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Micro-fragmented stromal-vascular fraction plus microfractures provides better clinical results than microfractures alone in symptomatic focal chondral lesions of the knee

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Abstract

Purpose To evaluate clinical outcomes over a 1-year period in patients affected by symptomatic focal chondral lesions of the knee treated with micro-fragmented stromal-vascular fraction plus microfractures compared to microfractures alone.

Methods Two groups of 20 patients were arthroscopically treated with microfractures for a symptomatic focal chondral defect of the knee. At the end of surgery, in the experimental group, micro-fragmented stromal-vascular fraction was injected into the joint. Primary end point was WOMAC score at 12 months. Secondary end points were any adverse events, Oxford Knee Score, EQ-5D score, VAS for pain, analgesic and anti-inflammatory consumption.

Results All the patients were evaluated at 12-month follow-up. No adverse reactions were noted. Analgesic and anti-inflammatory consumption was similar in both groups. At 1-month follow-up, no differences were noted between groups when compared to pre-operative scores. At 3-month follow-up, patients in both groups improved from the baseline in all variables. Significantly lower VAS scores were found in the experimental group (4.2 ± 3.2 vs. 5.9 ± 1.7, $p = 0.04$). At 6- and 12-month follow-ups, patients in the experimental group scored better in all outcomes with a moderate effect size; in particular, better WOMAC scores were obtained at 12 months, achieving the primary end-point of the study (17.7 ± 11.1 vs. 25.5 ± 12.7; $p = 0.03$).

Conclusions Injection of micro-fragmented stromal-vascular fraction is safe and, when associated with microfractures, is more effective in clinical terms than microfractures alone in patients affected by symptomatic focal chondral lesions of the knee. Results of the current study provide information that could help physicians to improve their counseling for patients concerning ADMSCs.

Level of evidence Level 1—therapeutic study.

Keywords Stromal-vascular fraction · Adipose-derived mesenchymal stem cell · Chondral lesion · Knee

Abbreviations

ADMSCs	Adipose-derived mesenchymal stem cells
BMI	Body mass index
CONSORT	Consolidated Standards of Reporting Trials
EQ-5D	EuroQol-5D
PRP	Platelet-Rich Plasma
VAS	Visual Analogue Scale
WOMAC	Western Ontario & McMaster Universities Osteoarthritis Index
MCID	Minimal Clinical Important Difference
MOCART	Magnetic Resonance Observation of Cartilage Repair Tissue

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Introduction

Cartilage lesions are difficult to treat because of their limited healing potential due to lack of vascularity and innervation [6]. Many treatments have been proposed to improve these patients' quality of life and help them maintain an active lifestyle. In particular, with the recent developments in regenerative medicine, many papers have focused on mesenchymal stem cells (MSCs). Adipose

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tissue has gained interest as a source of stem cells thanks to of its availability, its ease of access, and its abundance of stem cells (1% vs. 0.01% of the bone marrow) [3]. Furthermore, the number of adipose-derived mesenchymal stem cells (ADMSCs) is not related to ageing [2, 44]. ADMSCs are traditionally obtained after enzymatic treatment and prolonged expansion in vitro which both lead to significant senescence and reduction in stem potential [37]. However, this technique is difficult to application owing to complicated legislation; MSCs expansion in vitro is considered for the European regulation as a drug, thus necessitating a clinical trial before it can be accepted for human administration under compassionate use. Another drawback is that only homologous use has been approved. Recently, micro-fragmentation has been introduced as a new isolation method for ADMSCs, reducing costs and avoiding advanced cell therapies and regulatory problems [1, 42, 45]. Micro-fragmentation is based on the principle that ADMSCs are contained in the stromal-vascular fraction (niche) and do not need to be isolated to be activated; indeed, the niche represents the ideal environment for cell preservation and activation. Micro-fragmented stromal-vascular fraction is a complex structure formed by a scaffold (niche), with a high concentration of stem cells, and growth factors [5, 10, 12, 46] inducing host cells to heal the injury through a paracrine effect via the release of cytokines and chemokines, this is independent from the absolute number of ADMSCs adopted [7–9].

Intra-articular administration of ADMSCs has been shown to be safe and effective in animal models [13, 17, 24, 47, 48] and in humans [41]. The majority of publications deal with the treatment of osteoarthritis (especially in the knee) [11, 21–23, 32, 40, 41, 43]. Few comparative studies have been published on this topic [26–28, 33, 36], and are flawed by confounding factors, such as the association with other treatments (i.e., osteotomies and Platelet-Rich Plasma—PRP). Furthermore, adipose tissue has already been harvested the day before surgery and subsequently enzymatically processed [36], or centrifuged in the lab [26–29, 33]. To the best of our knowledge, there are only two comparative papers on the treatment of focal cartilage lesions of the knee with or without ADMSCs. In both these studies, ADMSCs were harvested the day before surgery, were then enzymatically processed and eluted in PRP in one study [36], or centrifuged and mixed to fibrin glue in the other [34].

