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Knee intra-articular administration of stromal vascular fraction obtained from adipose tissue: A systematic review

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ABSTRACT

Osteoarthritis is a debilitating chronic degenerative disease of cartilage joint surfaces and the knee is the weight-bearing joint most frequently plagued. Intra-articular cell therapies have recently emerged as a method to manage knee osteoarthritis. A literature search identifying all articles involving use of SVF to treat knee osteoarthritis was performed, consulting several databases. In conclusion, 24 clinical trials analysed report good to excellent clinical and radiographic results for the treatment of knee OA with the use of intraarticular administration of SVF.

1. Introduction

Osteoarthritis (OA) is a debilitating chronic degenerative disease of cartilage joint surfaces affecting approximately 9.6% of men and 18% of women aged ≥60 years.[1]

The knee is the weight-bearing joint most frequently plagued by OA. Conservative management of OA consists of physical exercises, body weight reduction, drugs, hyaluronic acid injections with or without corticosteroid, and platelet rich plasma (PRP) injection. The ideal treatment for OA should restore the biomechanical and biochemical properties of damaged cartilage focusing on cartilage repair and restoration.[2]

Intra-articular cell therapies have recently emerged as a method to manage knee osteoarthritis (KOA). Mesenchymal stem cells (MSCs) can be considered a potential biological approach to articular cartilage restoration given their properties of multi-lineage differentiation potential, self-renewal, and immunomodulatory capacity.[3] MSCs may be able to produce new cartilage, releasing factors that stimulate cartilage formation by resident chondrocytes or other cells in the joint, and inhibit joint inflammation.[4] MSC release growth factors and anti-inflammatory cytokines which inhibit ischemia-caused apoptosis, stimulate endogenous cell proliferation and repair, and stimulate angiogenesis and vessel stability, improving blood flow in the affected joint by contributing to endogenous tissue repair.[2] Most of the effects of these cells are consequent to their paracrine effect rather than their potential differentiation into chondrocytes.[5]

MSCshave been identified as an ideal cell source for OA therapy because they are easily expanded in culture and can be readily collected from different tissue sources. Comparing the adipose tissue samples extracted from the thigh and abdomen, the former provides a higher number of adipose-derived stromal cells (ADSCs).[6] Infrapatellar fat pad (IPFP) is another source of MSCs.[7] Autologous cells induce no rejection, carry no risk of disease transmission, and are less tumorigenic than embryonic stem cells.[8] Bone marrow and adipose tissue are the main sources of MSCs: they can be harvested in a minimally invasive fashion and can be minimally manipulated intra-operatively with sterile devices.[9] In particular, adipose/fat tissue is rich in vascular niches and contains a greater concentration of MSCs than bone marrow. Multipotent

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ADSCs are obtained by mechanical or enzymatic treatment which involve many processing steps, high economic burden, and restrictions associated with cell expansion and extensive manipulation.[10] To reduce processing times for immediate clinical use, numerous devices for the non-enzymatic mechanical processing of harvested adipose tissue were developed. These protocols are characterized by three common steps: harvesting, processing, and reinjection.[11] Unlike ADSC, stromal vascular fraction (SVF) can be readily obtained from lipoaspirate without the need for cell separation or culturing, which makes SVF more cost efficient and convenient.[12]

Although MSCs have shown efficacy in clinical studies, evidence on their efficacy in KOA remains unclear, given cell heterogeneity and concomitant procedures. The purpose of this systematic review was to evaluate SVF therapy in knee OA.

2. Materials and methods

We performed a systematic literature review according to Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines, considering articles published up to October 2021. A literature search identifying all articles involving use of SVF to treat knee osteoarthritis was performed, consulting the PubMed, Medline, CINAHL, Embase and the Cochrane Central Register of Controlled Trials databases. Key words included the terms "adipose derived stem cells "/"adipose derived stromal cells"/ "ADSC" OR/AND "stromal vascular fraction"/"SVF"/"tSVF" OR/AND "microfragmented Adipose tissue"/"MFAT" AND "knee" AND "osteoarthritis"/"OA" were differently combined. After removing duplicates and identifying the relevant studies through abstract information, the full text was examined, applying preestabilished inclusion and exclusion criteria.

The inclusion criteria were peer-reviewed studies, written in English; comparative studies, controlled clinical trials, observational studies and randomized clinical trials based on the intra-articular injection of minimally manipulated SVF and on the evaluation of the therapeutic response aimed at regenerating the articular cartilage in humans. Systematic review, case reports, conference presentations, narrative reviews, letters to the editors, editorials, and expert opinion were excluded. The exclusion criteria subsequently applied were: articles which did not use MSCs to directly treat OA patients, which did not use subcutaneous as source of adipose derived stem cells, which studied stem cells in vitro and which presented their research in a language other than English. Furthermore, if no statistical analyses were reported in the studies, the articles were excluded. All data were collected in a datasheet and included study authors, publication year, mean follow-up, mean age of patients, imaging data, concomitant procedures, failures and reported functional outcomes. Clinical outcomes were highlighted and some considerations were made on the technical instruments and procedures applied. A PRISMA flowchart of the selection method is reported in Fig. 1.

