. At second-look arthroscopy, performed in 16 patients, the articular cartilage maintained the preoperative grade or improved (87.5%).

Nguyen *et al.* recently reported the clinical and radiological results in 30 patients with knee OA (Grade 2 or 3 Kellgren–Lawrence scale), dividing the partecipants in two groups:

- 15 patients underwent isolated arthroscopic microfracture (Placebo group) and
- 15 patients recieved arthroscopic microfracture in association with ADSCs injection, harvested from the abdomen and suspended in PRP (treatment group).[65]

All patients were re-evaluated at 6, 12 and 18 months after treatment. The treatment group showed significantly differences compared with the Placebo group in WOMAC, VAS and Lysholm scores. Outerbridge classification, measured with MRI, showed non-differences between the two group, but Outerbridge score scores increased in the placebo group over time, but decreased in the treatment group. No adverse events reletad to the treatment were recorded in either group.

Michalek *et al.* in 2015 evaluated 1128 patients (1856 joints), at a mean follow-up of 17.2 months, with Grade 2–4 degenerative OA treated with a single injection of freshly isolated autologous ADSCs.[62] ADSCs, obtained after standard liposuction, were isolated and prepared for application into one to four major joints. No serious side effects were reported throughout the follow-up. Significant improvement was reported between 3 and 12 months after treatment. Almost all patients (91%) reported an improvement of at least 50% of the clinical score at 1 year after treatment.

Bone defects

Dufran *et al.* treated six patients with severe bone loss using a human autologous scaffold-free osteogenic 3-dimensional (3D) graft derived from autologous

ADSCs.[44] The final product obtained was stable and resistant, and easy to implant directly into the bone defect. The scaffold was used to treat three bone cancer and three patients with pseudarthrosis. No adverse effects were reported 4 years after implantation.

Safety

At a mean of almost three years, 91 patients treated with autologous ADSCs, harvested from the patient's abdomen using a tumescent solution, in association with PRP for different orthopaedic conditions were assessed.[63] ADSCs in association with PRP were injected into various joints ($n = 100$). VAS showed improvement after 1 month of follow-up (6.55), with further improvement at the final follow-up of 3 months (4.43). Minor self-limited complications such as swelling, tenosynovitis and tendinopathy were reported by most elderly patients; no complications related to tumour or cancer were detected.

Discussion

This systematic review evaluated the current literature on the clinical application of ADSCs in humans in a orthopaedic pathologies to assess the efficacy, tolerability, safety and possible future developments for the management of several orthopaedic conditions. Clinical trials which evaluate the effect of ADSCs can be considered a choice of treatment in addition to other widely studied regenerative medicine procedures such as bone marrow mesenchymal stem cells injections.

The main reasons for which the ADSCs have been the subject of numerous clinical and pre-clinical studies in recent years is attributable to their high number in the human body (ADSCs are 5% of nucleated cells in adipose tissue), the simplicity of harvesting with a lower donor-site morbidity, and their rapid expansion and high proliferative potential.

Moreover, ADSCs are able to maintain their own features even if manipulated through different cultures compared to other cellular lines.[31,68,69] ADSCs are easily obtained from adipose tissue under the skin.

Several techniques have been reported for fat harvesting, such as Coleman's technique, liposuction and direct excision of the fat, all aiming to improve the quality and quantity of ADSCs.[70]

Currently, Coleman's technique is the most popular, and was first described in 1994.[71] Liposuction techniques include conventional liposuction (tumescent) and ultrasound-assisted liposuction.[72,73] Tumescent liposuction is undertaken after injection of Klein's solution into the subcutaneous tissue, followed by suction-assisted aspiration of ADSCs using a microcannula.

Ultrasound-assisted liposuction was designed to injury subcutaneous adipose tissue and to ease the harvesting process.[73]

Adipose tissue is largely represented, and the area of fat harvesting influences the stem cell yield.[68] Normally, adipose tissue can be harvested from the belly or the hip/thigh region.[73–75] Patient's age can affect ADSCs yield. All age groups present similar ADSCs and osteogenic paracrine activities.[20,24,76] In contrast, ADSCs from newborns present higher angiogenic and osteogenic capabilities than those from adults. Proliferative activity, colony-differential potential, and population doubling differ between young adults (>20 years old) and more elderly subjects (50–70 years old).[77]

Only in one study were allogeneic cells used.[61] In the study of Lee *et al.* allo-ADSCs were obtained and processed from healthy donors, prior informed consent, and were then injected under ultrasound guidance. Further studies will be conducted on the benefits of allotransplant, as this procedure blends the ease in handling harvested cells with standardization of the process.