The aim of the current study was to evaluate whether the intra-articular administration of the stromal-vascular fraction can improve clinical findings in patients operated on for focal chondral lesions of the knee.

The hypothesis of the current study is that patients in the experimental group will have better clinical results at final follow-up.

Materials and methods

This study conforms to the Declaration of Helsinki and its subsequent modifications. The Institutional Review Board/Ethics Committee at the authors' institution approved the study protocol (LIPO 2—approval number 8/16). The study also meets CONSORT (Consolidated Standards of Reporting Trials) guidelines for randomized controlled trials, its being a prospective randomized controlled single blind (blind observer) clinical trial including 40 patients (40 knees) with a symptomatic focal chondral lesion of a femoral condyle (grades III–IV according to Outerbridge classification [39]). A computer-generated method randomly assigned patients to the experimental or control group with a 1:1 ratio.

Between May 2016 and October 2017, all the patients affected by symptomatic focal chondral lesions of the knee at the authors' institution were identified and selected according to the inclusion and exclusion criteria, as listed in Table 1. Sixteen patients were excluded (two were older than 70, 2 had lesions > 4 cm^2, two were surgically treated for the same reason, one had an anterior cruciate ligament tear, three had thyroid or metabolic disorders, one had a varus $> 10°$, and five had knee osteoarthritis ≥ 3 according to Kellgren–Lawrence [25]). Another five patients opted not to participate to the study) (Fig. 1).

Enrolled patients were evaluated before and after surgery according to the protocol, as reported in Table 2. At every follow-up visit, we administered a questionnaire containing Western Ontario & McMaster Universities Osteoarthritis Index (WOMAC) score [4], Oxford Knee Score [16], EuroQol-5D (EQ-5D) [18], Visual Analogue Scale (VAS) for pain [19], analgesic and anti-inflammatory drug consumption.

The primary end-point was WOMAC score at 12-month follow-up. Secondary end-points were any adverse events, WOMAC score at 3 and 6 months, Oxford Knee Score, EQ-5D, VAS for pain, and consumption of analgesics and anti-inflammatory drugs.

The experimental group consisted of 20 patients (8 women and 12 men) with a mean age of 49.8 ± 10.9 years (range 25–70). The control group consisted of 20 patients (7 women and 13 men) with a mean age of 46.1 ± 14.7 years (range 22–68). The two groups were homogeneous in terms of age, gender, body mass index (BMI), associated co-morbidities, severity of pre-operative knee osteoarthritis, consumption of analgesic and anti-inflammatory drugs, pre-operative values of analyzed outcomes, size of cartilage lesions, and associated procedures (Table 3).

Patients of both groups underwent knee arthroscopy by the same surgeon to confirm inclusion and exclusion

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Written informed consent	Patients unable to consent
Age 18–70	Previous surgery for the same reason
Focal chondral lesion (Outerbridge grade III–IV) [31]	ACL and/or PCL tear
Lesion size 1–4 cm^2	Malignancies
Failed conservative treatment for at least 3 months	Thyroid or metabolic disorders
Willing to participate to rehabilitation protocol and follow-up visits	History of alcohol and or drug abuse
	Synovits
	Malalignment (> 10° in varus or valgus)
	BMI > 30
	History of trauma in the 6 months before treatment
	Knee osteoarthritis (Kellgren-Laurence ≥ 3 [36]
	Subtotal or total meniscectomy

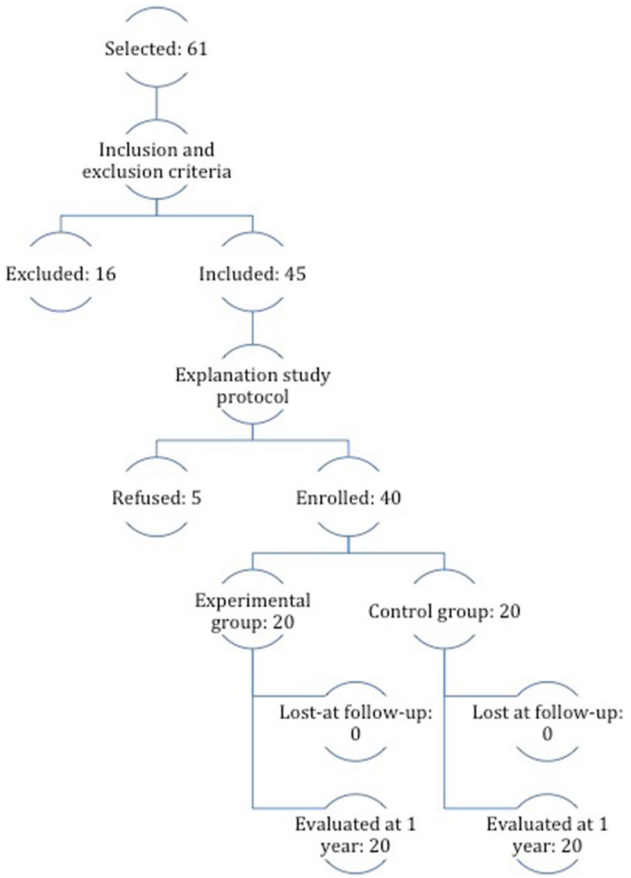

Fig. 1 CONSORT flow diagram. Diagram showing the patients selected, excluded, enrolled, and evaluated during the study