3. Results

After the duplicates were removed, 366 studies were reviewed. 61 human trials (16%) were selected applyizing the inclusion criteria. 24 studies (6%) remained after application of the exclusion criteria(6,10,13−30,30−33). Table 1 summarizes the mean age of patients, no. of cases, follow up in months, OA grade before injection, concomitant procedures, method of injection, principal outcomes and conclusions obtained.

3.1. Study characteristics

The sample size ranged from 6 to 182 patients, with a total of 914 patients. The mean age of patients was 61.01 years (range: 43−72), there were more females than males (55% vs 45%). Follow up ranged from 6 to 24 months, with a mean of 14 months.

In 2 studies[16,27] SVF was injected in patients with low grade (0−2) KOA, while in 4 studies[6,14,22,31] SVF was used in patients with mid grades of KOA[2,3] and in 3 studies SVF was injected in patients with low and mid grades.[1−3] High grades KOA patients were evaluated after SVF injections in 8 studies.[10,15,20,23,24,29,30,34] When Kellgren Lawrence classification was not stratified for patient, we consider that SVF was injected without grading OA stages before.[25,28,32,33] In two studies KL was not used to identify the severity of KOA, Castellarin et Al.[13] preferred Outterbridge Classification while Bansal et Al[18] preferred Brandt Radiographic Grading Scale of Osteoarthritis.

SVF injection may be the only procedure used to treat patients with KOA, but some authors used concomitant procedures such as PRP injections,[18,21] arthroscopic debridement alone or with microfractures,[6,16,17,22,27,29,33] or arthroscopy with ACL/LCL reconstruction or meniscectomy or tibial osteotomy[17]

3.2. Method of SVF production

SVF can be obtained through different strategies (Table 2).

Lipogems® was the most commonly used device[10,15−17,23−25,27,28]: it is a mechanical device that allows the fragmentation of the lipoaspirate by means of steel balls. The product obtained is filtered and injected directly in the joint, without centrifugation.

The Lipocell device was used in three studies[13,31,32]: 60−90 mL of lipoaspirated fat are collected from the abdominal subcutaneous fat and transferred into a device where it is dialized with a filter and washed with 500 ml of Ringer lactate or NaCl 0.9%.

Three studies used a centrifugation system,[21,30,33] Pintat et Al.[21] used a Proteal lipo Pras 20 kit to centrifugate the lipoaspirate for 3 min at 1200 g, Yokota et al.[30] reported the use of the Cellution® centrifuge which integrates mechanical manipulation with enzymatic processing, replacing collagenase with two process reagents and centrifugation performed at 400 g for 10 min. Roato et al.,[33] instead, used a simple centrifugation at 3000 rpm for 3 min to separate the oily supernatant from the SVF.

Fodor et al.[19] and Garza et al.[14]used GID SVF1® and GID SVF-2® respectively. They are devices that differ only in the maximum volume of adipose tissue that can be processed in a single run (for SVF-1, up to 300 mls; for SVF-2, up to 125 mls). The devices allow selective capture of tissue fragments in an inner mesh filter compartment, while waste fluid readily passes through and is immediately aspirated into a waste container. The GID SVF device is then insulated with heating packs for transportation to the laboratory, where the adipose tissue is processed and disaggregated using Type I collagenase CLS-1. The GID SVF1 device allows centrifugation at 800*g* for 10 min using a standard laboratory centrifuge. The GID SVF-2 allows centrifugation at 600 × 3g for 6 min, the adipose tissue is stirred with the internal impeller, and then centrifugated for another 4 min for a total of 10 min of centrifugation time. Other studies used centrifugation combined to enzyme digestion.[18,20,22,29] In particular, Bansal et al.[18] used centrifugation and enzyme digestion with collagenase at 37 °C for 30 min with agitation at 5-min intervals. The suspension was centrifuged again at 500×*g* for 5 min to collect the SVF as a pellet. The pellet was washed, resuspended in phosphate buffered saline (PBS), filtered through a 100 μm cell strainer and then recentrifuged at 500×*g* for 5 min to discard the supernatant.

