Knee Surgery, Sports Traumatology, Arthroscopy
https://doi.org/10.1007/s00167-019-05688-9

KNEE

Good mid-term outcomes after adipose-derived culture-expanded mesenchymal stem cells implantation in knee focal cartilage defects

Theofylaktos Kyriakidis[1] · Michael Iosifidis[2] · Efstathios Michalopoulos[3] · Ioannes Melas[2] · Catherine Stavropoulos-Giokas[3] · René Verdonk[1]

Received: 29 April 2019 / Accepted: 21 August 2019

Abstract

Purpose The purpose of the present study was to investigate the mid-term outcomes of a single-stage cell-based procedure in patients with knee focal symptomatic cartilage defects using matrix-induced culture-expanded autologous AD-MSCs. It was hypothesised that the increased number of autologous AD-MSCs after culture expansion is a safe and efficient cartilage repair procedure, which improves overtime chondrogenesis in cartilage lesions

Methods Twenty-five consecutive patients treated for a symptomatic cartilage defect were prospectively followed for 3 years. The median age of patients was 30.5 (range 16–43) with a median BMI of 23.6 kg/m^2 (range 19–29) and an average size of the lesion of 3.5 cm^2 (range 2–6). All patients underwent a single-stage procedure consisting in filling each defect with autologous culture-expanded mesenchymal stem cells embedded in a trimmed-to-fit commercially available biodegradable matrix. Pre-operative and post-operative evaluation included knee-related clinical and functional evaluation based on objective and subjective scores at 6, 12, 24 and 36 months and MRI evaluation of the repair tissue using the MOCART score at 12 and 24 months.

Results Clinical outcomes recorded significant improvements ($p < 0.05$) at the final follow-up compared with baseline as following: all subcategories of KOOS Score, the IKDC subjective from 40.9 (range 20.7–65.6) to 76.9 (range 42–90.3), Tegner Activity Score from 3 (range 2–4) to 4 (range 3–4), VAS for pain from 6 (range 4–8) to 1 (range 0–3). All patients improve significantly their IKDC objective scores. The MRI findings showed complete filling of the defect and integration to the border zone for 65% of the patients. Two patients underwent post-operative biopsies and the histological analysis demonstrated the presence of hyaline-like tissue.

Conclusions Adipose-derived culture-expanded mesenchymal stem cells were shown to be an efficient and safe single-stage cell-based procedure for symptomatic, full-thickness knee chondral lesions. The findings of the present study demonstrate that all patients presented significant mid-term clinical, functional and radiological improvement.

Level of evidence IV.

Keywords Chondral lesion · Adipose-derived stem cells · Cartilage repair · Regenerative medicine · Tissue engineering · Scaffolds · Cell culture expansion

Theofylaktos Kyriakidis and Michael Iosifidis have contributed equally to this article and share first authorship.

✉ Michael Iosifidis
info@orthobiologics.gr; iosfyl@icloud.com

[1] Department of Orthopaedic Surgery and Traumatology, Erasme University Hospital, Université Libre de Bruxelles, Route de Lennik 808, 1070 Brussels, Belgium

[2] OrthoBiology Surgery Center, Tsimiski 43, 54623 Thessaloníki, Greece

[3] Hellenic Cord Blood Bank, Biomedical Research Foundation Academy of Athens, 4 Soranou Ephessiou Street, 115 27 Athens, Greece

Introduction

Management of cartilage defects is challenging due to the limited self-repair capacity of chondral tissue. Hence, several treatment modalities have been used to enhance healing and promote regeneration. Biological applications have gained more attention generating conditions that allow natural healing and tissue rebuilding.

Brittberg et al. [3] introduced the first cell therapy known as autologous chondrocyte implantation (ACI). Several studies on ACI [18, 21, 25, 26] have reported good

Published online: 06 September 2019

mid- and long-term clinical results, while they have also successfully demonstrated the coverage of the defect with almost identical tissue to the original. The technique was further improved with the use of three-dimensional scaffolds again with good clinical results. Besides the successful outcomes of the previous techniques, their main disadvantages are the two-stage procedure and the dedifferentiation of the chondrocytes to a fibroblast-like phenotype during cultivation.