criteria and sizing of cartilage lesions. In the case of meniscal tears, repair or selective meniscectomy was performed as appropriate, leaving at least 2/3 of the meniscus intact. Margins of the cartilage lesions were debrided with a shaver to obtain a stable rim. Microfractures were created with an arthroscopic awl 3–4 mm apart; intra-articular pressure was reduced to confirm blood leakage. During the same arthroscopic procedure, patients in the experimental group had their abdomen prepared and draped in a sterile fashion. After subcutaneous infiltration with Klein's solution (adrenalin 2 µg/ml and lidocaine 0.02%), two small incisions were made with a scalpel blade on both sides to insert a small cannula (13 gauge) with a blunt tip connected to a syringe with a negative pressure. With gentle longitudinal movements, adipose tissue was harvested in variable amounts (15–50 cc). At the same time, the device used for micro-fragmentation of the stromal-vascular fraction (Lipogems®, Lipogems International SpA, Milan, Italy), was filled with saline solution. Lipoaspirate was injected through the first filter to reduce the size of the adipose clusters and to eliminate fibrous tissue. The device has five stainless steel spheres to emulsify the adipose component when shacked. Continuous saline flow was adopted to eliminate debris in the discharge bag, while the two filters maintained the stromal-vascular fraction inside the device. As soon as the fluid was yellow and transparent, a second volumetric reduction of the adipose clusters was carried out through the second filter. About 10 cc of micro-fragmented stromal-vascular fraction were injected under arthroscopic control into the knee in patients in the experimental group.

Statistical analysis

A review of the literature showed that in patients with focal cartilage lesions of the knee with features similar to those of the patients enrolled in this study, WOMAC scores are normally distributed with a standard deviation of 17 points and a Minimal Clinical Important Difference (MCID) of 15 points. Given an error (α) = 5% and a power ($1-\beta$) = 80%, considering a dropout rate of 10% at follow-up, at least 20

Table 2 Evaluation protocol

	Baseline V1	Surgery V2	1-month visit V3	3-month visit V4	6-month visit V5	12-month visit V6
Inclusion and exclusion criteria	X					
Written informed consent	X					
Demographic file	X					
MRI	X					
WOMAC score	X			X	X	X
Oxford knee score	X			X	X	X
EQ-5D Current health assessment	X			X	X	X
VAS pain score	X		X	X	X	X
Kellgren-Lawrence grading scale (Rx)	X					X
Arthroscopic evaluation of the lesion		X				
Adverse event reporting		X	X	X	X	X
Satisfaction				X	X	X

Table 3 Pre- and intra-operative variables in the experimental and the control groups

	Experimental group	Control group
WOMAC	55.2 ± 17.3	55.7 ± 21.8
Oxford Knee Score	26.4 ± 8.9	24.0 ± 11.3
EQ-5D	17.2 ± 16.6	12.0 ± 16.7
VAS for pain	6.2 ± 1.9	7.1 ± 2.3
BMI	24.8 ± 3.2	26.1 ± 3.0
Prior surgical procedures to the index knee		
Meniscectomy—*n* (%)	3 (15)	4 (20)
Osteoarthritis—*n* (%)		
Grade 0	3 (15)	3 (15)
Grade 1	11 (55)	10 (50)
Grade 2	6 (30)	7 (35)
Knee axis (positive values mean valgus)	4.1 ± 3.0	4.9 ± 2.8
Size of the lesion after arthroscopic debridement (cm^2)	3.2 ± 1.7	3.1 ± 1.5
Meniscal lesions—*n*		
Repair	4	3
Meniscectomy	2	2

patients per group were needed for the purposes of this study.

Descriptive statistics were used to summarize the characteristics of the study groups. Data were visually inspected for normal distribution and outliers. Results were rounded to the first decimal digit. Transformation of data was adopted as needed. Student *t* test was used to compare all the continuous variables. Categorical variables were analyzed using Chi-square test (or Fisher exact test as needed), Wilcoxon signed rank test or Wilcoxon paired rank test, as appropriate. Two-sided statistical significance was defined as $p < 0.05$. For statistically significant differences, effect size was evaluated using Cohen's d coefficient [15] and rated as very small (< 0.20), small (0.20–0.50), medium (0.50–0.80), large (0.80–1.30), very large (> 1.30). Statistical analyses were performed with SPSS v.15.0 (SPSS Inc., an IBM Company, Chicago, IL, USA).

Results

All the patients were evaluated 12 months after surgery. There were no adverse events related to micro-fragmented stromal-vascular fraction injection. There was a case of knee effusion 3 days after surgery in the control group. Consumption of analgesics and anti-inflammatories was similar in both groups after surgery.