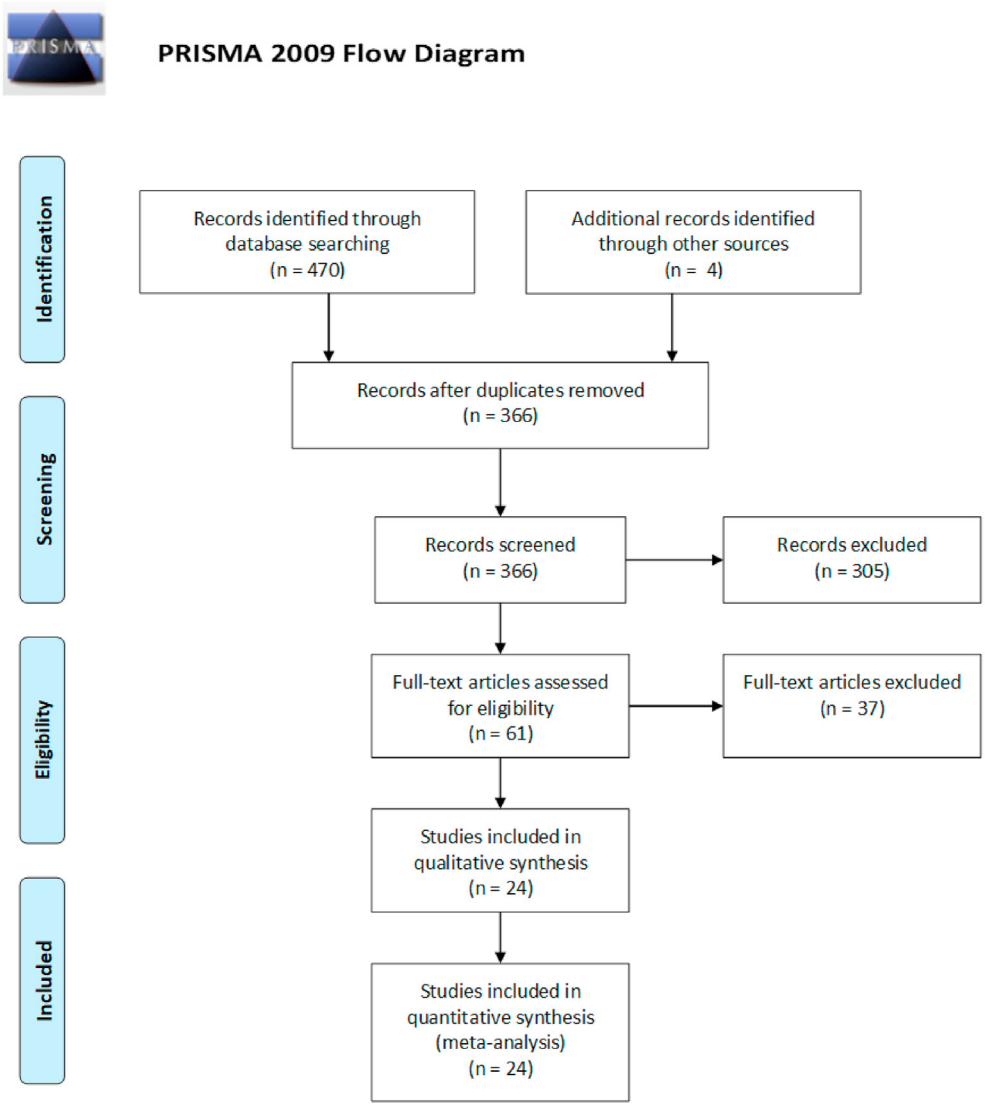

Fig. 1. PRISMA flowchart.

Lapuente et al.[20] used the ADSC System commercial kit (Lyposmol Biotech, Madrid, Spain). The protocol consists of centrifugation, enzyme digestion with collagenase I and II, and agitation. Two studies[26,34] did not specify which procedure they used to harvest and process lipoaspirate.

3.3. Clinical outcomes

Clinical evaluations were performed in a non-homogeneous way, and different analysis scales were used: Knee Injury and Osteoarthritis Outcome Score (KOOS)[16,17,23,25,28,29,34]; Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[6,13,14,18–23,26,30–33]; Visual Analog Scale (VAS)[10,13,15,17,19,20,22,23,25–27,29,31–34]; Tegner Lysholm Knee (TLK)[17,29]; International Knee Documentation Committee (IKDC)[17]; Emory Quality of Life (EQOL)[25]; Japanese Knee Osteoarthritis Measure(JKOM)[30]; International Knee Society (IKS) knee and function scores,[27] Range of motion (ROM)[6,19] and Lequesne index(20,31).

Nuclear magnetic resonance (MRI) imaging was also performed in 13 studies[6,10,13,13–15,18,19,21–23,26,28] and advanced MRI techniques included dGEMRIC(10,15), WORMS and MOCART.[6]

Hudetz et al.(10) also evaluated the presence of GAG in the synovial fluid after injection.

Elnhal et al.[26] evaluated immunohistochemistry (IHC), while Roato et al.[33] evaluated, in addition to IHC, also scanning electron microscopy (SEM).

Adriani et al.[32] considered consumption of non steroideal drugs after management of knee OA with SVF injection.

The present work included 17 prospective non-randomized trials,[10,13,15,18,19,21–31,34] 2 prospective randomized trials[6,14] and 4 retrospective[16,17,20,32]trials. Mautner et al. [33] included the largest number of knees in their study (106 knees, 76 patients). In all studies, the clinical outcomes highlight a significant decrease in the patient's symptomatic distress, with improvement in joint function and reduction of pain. Specifically, 16 out of 24 studies used the VAS pain scale to evaluate clinical outcomes.

Mautner et Al.,[25] Adriani et al. and Koh et al.[29] showed that the mean VAS decreased from 4.3 ± 0.385 at preprocedure to 2.8 ± 0.376 at postprocedure, from 7.7 ± 1.2 at baseline to 5.2 ± 0.2 at 1 month and 4.3 ± 1 at 3 months (with a slight deterioration at 1 year), from 4.7 ± 1.6 preoperatively to 1.7 ± 1.4 at 2 years,

Table 1
Summary of the mean data obtained from the studies included.