As a consequence, the research field was oriented to different approaches to overpass these limitations with principal interest in one-stage procedure. Mesenchymal stem cells (MSCs) hold considerable place in this direction due to their ability of self-renewal and plasticity [1]. Up to date, several clinical studies explored the efficacy of MSCs impregnated to scaffolds with encouraging results [9, 10].

Commonly, bone marrow-derived MSCs were used, either in the form of bone marrow aspirate concentrates or culture expanded. Moving towards to less invasive procedures, new cell sources were revealed, such as the adipose tissue. It was found to contain stem cells with equal potential differentiation to that of bone marrow. Additionally, its easy access and simple and reproducible isolation procedure are clear advantages [27].

Various studies have been conducted using adipose-derived mesenchymal stem cells (AD-MSCs) for knee osteoarthritis and concluded that they are safe, with good clinical and arthroscopic results [4, 14–16, 22, 23]. However, many of these studies prepared AD-MSCs in the form of the stromal vascular fraction (SVF). This rising concerns as SVF contains different proportions of AD-MSCs, and other components (e.g. pericytes, vascular adventitia cells, fibroblasts, preadipocytes, monocytes, macrophages and red blood cells). Subsequently, there is limited ability to evaluate only the stem cells efficacy, and in consequence these clinical studies cannot clarify the specific role of each SVF component [8, 11].

Existing literature lacks evidence on the effectiveness of AD-MSCs in the symptomatic chondral defects. Although its use is deemed to be promising, it has not yet been acknowledged as the main treatment option. Especially AD-MSCs can easily be harvested and, after culture expansion, both the high number and quality of the cells are guaranteed and would be possibly a stable and reproducible cell therapy for chondral tissue restoring.

In this regard, the purpose of the present study was to investigate the mid-term outcomes of a single-stage cell-based procedure in patients with knee focal symptomatic cartilage defects using matrix-induced culture-expanded autologous AD-MSCs. It was hypothesised that the increased number of autologous AD-MSCs after culture expansion is a safe and efficient cartilage repair procedure, which improves over time chondrogenesis in cartilage lesions.

Materials and methods

Between April 2013 and December 2015, 25 consecutive patients were treated with culture-expanded AD-MSCs embedded in a biodegradable scaffold for symptomatic full-thickness cartilage defects of the knee and prospectively followed at 6, 12, 24 and 36 months (range 36–62 months). The diagnosis was based on clinical and radiological features and confirmed during arthroscopy. All patients reported clinical semiology of pain, swelling, stiffness, clicking or locking. Coexisting lesions were treated at the same surgery. The inclusion and exclusion criteria are listed in Table 1, while demographic and defects characteristics are provided in Table 2. Preliminary results of a subgroup of this cohort have been reported

Table 1 Inclusion and exclusion criteria

Inclusion criteria
1. International Cartilage Repair Society (ICRS) grade 3 and 4 focal, symptomatic cartilage defects of the knee
2. Male and female gender between the age of 16 and 45
3. Cartilage defect > 2 cm^2
4. Body mass index (BMI) ≤ 30 kg/m^2)
5. Clinical symptoms of pain, swelling, stiffness, clicking or locking
6. Minimum of 3 years of follow-up
Exclusion criteria
1. Any previous knee operation within 6 months before screening
2. Varus or valgus malalignment exceeding 5°
3. Inflammatory joint disease, septic arthritis
4. Osteochondritis dissecans (OCD) and osteonecrosis
5. Previous cartilage repair procedure
6. Intra-articular injections (corticosteroid, hyaluronic acid, PRP, etc.) within 90 days before enrolment

Table 2 Patient's demographic and defects characteristics

Characteristics	Patient data
Sex	Female, $n = 10$ Male, $n = 15$
Age	30.5 years (range 16–43)
BMI	23.6 kg/m^2 (range 19–29)
Side	Right, $n = 15$ Left, $n = 10$
Location	MFC, $n = 9$ LFC, $n = 7$ Patella, $n = 5$ Trochlea, $n = 4$
ICRS classification	Grade III, $n = 9$ Grade IV, $n = 16$
Defect size	3.5 cm^2 (range 2–6)
Concomitant procedures	ACLR, $n = 10$

MFC medial femoral condyle, *LFC* lateral femoral condyle

previously. Neither complications nor treatment-related adverse events were observed.