At 1-month evaluation, only VAS score was analyzed. There were no statistically significant differences between groups or compared to the baseline (5.1 ± 3.1 in experimental group vs. 6.4 ± 1.5 in control group; p = n.s.).

At 3-month follow-up, patients in both groups significantly improved compared to baseline ($p < 0.05$). Patients in the experimental group obtained significantly lower VAS for pain scores ($p = 0.04$). There were no differences in all the other variables (Table 4).

Patients in both groups further improved between 3 and 6 months, obtaining statistically significant differences in all variables compared to pre-operative values ($p < 0.001$). Patients in the experimental group obtained statistically better scores compared to the control in all the variables (Table 5).

At 12-month follow-up, patients in both groups obtained statistically significant differences compared to baseline values ($p < 0.001$). The primary end-point of this study was achieved; in fact, patients in the experimental group reported significantly better WOMAC scores compared to controls. Furthermore, statistically significant differences were also found in all the other variables favoring the experimental group (Table 6).

Temporal trends of the scores for all analyzed variables in the experimental and control groups are reported in Fig. 2.

Discussion

The most important finding of the current study was that the injection of micro-fragmented stromal-vascular fraction was found to be more effective in clinical terms than microfractures alone in patients affected by symptomatic focal chondral lesions of the knee. The primary end-point of this study was matched; in fact, at 12-month follow-up patients in the experimental group obtained statistically better WOMAC scores, with a medium effect size. Better VAS for pain scores were observed in the experimental group already at 3-month follow-up, while at 6-month follow-up, there were statistically significant differences in all considered variables that were maintained for up to 1 year after surgery with a medium effect size (according to Cohen's d coefficient).

To the best of our knowledge, there are only two papers on the treatment of focal cartilage lesions of the knee with microfractures with or without ADMSCs [34, 36]; however, they are biased by the inclusion of other variables in the treatment that can influence the interpretation of the results.

Nguyen et al. [36] evaluated two groups of 15 patients with an average age of 58 years affected by medium-moderate knee osteoarthritis, and who had already been treated with the previous autologous cartilage transplantation,

Table 4 Comparison at 3-month follow-up between the experimental and the control groups

	Experimental group	Control group	p value	Cohen d
WOMAC	41.0 ± 19.8	44.4 ± 18.5	n.s	
Oxford knee score	32.4 ± 8.4	31.3 ± 10.3	n.s.	
EQ-5D	30.0 ± 17.9	26.3 ± 21.7	n.s.	
VAS for pain	4.2 ± 3.2	5.9 ± 1.7	0.04	0.69
Satisfaction	2.6 ± 1.2	1.9 ± 1.0	0.05	0.63

n.s non significant

Table 5 Comparison at 6-month follow-up between the experimental and the control groups

	Experimental group	Control group	p value	Cohen d
WOMAC	19.3 ± 17.1	32.6 ± 21.4	0.03	0.69
Oxford knee score	43.6 ± 11.4	36.2 ± 9.5	0.03	0.71
EQ-5D	60.3 ± 19.1	45.7 ± 21.3	0.02	0.72
VAS for pain	3.4 ± 2.9	5.1 ± 1.5	0.02	0.77
Satisfaction	2.8 ± 1.3	1.5 ± 0.9	0.001	1.18

Table 6 Comparison at 12-month follow-up between the experimental and the control group

	Experimental group	Control group	p value	Cohen d
WOMAC	17.7 ± 11.1	25.5 ± 12.7	0.03	0.65
Oxford knee score	45.1 ± 3.7	38.9 ± 12.8	0.02	0.75
EQ-5D	72.6 ± 5.5	58.7 ± 24.9	0.02	0.91
VAS for pain	2.6 ± 1.8	4.3 ± 2.7	0.02	0.75
Satisfaction	3.2 ± 1.1	1.4 ± 1.7	0.0003	1.28