Author	Age (mean)	No. of cases	Follow up (months)	OA grade	Concomitant procedures	Method of injection	Outcomes	Conclusions
Garza[21]	59	39: randomized to high-dose SVF, low-dose SVF, or placebo	12	2-3 KL		Liposuction from abdomen, processed with **GID SVF-2 device**	WOMAC MRI	Improvement of symptoms. High-dose group had a large effect size than low-group.
Boric[22]	69	10	24	3-4 KL		Liposuction from abdomen, processed with **Lipogems® device**	VAS dGEMRIC MRI	GAG content improvement VAS decrement
Cattaneo[23]	54	35	12	1-2 KL	Arthroscopy	Liposuction from abdomen, processed with **Lipogems® device**	KOOS Physical examination	Improvement of symptoms
Russo[24]	43	30	12	1-3 KL	24: ACL/LCL reconstruction, high tibial osteotomy or meniscectomy 6: arthroscopy alone	Liposuction from abdomen, processed with **Lipogems® device**	KOOS IKDC-subjective TLK VAS	Improvement of symptoms
Bansal[25]	58.4	10	24	Brandt Radiographic Grading Scale of Osteoarthritis grade 1 and 2	PRP	Liposuction from abdomen, isolated using an **enzyme digestion** and resuspended in PRP for intra-articular injection in the knee.	WOMAC MRI	Improvement of symptoms Increased cartilage thickness >0.2 mm in six patients
Fodor[26]	59	6 (8 knees)	12	1-3 KL		Adipose-derived SVF cells were obtained through **enzymatic disaggregation** of lipoaspirate harvested from the abdomen, flanks, and or lateral thighs. **GID SVF1**	VAS WOMAC ROM MRI	Improved clinical scores No changes in MRI
Lapuente[27]	69.5	N = 50 bilateral patients (100 knees)	12	3-4 KL		Adipose-derived SVF cells were obtained through **enzymatic disaggregation** of lipoaspirate harvested from the abdomen, flanks, and or lateral thighs.	Lequesne WOMAC VAS biomarkers in synovial fluid	Significant improvement in clinical outcomes In synovial fluid: decreased MMP-2, IL-1b, IL-6 and IL-8; while IGF-1 and IL-10 increased compared to baseline
Pintat[28]	43.1	19	12		PRP	Adipose-derived SVF cells were obtained through **centrifugation** of lipoaspirate harvested from subcutaneous medial knee fat	WOMAC MRI T2 ICRS-like classification	Functional improvement no differences in MRI
Tran[29]	59	N = 33, SVF versus placebo	24	2-3 KL	Arthroscopy	Adipose-derived SVF cells were obtained through **enzymatic disaggregation** of lipoaspirate harvested from the abdomen, flanks, and or lateral thighs	VAS WOMAC MRI Outerbridge BME	Better clinical outcomes in KL3 than KL2 Decreased bone marrow edema Better efficacy of SVF with the microfracture method.
Hudetz[16] 2017	69	N = 17, 32 knees	12	2-4 KL		Liposuction from abdomen, processed with **Lipogems® device**	VAS MRI dGEMRIC CRP	Pain and function improvement GAG improvement in cartilage No changes in CRP No adverse events

Table 1 (*continued*)

Author	Age (mean)	No. of cases	Follow up (months)	OA grade	Concomitant procedures	Method of injection	Outcomes	Conclusions
Hudetz[30]	62.5	N = 20	12	3-4 KL		Liposuction from abdomen, processed with **Lipogems® device**	MRI VAS WOMAC KOOS	Pain and function improvement Three patients followed TKR
Panchal[31]	72	N = 17 (26 Knees)	12	3–4 KL		Liposuction from abdomen, processed with **Lipogems® device**	Pain and function NPRS LEAS	No serious adverse events Minimal clinical important differences in pain, function and quality of life
Mautner[32]	BMAC group: 59 MFAT group 63	76 patients (BMAC 41, MFAT 35 inject) and 106 knees (BMAC 58, MFAT 48 inject).	12	1-4 KL		BMAC was harvested from the posterior superior iliac spine and then processed on location in an Emcyte centrifuge. The MFAT:Liposuction from abdomen, processed with **Lipogems® device**	KOOS EQOL VAS	For both groups: improvement in EQOL, VAS, and all KOOS parameters without a significant difference when comparing the two autologous tissue sources
Elnhal[33]		20	6	1-3 KL		Adipose-derived SVF cells were obtained through **enzymatic disaggregation** of lipoaspirate harvested from the abdomen	WOMAC VAS MRI IHC	Improvement in WOMAC and VAS
Casellarin[4]	52	92	12 months	2-3 Outerbridge classification		Liposuction from abdomen, processed with **Lipocell device**	VAS WOMAC MRI	Pain relief and functional recovery. reduction or even disappearance of peri-lesional subchondral edema.
Schiavone Panni[34]	67.3	52	24 months	0-2 KL	Arthroscopy	Liposuction from abdomen, processed with **Lipogems® device**	IKS VAS	Improvement of clinical scores, especially those with higher pre-operative VAS scores.
Van Genechten[35]	54.2	56	12	1-4 K-L		Liposuction from abdomen, processed with **Lipogems® device**	KOOS NRS UCLA EQ5D MRI	Early clinical improvement Patients with mild bone marrow lesions had a significantly higher therapeutic response on MRI
Koh[36]	70.3	30	24	KL 2-4	Arthroscopy	Adipose-derived SVF cells were obtained through centrifugation and **enzymatic disaggregation** of lipoaspirate from the patients' buttocks	KOOS VAS TLK	Significant improvement in all clinical outcomes On second-look arthroscopy, 87.5% of elderly patients improved or maintained cartilage status at least 2 years postoperatively. None of the patients underwent total knee arthroplasty during this 2-year period.
Yokota[37] 2017	74.5	13 (26 knees)	6	K-L 3-4		Adipose-derived SVF cells were obtained through **centrifugation** of	VAS JKOM WOMAC	Overall improvement of clinical outcomes.