Moreover, the liposuction and processing step would be eliminated, reducing surgical time, and a pre-selection of healthy donors, according to their cytokine and cellular lines, could improve the treatment with ADSCs.[78]

In all the other studies, ADSCs were obtained from autologous subcutaneous adipose tissue from the buttocks or retropatellar fat.

Almost all studies (82.6%) regards knee or ankle disorders,[45–60,64–66] in particular to treat cartilage defect or osteoarthritis[45–60,64,65]: if ADSCs are

cultured in high density culture in association with transforming growth factor beta (TGFb), growth hormone and fibroblast growth factor-2 (FGF-2), and located in a fibrin glue scaffold placed into the lesion, they have the potential to form tissue with hyaline cartilage phenotype.[79]

Only two studies used the ADSCs in the management of tendinopathy.[61,66] ADSCs in fact are able to enhance gene expression profile of an extracellular matrix protein primarily present in cartilage (cartilage oligomeric matrix protein—COMP). COMP are crucial to bind and organize collagen fibrils.[38,80–82]

Dufrane *et al.* was the first to apply adipose cells to treat bone defects with good results.[44] *In vitro*, ADSCs are able to induce osteogenesis within 3–4 week if enriched in culture with dexamethasone, b-glycerophosphate, L-glutamine and ascorbate.[83] This process leads to the formation of normal bone components, such as calcium phosphate, osteocalcin, and collagen type I, and are also stimulated genes that promote bone formation (Run2 and Osterix). Furthermore, FGF2 is another factor that enhance osteogenesis if added to MSC.[84] Once these stem cells have differed into osteoblasts, are able to produce proteins typical of the bone osteoblastic cellular line.

ADSCs seem to produce excellent clinical results, although with different follow-up in the treatment of various diseases. In fact, all the studies report subjective and objective clinical improvements with minimal complication rates. Analysing the complications rates on 1746 patients treated with mesenchymal cells of adipose origin, none suffered serious complications, and none developed a tumour. The total number of complications identified in the present review is 254/1746 (14.55%), most of which were minor, such as joint pain or other complications not directly related to the treatment.

There are several ways to deliver ADSCs to diseased or injured tissues. The systemic delivery of ADSCs is dependent on the 'native' homing of ADSCs to the injured site. On their surface ADSCs express different receptors for cytokine and chemokine which helps them to be recruited to the suffering areas thanks to a chemotactic gradient secreted from the same injured tissue. Among the included studies, it is also possible to observe a difference in the delivery way of ADSCs: in six studies ADSCs (26.0%) were used by direct intra- or peri-articular or tendinous injection to the injuried site; in 15 studies (65.3%) ADSCs were applied during surgery, and in the remaining two (8.7%) injection of the ADSCs was performed the same day of the arthroscopy after stem cell preparations (3–4 h).

Intra-articular injection of ADSCs can lead to a significant improvement of the cartilage and subchondral bone, protecting against arthritic processes, regardless of the source of harvesting.[85]

On the other hand, scaffolds may affect the proliferation and differentiation of stem cells by controlling chemical compositions and physical properties. The ideal scaffold should stimulate cell attachment, growth and differentiation, as well as the formation and organization of new tissue. Scaffolds can be permanent, in this case with the purpose of ensuring a reliable support for cells andnew-tissue, or temporary as a means of cell delivery system.[80,81,86]

Furthermore, in some studies ADSCs have been used in combination with PRP[56,60,63,65] co-administration of ADSCs and other chemical components can enhance the effect, allowing to use a smaller amount of drugs or devices and possibly decreasing adverse effects.[87–91] Tumours release of a number of chemical signals such as cytokines, which recruit ADSCs, enabling ADSCs as transporter for cancer drugs; moreover, ADSCs could have a carcinogenic effect promoting angiogenesis and improving the tumour environment.[92–93]

None of the studies identified is a randomized double-blinded trial, and most of the selected studies present major limitations, and different methods, counfounding the results of our review.

First of all, in many studies ADSCs were in association with PRP. In this case, it is not possibile to establish the effects of ADSCs alone from those of PRP. Furthermore, the association with surgical procedures, such as debridement, can lead to clinically relevant improvement as regards pain in the short term. Finally, the lack of a control to confirm the efficacy of ADSCs in orthopeadic conditions.

Conclusions

Although the literature is scarce regarding the use of ADSCs in humans for orthopaedic pathologies, preliminary outcomes are very encouraging, with a low rate of complications. Different delivery systems for these stem cells are being tested. ADSCs can be administered either with a simple injection or during surgical procedure. Clinical research regarding the use of ADSCs is very limited and, at present, long-term safety is the biggest challenge of ADSCs based regenerative medicine. It is necessary to conduct more and better studies to ascertain whether ADSCs really play a role in modern orthopaedic surgery.

Conflict of interest statement

The authors have no potential conflicts of interest.

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