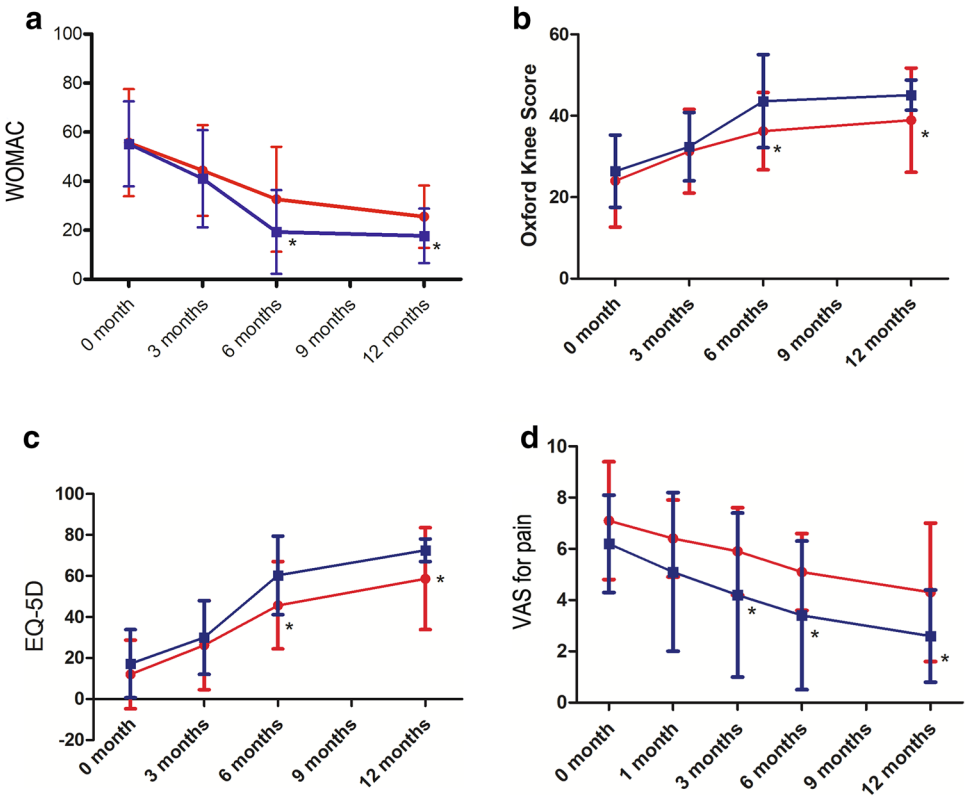

Fig. 2 Temporal trends of the scores (mean and standard deviation) for WOMAC (**a**), Oxford Knee Score (**b**), EQ-5D (**c**), and VAS for pain (**d**) in the experimental group (blue line) and control group (red line). Statistically significant differences have been reported with an asterisk

and were committed with an arthroplasty condition. The experimental group was treated with microfractures and ADMSCs isolated in the lab through enzymatic digestion and eluted in PRP before being injected into the joint. The control group was treated with microfractures and saline infiltration. At 18-month follow-up the experimental group achieved better MRI scores (bone edema) and better WOMAC, Lysholm e VAS for pain scores. The authors reported patients in the control group returned to pre-operative scores between 12 and 18 months, while patients in the experimental group further improved.

Koh et al. [34] evaluated in a randomized controlled trial two groups of 40 patients aged between 10 and 50 years with a focal cartilage lesion greater than 3 cm^2. The experimental group was treated with microfractures and with ADMSCs, the latter centrifuged, mixed with fibrin glue, and sealed to the cartilage lesion. The control group was treated with microfractures alone. At 2-year follow-up, patients in the experimental group had better MRI scores (Magnetic Resonance Observation of Cartilage Repair Tissue—MOCART [31]) and KOOS for pain and symptoms. The authors did not note any difference in activity level, sport or quality of life between the two groups. Some of the patients from both groups had a second arthroscopy (30/40 in the experimental and 27/40 in control groups) to evaluate the quality of cartilage tissue and to perform a biopsy (18 in the experimental and 16 in the control groups). At histology, there was no difference in tissue quality.

The clinical results of the current study agree with the previous publications, albeit a different population was analyzed and ADMSCs were obtained with minimal manipulation [34, 36]. In fact, better pain scores were observed in the experimental group 3 months after surgery, possibly indicating the immuno-modulatory and anti-inflammatory function of the stromal vascular fraction on synovial membrane and sub-chondral bone rich in nerve terminations and blood vessels. At 6-month follow-up the clinical difference toward the experimental group was evident in all considered variables and was maintained up to 1 year. This may be due to the response of resident cells (chondrocytes and chondroblasts) to the trophic effect induced by cytokines and growth factors released by ADMSCs. Effect size was medium for all variables, indicating a moderate difference between groups.

In the current study, in agreement with the previous publications, no adverse events related to ADMSCs were observed, on the operated knee or in the liposuction area. No major complications or infection related to ADMSCs have yet been reported, strongly suggesting that they constitute safe therapy [46]. In agreement with the previous studies, patients in control group in this study significantly improved in terms of pain and function after arthroscopic microfractures up to 1 year. Microfractures were shown to be mainly indicated in small cartilage lesions in young patients with

low functional demands. In these patients, good results can be obtained up to 2 years after surgery, with a worsening at 5 years [20], even though recent studies reported failure rates similar to autologous chondrocytes implantation also in large lesions in the medium- and long-term [30, 31, 38]. Furthermore, about a third of the patients at 5 years and half of the patients at 15-year follow-up (microfractures or autologous chondrocytes implantation) develop knee osteoarthritis grade 2 or greater according to Kellgren-Laurence [30, 31].

Treatment of focal cartilage lesions is challenging for the orthopaedic surgeon. These patients are young, have an active lifestyle and high expectations. Several treatments have been proposed to reduce pain and disability, improve joint function in the short-term, but especially to decrease failure rates, revisions and prevent knee osteoarthritis in the long-term. Unfortunately, in the cases with severe osteoarthritis, joint replacement is the only treatment to relieve pain and improve function.

This is one of the few prospective randomized trials. Strict inclusion and exclusion criteria were adopted; sample size was calculated "a priori" and based on primary outcome of the study; both patient-reported outcomes and objective measurements (consumption of analgesics and anti-inflammatories) were analyzed, and all patients were evaluated at follow-up.