(*continued on next page*)

Table 1 (*continued*)

Author	Age (mean)	No. of cases	Follow up (months)	OA grade	Concomitant procedures	Method of injection	Outcomes	Conclusions
						lipoaspirate harvested from lower abdomen. The adipose tissue harvested was processed using the **Celution** Centrifuge IV		
yokota[41]	ASC: 70 SVF: 73	42 patients (59 knees) receiving intra-articular injection of ASCs and 38 patients (69 knees) receiving a SVF	6	2-4 KL		The method for harvesting the SVF was similar to that for the ASC, except that the cells were not cultured, but isolated and injected on the same day.	VAS KOOS OMERACT-OARSI	The SVF group had a higher frequency of knee effusion and minor complications related to the harvest site. Improvements in VAS and KOOS. ASC group, symptoms improved earlier and pain VAS decreased to a greater degree compared with the SVF group. The proportion of OMERACT-OARSI responders in the ASC group was slightly higher Both ASCs and SVF resulted in clinical improvement, but that ASCs outperform SVF in the early reduction of symptoms and pain with less comorbidity
Hong[12]	52	16: randomized into two groups. Each patient received autologous adipose-derived SVF treatment in one side of knee joints and a single dose of hyaluronic acid treatment (group control) in the other side	12	2-3 KL	Arthroscopy	Adipose-derived SVF cells were obtained **through enzymatic disaggregation** of lipoaspirate from the abdomen.	VAS WOMAC ROM WORMS MOCART	VAS, WOMAC and ROM improved in the SVF-treated knee and not in the contralateral control. Significant reduction in pain and WOMAC pain and stiffness in the SVF group
Caforio[38]	60	30	12	2-4 KL		Lipocell	VAS Womac Lequesne questionnaires	VAS, WOMAC and ROM improvement
Adriani[39]	63.3	30	12	1-4 KL		Lipocell	VAS WOMAC Consumption of non steroidal drugs	VAS, WOMAC and ROM improvement
Roato40	59.6	20	18	1-4 KL	Arthroscopy	Low-pressure liposuction with fenestrated blunt cannula was used to harvest adipose tissue. Centrifugation	VAS WOMAC IHC SEM	Pain reduction and increased functionality Biopsy: layer of newly formed tissue.

ACL (Anterior Cruciate Ligament), **BME** (Bone marrow edema), **BMAC** (Bone marrow aspirate concentrate), **dGEMRIC** (delayed gadolinium-enhanced MRI of cartilage), **ICRS** (International Cartilage Repair Society), **IHC** (Immunohistochemistry)(**IKS** (International Knee Society), **IKDC** (International Knee Documentation Committee), **KOOS** (Knee Injury and Osteoarthritis Outcome Score), **KL** (Kellgren Lawrence), **LEAS** (lower extremity activity scale) **LCL** (Lateral Cruciate Ligament), **MFAT** (Microfragmented adipose tissue), **MRI** (Magnetic resonance imaging), NPRS (Numeric Pain Rating Scale), **OA** (osteoarthritis), **ROM** (Range of Motion), **SEM** scanning electron microscopy **SVF** (stromal vascular fraction), **TLK** (tegner Lysholm Score), **UCLA** (the University of California in Los Angeles score for activity) **VAS** (visual analog score), **WOMAC** (Western Ontario and McMaster Universities Arthritis Index), **WORMS** whole-organ magnetic resonance imaging score, **MOCART** (magnetic resonance observation of cartilage repair tissue).

respectively. Schiavone Panni et al.[27] showed that the mean VAS score decreased from 8.5 pre-operatively to 5.1 at the latest follow-up and, therefore, patients with a pre-operative VAS score greater than 8 showed greater clinical and functional benefits compared with patients with VAS score lower than 8. Caforio et al.[31] reported VAS significantly decreased, showing a reduction of 53% after 1 month and 83% after a year, while Elnhal et al.(26) showed 32% improvement at six months. Roato et al.(33) reported that the mean VAS score was significantly reduced (7.053 ± 0.4) compared to the three post-operative time points examined: 3.321 ± 0.49; 3.011 ± 0.5 and 3.337 ± 0.6.

Three studies[10,15,23] divided VAS score obtained during activity and during resting. Hudetz et al.[10] and Boric et al.[15]reported an improvement in activity VAS from 7.33 ± 1.72 to 3.17 ± 1.98 at 12 months and from 7.73 ± 1.35 to 3.40 ± 1.65 at 24 months, respectively. Resting VAS decreased from 3.94 ± 2.56 to 0.56 ± 1.2 at 12 months for Hudetz(10); from 4.45 ± 2.42 to 0.55 ± 1.04 at 24 months for Boric.[15] In 2019, Hudetz et al.(23) reported a degreasing resting VAS from a baseline of 4.06 + 2.35 to 0.75 + 1.65 and activity VAS 7.38 + 1.41 to 3.38 + 1.89 at 12 months.

Fodor et al.,[19] showed statistically significant improvement in WOMAC and VAS scores after 3 months postoperatively, a result which was maintained at 1 year. The VAS score decreased from a preoperative mean of 5.9 to a postoperative mean of 1.8 at 3 months and 2.1 at 1 year, respectively. The WOMAC score decreased from a preoperative mean of 32.9 to a postoperative mean of 10.8 at 3 months and 9.4 at 1 year. Caforio et al.[31] al and Elnhal et al.[26] measured WOMAC with an improvement of 84% after 1 year and of 20.37% after six month post injection, respectively.

Castellarin et al. showed that the WOMAC score ameliorated from 79.5 points before the treatment to 61.5 one month after the injection, while Bansal et al. from 64 at baseline to 52 at 3 months, 46 at 6 months, 42 at 1 year, 38 at 1.5 years, and 41 at 2 years.[18]

Garza et al.[14] showed that the median percentage change in WOMAC score at 6 months after injection were dose dependent: for the high-dose it was 83.9%, for the low-dose it was 51.5%, and for the placebo groups it was 25.0%. The high- and low-dose groups had statistically significant changes in WOMAC scores when compared with the placebo group. The median percentage change in WOMAC score from baseline to 1 year after injection for the high-dose, low-dose, and placebo groups was 89.5%, 68.2%, and 0%, respectively.

Hudetz et al.[23] reported a baseline WOMAC for pain from 11.88 + 3.76 to 6.5 + 3.35, WOMAC stiffness from 4.31 + 1.89 to 2.56 + 1.46, WOMAC physical function from 39.19 + 14.2 to 23.19 + 10.85, WOMAC total score from 55.38 to 32.25 + 14.62.

In Adriani et al.' s study, the total WOMAC score was 89.9 ± 1.7 at baseline, 66.3 ± 1 at 1-month follow-up, 68.6 ± 1.7 at 3 months, and 73.2 ± 1.8 at 12 months of follow-up. The analysis of the WOMAC subscores confirmed evidence of a relative deterioration of pain over time (17.8 ± 0.3 at baseline, 11.7 ± 0.3 at 1 month, and 14.1 ± at 12 months), which is also accompanied by a deterioration of joint stiffness (7.1 ± 0.2 at baseline, 3.4 ± 0.3 at 1 month, and 4.8 ± 0.2 at 12 months) and function (63.7 ± 1.3 at baseline, 51.2 ± 1.4 at 1 month, and 55.8 ± 1.3 at 12 months).[32]

Roato et al.[33] and Yokota et al.[30] observed that the mean WOMAC scores were reduced from 45.91 ± 2.8 and 49.6 ± 20.4 pre-operatively, respectively, to 27.47 ± 3.02 (12th month) and 33.8 ± 20.9 (6th month) post-operatively.

In Pintat et al.'s study,[21] the mean WOMAC scores decreased from 34.3 to 14.2. after intrarticular injection of MSCs and PRP at 12-months compared with baseline.

In Lapuente et al.'s study[20] the mean total WOMAC score was 41.04 for patients with grade III osteoarthritis and 52.8 for those with grade IV, obtaining a final average 12 months after SVF implantation of 6.18 for patients with OA grade III and 23.8 for grade IV.

Tran et al. reported that after 12 months, no significant difference was found between the VAS scores of the SVF treatment and placebo groups (5.1 ± 2.5 vs. 4.9 ± 2.4). The score at 24 months was significantly reduced from 5.1 ± 1.2 to 3.4 ± 1.8. On the contrary, the score of the placebo group at 24 months increased to 5.9 ± 2.47, but it was not significant. Meanwhile, the WOMAC score in the treated group decreased sharply after 12 months from 44.7 ± 15.4 to 16.4 ± 12.1 and further declined significantly to 11.1 ± 11.9 at 24 months. A similar trend was also observed for the WOMAC score in the placebo group, which was significantly decreased after 12 months of treatment from 47.3 ± 17.1 to 28.6 ± 12.7, but a significant increase was observed thereafter at 24 months (36.5 ± 20.3). Overall, at 24 months, both VAS and WOMAC scores in the placebo and treatment groups diminished compared with the scores before treatment. However, the decreasing trend in the treatment group was larger than in the placebo group, which is indicative of improvement after SVF therapy.[22]

Table 2
Different methods to obtain SVF.