This study has also some limitations. The main limitation is the short follow-up of 1 year; however, it is similar to the previous studies: Koh et al. [34] evaluated their patients at 2 years, Nguyen et al. [36] evaluated their patients at 18 months. Another important limitation is the small sample size of 20 patients per group, although this is comparable to the studies from Koh et al. (40 patients per group) [34] and Nguyen et al. (15 patients per group) [36]. Lipoaspiration was not performed in the control group, because it was believed unethical. Klein solution was not injected, skin of the abdomen was not incised, a compressive bandage was not applied and a post-operative elastic belly band was not prescribed to patients in control group. For these reasons, patients were not blind, and this may have influenced the clinical scores. In fact, it is well known that placebo is an important factor regarding pain evaluation. This may explain the better results and satisfaction observed in the experimental group. Even in the study by Koh et al. [34] liposuction was not performed, while Nuyen et al. [36] did not specify how the control group was treated. In the previous retrospective studies on ADMSCs injection in patients with knee and ankle osteoarthritis, liposuction was not performed in the control group [26–29], while another study (prospective) stated that liposuction was performed in all patients [33]. For ethical reasons, a second arthroscopy was not included in the protocol; none of the patients needed a second surgery on the index knee, a procedure that may have provided some information on healing of the cartilage. By contrast, Koh et al. [34] did perform a second arthroscopy in 30 patients in the experimental group and in 27 patients in control group, and a biopsy in 18 patients in the experimental group and 16 patients in control group. For economic reasons, MRI was not performed at follow-up. This may have provided some indirect information on the healing of the cartilage through MOCART score [35] and/or evaluation of bone edema. In the previous studies, Nguyen et al. [36] analyzed bone edema, while Koh et al. [34] reported MOCART scores at follow-up indicating better results in patients treated with ADMSCs. Histology, cytology and biochemical analysis of ADMSCs and growth factors of the processed lipoaspirate and of the fluid of the discharge bag were not performed in this study. Some studies have already reported the features of the micro-fragmented lipoaspirate and its cells [5, 10], but these data need further research. Furthermore, a recent publication reported on mesenchymal stromal cells in the fluid of the discharge bag of the device that might be used for regenerative purposes or drug delivery [14]. The results of the current study should also be analyzed in terms of treatment benefit compared to cost of acquiring the device. In particular, the cost of the device at our Institution is 1000 euros (VAT not included), while the cost of the post-operative elastic belly band is about 20 euros to the patient.

Conclusions

Results of the current study confirm the safety profile of ADMSCs injection into the knee joint. Of further interest, in patients affected by symptomatic focal cartilage lesions of the knee, the addiction of the micro-fragmented stromal-vascular fraction to microfractures provided statistically clinical better results compared to microfractures alone, together with a medium effect size.

Author contributions SB has been involved in conception and design, analysis and interpretation of data, and drafting the manuscript. CT has been involved in conception and design, performed all the surgeries, and revised the manuscript critically for important intellectual content. GB and SMP have made substantial contributions to acquisition of data. All the authors have given final approval of the version to be published. They agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Compliance with ethical standards

Conflict of interest The authors have no potential conflict of interest.

Ethical approval Ethical approval was received from the University of Rome Tor Vergata Ethics Committee (Protocol LIPO 2 - approval number 8/16).