Author	Method of injection
Garza[21]	GID
	SVF-2 device
Fodor[26]	GID SVF1
Boric[22]	Lipogems® device
Cattaneo[23]	Lipogems® device
Russo[24]	Lipogems® device
Hudetz[16] 2017	Lipogems® device
Hudetz[30]	Lipogems® device
Panchal[31]	Lipogems device
Van Genechten[35]	Lipogems® device
Schaivone Panni[34]	Lipogems® device
Castellarin4	Lipocell device
Caforio[38]	Lipocell device
Adriani[39]	Lipocell device
Mautner[32]	BMAC:Emcyte centrifuge.
	The MFAT: Lipogems® device
Elnhal[33]	Enzymatic digestion
Bansal[25]	Enzymatic digestion
Lapuente[27]	Enzymatic digestion
Tran[29]	Enzymatic digestion
Koh[36]	Enzymatic digestion
Hong[12]	Enzymatic digestion
Roato[40]	Centrifugation
Pintat[28]	Centrifugation
Yokota[37] 2017	Celution Centrifuge IV

3.4. Imaging and immunochemistry findings

The results of MRI imaging are controversial. In four studies[14,19,21,33] no changes in cartilage thickness after treatment were observed, with no significant differences in grade, surface, or T2 value of the chondral lesions. Bansal et al.,[18] instead, revealed an improvement of cartilage thickness at least 0.2 mm in six patients, while, in two patients, the thickness remained unchanged and, in other two patients, it decreased by at least 0.2 mm. Castellarin et al. observed in 8 patients an evident reduction or even disappearance of peri-lesional subchondral edema; in 9 patients there was no evidence of hemarthrosis, and 1 patient showed a mild effusion; 1 patient showed decrease of the chondral lesion.[13] Van Genechten et al.[28] showed a significantly therapeutic response in patients with mild bone marrow lesions.

Tran et al.[22] showed that, 24 months after treatment, bone marrow edema was decreased in both the placebo and the SVF treatment groups; however, the decrease in bone marrow edema in

the SVF treatment group was larger (22 mm vs. 8 mm) than in the placebo group (20 mm vs. 12 mm). Similarly, the Outbridge score was decreased from 4 (at 0 months) to 3 (at 12 months) and 1 (at 24 months), implying a considerable improvement in cartilage regeneration in the SVF-treated group.

Magnetic resonance sequence in dGEMRIC relies on the infiltration of the anionic, negatively charged contrast gadopentetate dimeglumine (Gd-DTPA2−) into the cartilage. It indicates that the contents of cartilage glycosaminoglycans significantly increased in specific areas of the treated knee joint. Boric et al.'s[15] and Hudetz et Al's[10] results suggest that intra-articular injection of autologous microfragmented adipose tissue improves GAG content, with over half of the measurements suggesting relevant improvement 24 months in opposed to the decrease in GAG content, over the natural course of the disease.

Only few studies analyse the synovial fluid and IHC, Lapuente et al.[20]observed that the levels of metalloproteinase 2 (MMP2) and insulin-like growth factor type 1 (IGF1) decreased 80.24% and increased 330.64%, respectively. Likewise, analysing the pro-inflammatory and anti-inflammatory profiles observed by evaluation of pro-inflammatory cytokines (IL1β, IL6, and IL8) and anti-inflammatory cytokines (IL10), decreased by 32.26% in the case of IL1β, 58.25% in the case of IL6, and 36.77% in the case of IL8 and increased 70.80% in the case of IL10.

Knee joint immunohistochemistry showed strong bone remodeling in two studies,[26,33] with the presence of new tissue formation starting from the bone side of the osteochondral lesion.

4. Discussion

Recently, clinicians and researchers are focusing on the prevention of progression of KOA through the use of mesenchymal stromal stem cells (MSCs). These cells, with their chondrogenic and anti-inflammatory properties, may well reverse the first stages of KOA, reducing synovitis, cartilage degeneration and osteophyte formation.[35] The SVF contains pre-adipocytes, endothelial cells, smooth muscle cells, pericytes, macrophages, fibroblasts and ADSCs, but does not contain adipocytes; it has a very low concentration of leukocytes and a very low presence of extracellular matrix. These features impart to SVF the potential to differentiate into adipogenic, osteogenic, chondrogenic, and other mesenchymal lineages.[36] Furthermore intra-articular injection of autologous micro fragmented adipose tissue in patients with KOA increased glycosaminoglycan content in hyaline cartilage.[10] The characteristics of SVF, mainly cell availability, vary depending on whether the preparation protocol involves enzymatic digestion or mechanical breakdown of the lipoaspirate; the former is termed cellular stromal vascular fraction of adipose tissue (cSVF), and the latter tissue stromal vascular fraction of adipose tissue (tSVF) or microfragmented adipose tissue (MFAT). ADSCs are able to maintain their own features even if manipulated through different cultures and, on their surface, express different receptors for cytokine and chemokine which helps them to be recruited to the injured areas thanks to a chemotactic gradient secreted from the same suffering tissue. Unlike ADSC, the stromal vascular fraction (SVF) can be readily obtained from the lipoaspirate without the need for cell separation or culturing, which makes SVF more cost efficient and convenient.[12] SVF presents many advantages compared to ADSCs: 1) SVF is promptly accessible from lipoaspirate without separation and cell culturing, 2) SVF is cheaper and faster; 3) the injection can be performed on the same day of the surgical procedure. Concomitant strategies could be implemented in conjunction with SVF injection treatments: high tibial osteotomy, microfractures, platelet-rich plasma, and hyaluronic acid injections.[37] Biological treatments are obviously more expensive than other conservative treatment, but adipose derived stem cells have been the subject of numerous clinical studies because traditional treatments are often only palliative. Biological treatments offer the opportunity to regenerate tissues and delay the progression of KOA.[38]

In this systematic review, we evaluated the current literature on the clinical application of SVF in patients with knee OA to assess their safety and efficacy. From a clinical point of view, the treatments significantly increased clinical outcomes in all these studies. VAS and WOMAC were the most commonly used scores to analyse clinical outcomes of these patients. The most important finding from this systematic review was that SVF injection is a safe procedure for the management of knee OA, with good clinical and imaging outcomes in the early follow-up period (6–24 months). Intra-articular injection of SVFs can lead to a significant improvement of the cartilage and subchondral bone, protecting against arthritic processes. Koh et al.[29] showed that, through second-look arthroscopy, clinical improvements persisted for more than 2 years and that 87.5% of elderly patients improved or maintained cartilage healing status at 2 years postoperatively. Isolation of the SVF is a relatively simple procedure, with the addition of approximately 60–70 min of time spent on processing and injecting 1 or 2 knees.[19]

However, limitations of radiography to visualize OA features, including insensitivity to early changes, non-specificity, absence of reproducibility in longitudinal studies and challenges regarding positioning, significantly limits the utility of radiography to assess the efficacy of intraarticular therapy. Considering the articles analysed, we cannot assess whether SVF injections give better results on early or late stages of KOA. For example, in Tran et al.[22] SVF therapy was more effective in KL 3-grade compared with KL 2-grade OA patients, while in Lapuente et al.[20] the satisfaction rate of patients with KL 3-grade osteoarthritis was higher than that of patients with KL 4-grade. A more advanced disease stage could be reasonably more difficult to treat or respond to the treatment applied. Some studies used magnetic resonance imaging (MRI) to evaluate the real effectiveness of intraarticular SVF injections before and after treatment. Indeed, the use of more advanced imaging modalities such as MRI is important to assess biochemical compositional changes of articular and periarticular tissues. Boric et al.[15] used a single intra-articular injection of autologous microfragmented adipose tissue, and evaluated full-thickness cartilage layers and GAGs production through MRI. Their results suggest that a single intra-articular injection of autologous microfragmented adipose tissue improves GAG content in 24 months.

No serious adverse events were reported in the studies analysed, and only minor events were described, such as joint pain, hematoma, recurrent effusion and other complications not directly related to the treatment.

The results of the present review should be taken with caution because these clinical trials present major limitations, different methods, and confounding factors. Above all, the follow up of these studies ranged from 6 to 24 months, maybe too short to assess long term results of intraarticular injections of SVF. Not all studies reported how many patients underwent TKA after SVF injections. Only two studies[6,14] were randomized controlled trials and Tran et Al[22] instead used a control group consisting on the contralateral knee.

Another limit was that several techniques have been reported for fat harvesting. In the included studies, different devices were used to process harvested SVF. The most commonly evaluated procedure is Lipogems®, but the superiority of one method of preparation over another still remains unanswered. Reasonably, greater standardization of devices protocols will be desirable, but only obtainable with qualitatively optimal and dedicated clinical studies for each device. SVF is a mixture of pericytes, fibroblasts,

adipocytes, monocytes, macrophages, red blood cells and ADSCs (9–9.5%), but we cannot evaluate the effectiveness of the stem cell component of the fraction in isolation and how many cells have to be administered to achieve the best therapeutic effect. Garza et al.[14] obtained better results in symptoms and pain with high-dose of intra-articular SVF and further studies are necessary to identify the optimal cell concentration and environment needed for clinical application of intra-articular injection in knee OA.[39,40] The area of fat harvesting and patient age may influence the stem cell yield,[41] but not all studies report the site of harvesting. The most important limit is that in many studies patients underwent concomitant procedures such as arthroscopic debridement, microfractures, or high tibial osteotomy, thus preventing a clear understanding of the real contribution and clinical potential of stem cell-based treatment. Schiavone Panni et al.[27] proposed the concomitant use of adipose stem cells with knee arthroscopy debridement with significant improvement in clinical and functional scores in patients with early KOA for 6–24 months. In some studies[25]) SVF have been used in combination with PRP. Coadministration of SVF s and other devices can enhance the effect, allowing to use a smaller amount of drugs or devices, and possibly decreasing adverse effects. On the other hand, the use of concomitant therapies makes it impossible to establish the effects of SVF alone.

5. Conclusion

The reported excellent clinical and radiographic results for the treatment of knee OA of 24 clinical trials on intraarticular knee injection of SVF encourage to standardize new therapeutic protocols for knee osteoarthritis.

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