References

1. Arcidiacono JA, Blair JW, Benton KA (2012) US food and drug administration international collaborations for cellular therapy product regulation. Stem Cell Res Ther 3(5):38
2. Baer PC, Geiger H (2012) Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. Stem Cells Int. https://doi.org/10.1155/2012/812693
3. Becerra J, Andrades JA, Guerado E, Zamora-Navas P, López-Puertas JM, Reddi AH (2010) Articular cartilage: structure and regeneration. Tissue Eng Part B Rev 16(6):617–627
4. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW (1998) Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 15(12):1833–1840
5. Bianchi F, Maioli M, Leonardi E, Olivi E, Pasquinelli G, Valente S, Mendez AJ, Ricordi C, Raffaini M, Tremolada C, Ventura C (2013) A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. Cell Transpl 22(11):2063–2077
6. Buckwalter JA, Brown TD (2004) Joint injury, repair, and remodeling: roles in post-traumatic osteoarthritis. Clin Orthop Relat Res 423:7–16
7. Caplan AI (2009) Why are MSCs therapeutic? New Data: New Insight J Pathol 217(2):318–324
8. Caplan AI (2017) Mesenchymal stem cells: time to change the name! Stem Cells Transl Med 6(6):1445–1451
9. Caplan AI, Correa D (2011) The MSC: an injury drugstore. Cell Stem Cell 9(1):11–15
10. Carelli S, Messaggio F, Canazza A, Hebda DM, Caremoli F, Latorre E, Grimoldi MG, Colli M, Bulfamante G, Tremolada C, Di Giulio AM, Gorio A (2015) Characteristics and properties of mesenchymal stem cells derived from microfragmented adipose tissue. Cell Transpl 24(7):1233–1252
11. Cattaneo G, De Caro A, Napoli F, Chiapale D, Trada P, Camera A (2018) Micro-fragmented adipose tissue injection associated with arthroscopic procedures in patients with symptomatic knee osteoarthritis. BMC Musculoskelet Disord 19(1):176
12. Ceserani V, Ferri A, Berenzi A, Benetti A, Ciusani E, Pascucci L, Bazzucchi C, Coccè V, Bonomi A, Pessina A, Ghezzi E, Zeira O, Ceccarelli P, Versari S, Tremolada C, Alessandri G (2016) Angiogenic and anti-inflammatory properties of micro-fragmented fat tissue and its derived mesenchymal stromal cells. Vasc Cell 8:3
13. Choi S, Kim JH, Ha J, Jeong BI, Jung YC, Lee GS, Woo HM, Kang BJ (2018) Intra-articular injection of alginate-micro-encapsulated adipose tissue-derived mesenchymal stem cells for the treatment of osteoarthritis in rabbits. Stem Cells Int 2018:2791632
14. Coccè V, Brini A, Giannì AB, Sordi V, Berenzi A, Alessandri G, Tremolada C, Versari S, Bosetto A, Pessina A (2018) A nonenzymatic and automated closed-cycle process for the isolation of mesenchymal stromal cells in drug delivery applications. Stem Cells Int 10:11. https://doi.org/10.1155/2018/4098140
15. Cohen J (1988) Statistical power analysis for the behavioral sciences, 2nd edn. Lawrence Earlbaum Associates, Hillsdale, pp 20–26
16. Dawson J, Fitzpatrick R, Murray D, Carr A (1998) Questionnaire on the perceptions of patients about total knee replacement surgery. J Bone Joint Surg Br 80(1):63–69
17. Dragoo JL, Carlson G, McCormick F, Khan-Farooqi H, Zhu M, Zuk PA, Benhaim P (2007) Healing full-thickness cartilage defects using adipose-derived stem cells. Tissue Eng 13(7):1615–1621
18. EuroQol Group (1990) EuroQol ``a new facility for the measurement of health-related quality of life''. Health Policy 16(3):199–208
19. Gould D, Kelly D, Goldstone L, Gammon J (2001) Examining the validity of pressure ulcer risk assessment scales: developing and using illustrated patient simulations to collect the data. J Clin Nurs 10(5):697–706
20. Goyal D, Keyhani S, Lee EH, Hui JH (2013) Evidence-based status of microfracture technique: a systematic review of level I and II studies. Arthroscopy 29(9):1579–1588
21. Ha CW, Park YB, Kim SH, Lee HJ (2019) Intra-articular mesenchymal stem cells in osteoarthritis of the knee: a systematic review of clinical outcomes and evidence of cartilage repair. Arthroscopy 35(1):277.e2–288.e2
22. Hudetz D, Borić I, Rod E, Jeleč Ž, Radić A, Vrdoljak T, Skelin A, Lauc G, Trbojević-Akmačić I, Plečko M, Polašek O, Primorac D (2017) The effect of intra-articular injection of autologous microfragmented fat tissue on proteoglycan synthesis in patients with knee osteoarthritis. Genes (Basel) 8(10):270
23. Jayaram P, Ikpeama U, Rothenberg JB, Malanga GA (2019) Bone marrow-derived and adipose-derived mesenchymal stem cell therapy in primary knee osteoarthritis: a narrative review. PM R 11(2):177–191
24. Jurgens WJ, Kroeze RJ, Zandieh-Doulabi B, van Dijk A, Renders GA, Smit TH, van Milligen FJ, Ritt MJ, Helder MN (2013) One-step surgical procedure for the treatment of osteochondral defects with adipose-derived stem cells in a caprine knee defect: a pilot study. Biores Open Access 2(4):315–325
25. Kellgren JH, Lawrence JS (1952) Rheumatism in miners. II. X-ray study. Br J Ind Med 9(3):197–207
26. Kim YS, Lee M, Koh YG (2016) Additional mesenchymal stem cell injection improves the outcomes of marrow stimulation combined with supramalleolar osteotomy in varus ankle osteoarthritis: short-term clinical results with second-look arthroscopic evaluation. J Exp Orthop 3(1):12
27. Kim YS, Park EH, Kim YC, Koh YG (2013) Clinical outcomes of mesenchymal stem cell injection with arthroscopic treatment in older patients with osteochondral lesions of the talus. Am J Sports Med 41(5):1090–1099
28. Kim YS, Koh YG (2016) Injection of mesenchymal stem cells as a supplementary strategy of marrow stimulation improves cartilage regeneration after lateral sliding calcaneal osteotomy for varus ankle osteoarthritis: clinical and second-look arthroscopic results. Arthroscopy 32(5):878–889
29. Kim YS, Koh YG (2018) Comparative matched-pair analysis of open-wedge high tibial osteotomy with versus without an injection of adipose-derived mesenchymal stem cells for varus knee osteoarthritis: clinical and second-look arthroscopic results. Am J Sports Med 46(11):2669–2677
30. Knutsen G, Drogset JO, Engebretsen L, Grøntvedt T, Isaksen V, Ludvigsen TC, Roberts S, Solheim E, Strand T, Johansen O (2007) A randomized trial comparing autologous chondrocyte implantation with microfracture. Findings at 5 years. J Bone Joint Surg Am 89(10):2105–2112
31. Knutsen G, Drogset JO, Engebretsen L, Grøntvedt T, Ludvigsen TC, Løken S, Solheim E, Strand T, Johansen O (2016) A randomized multicenter trial comparing autologous chondrocyte implantation with microfracture: long-term follow-up at 14–15 years. J Bone Joint Surg Am 98(16):1332–1339
32. Koh YG, Choi YJ, Kwon OR, Kim YS (2014) Second-look arthroscopic evaluation of cartilage lesions after mesenchymal stem cell implantation in osteoarthritic knees. Am J Sports Med 42(7):1628–1637
33. Koh YG, Kwon OR, Kim YS, Choi YJ (2014) Comparative outcomes of open-wedge high tibial osteotomy with platelet-rich

plasma alone or in combination with mesenchymal stem cell treatment: a prospective study. Arthroscopy 30(11):1453–1460
34. Koh YG, Kwon OR, Kim YS, Choi YJ, Tak DH (2016) Adipose-derived mesenchymal stem cells with microfracture versus microfracture alone: 2-year follow-up of a prospective randomized trial. Arthroscopy 32(1):97–109
35. Marlovits S, Singer P, Zeller P, Mandl I, Haller J, Trattnig S (2006) Magnetic resonance observation of cartilage repair tissue (MOCART) for the evaluation of autologous chondrocyte transplantation: determination of interobserver variability and correlation to clinical outcome after 2 years. Eur J Radiol 57(1):16–23
36. Nguyen PD, Tran TD, Nguyen HT, Vu HT, Le PT, Phan NL, Vu NB, Phan NK, Van Pham P (2017) Comparative clinical observation of arthroscopic microfracture in the presence and absence of a stromal vascular fraction injection for osteoarthritis. Stem Cells Transl Med 6(1):187–195
37. Orozco L, Munar A, Soler R, Alberca M, Soler F, Huguet M, Sentis J, Sánchez A, García-Sancho J (2013) Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. Transplantation 95(12):1535–1541
38. Ossendorff R, Franke K, Erdle B, Uhl M, Südkamp NP, Salzmann GM (2019) Clinical and radiographical 10 years long-term outcome of microfracture vs. autologous chondrocyte implantation: a matched-pair analysis. Int Orthop 43(3):553–559
39. Outerbridge RE (1961) The etiology of chondromalacia patellae. J Bone Joint Surg Br 43B:752–757
40. Pak J (2011) Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: a case series. J Med Case Rep 5:296
41. Perdisa F, Gostyńska N, Filardo G, Marcacci M, Kon E (2015) Adipose-derived mesenchymal stem cells for the treatment of joint degeneration: a systematic review on preclinical and clinical evidence. Stem Cells Int 597652
42. Roseti L, Serra M, Tigani D, Brognara I, Lopriore A, Bassi A, Fornasari PM (2008) Cell manipulation in autologous chondrocyte implantation: from research to clean room. Chir Organi Mov 91(3):147–151
43. Russo A, Condello V, Madonna V, Guerriero M, Zorzi C (2017) Autologous and micro-fragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis. J Exp Orthop 4(1):33
44. Stolzing A, Jones E, McGonagle D, Scutt A (2008) Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. Mech Ageing Dev 129(3):163–173
45. Torre ML, Lucarelli E, Guidi S, Ferrari M, Alessandri G, De Girolamo L, Pessina A, Ferrero I (2015) Ex vivo expanded mesenchymal stromal cell minimal quality requirements for clinical application. Stem Cells Dev 24(6):677–685
46. Tremolada C, Colombo V, Ventura C (2016) Adipose tissue and mesenchymal stem cells: state of the art and Lipogems® technology development. Curr Stem Cell Rep 2:304–312
47. Van Pham P, Hong-Thien Bui K, Quoc Ngo D, Tan Khuat L, Kim Phan N (2013) Transplantation of nonexpanded adipose stromal vascular fraction and platelet-rich plasma for articular cartilage injury treatment in mice model. J Med Eng. https://doi.org/10.1155/2013/832396
48. Zeira O, Scaccia S, Pettinari L, Ghezzi E, Asiag N, Martinelli L, Zahirpour D, Dumas MP, Konar M, Lupi DM, Fiette L, Pascucci L, Leonardi L, Cliff A, Alessandri G, Pessina A, Spaziante D, Aralla M (2018) Intra-articular administration of autologous micro-fragmented adipose tissue in dogs with spontaneous osteoarthritis: safety, feasibility, and clinical outcomes. Stem Cells Transl Med 7(11):819–828