Isolation of AD-MSCs

A quantity of almost 1 g of subcutaneous adipose tissue was harvested from the patient's hypogastric region by a small incision (1 cm) under local anaesthesia in the outpatient clinic as described previously. The tissue block was then placed in a sterile tube containing natural saline and was stored in an isothermal kit at about 4 °C and transferred to the laboratory for culture expansion. At the end of cultivation, following three passages, identification of the mesenchymal cells was established according to the criteria of the International Society for Cellular Therapy [7]. The characterisation of AD-MSCs was indicated by microscopic morphological check and specific surface antigen markers expression such as CD90, CD 29, CD73 and CD105 and expression lack of CD3, CD14, CD19, CD31, CD34, HLA DR, CD62 and CD45 as measured by flow cytometry.

Surgical procedure and implantation of AD-MSCs

All surgeries were performed by the same senior orthopaedic surgeon as described previously. More precisely, patients were placed in supine position under general anaesthesia with tourniquet application. Standard knee arthroscopy was conducted to estimate the characteristics of the defect, i.e. type, size, location and to deal with any coexisting injury. The cartilage repair procedure was then performed either arthroscopically or by mini-arthrotomy depending on the characteristics of each lesion. Chondral defects were prepared and templated to receive a trimmed-to-fit biodegradable three-dimensional matrix (Hyalofast® Anika Therapeutics, Inc.). The matrix was embedded with autologous culture-expanded adipose-derived stem cells just before its application on the defect.

Evaluation of outcomes

All patients were clinically evaluated at 6, 12, 24 and 36 months using two validated subjective knee questionnaires, the Injury and Osteoarthritis Outcome Score (KOOS) [2] and the International Knee Documentation Committee (IKDC) Score [5], the Tegner Activity Scale, the Visual Analogue Scale (VAS) for pain and the IKDC examination form. Magnetic resonance imaging (MRI) was performed pre-operatively and at 12 and 24 months post-operatively. Evaluation of the MR images with the Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) [20] Score was done by one independent radiologist reviewer (AM) in a blinded manner. Pre-operative MRI was used as the baseline for evaluation of treatment effectiveness. MR images at the final follow-up were available for the 20 out of the 25 patients included in the study. All patients accepted to follow the same rehabilitation protocol. This study was approved by the Bioethics Committee of the Aristotle University of Thessaloniki (ID number: 1.4.2013/34).

Statistical analysis

All data were analysed by an independent statistician. Statistical analysis was conducted using SPSS 24.0 (IBM Corp, Armonk, NY). For all the values, the median and range are provided. The Kolmogorov–Smirnov test was used to check if data were normally distributed. The paired *t* test for normally and the Wilcoxon signed-rank test for non-normally distributed data were performed to analyse outcomes between pre-operative and post-operative findings, respectively. *p* values less than 0.05 were considered statistically significant. An a priori power analysis was performed to demonstrate a difference in KOOS assessments of 10 points with an expected standard deviation of 10. A sample of 13 patients was found adequate. Power analysis was conducted using STATA 13 (StataCorp LP, College Station, TX). Radiological features were performed twice by the same observer with an interval of 6 weeks. The intra-observer reliability of these measurements was evaluated by an intraclass correlation coefficient. The intra-observer reliability was 0.96.

Results

Clinical, radiological and histological outcomes

Clinical outcomes assessed subjective and objective evaluation scores including the KOOS and the IKDC scores, the Tegner Activity Scale, the Visual Analogue Scale (VAS) for pain and the IKDC examination form. Patients presented significant improvement in all values at the final follow-up ($p < 0.05$) as shown in Tables 3 and 4.

Twenty patients performed MRI at 12 and 24 months after procedure. The analytical results of the MOCART evaluation are presented in Table 4.

Two patients underwent a further arthroscopic procedure for independent reasons to the initial surgery (meniscal tear and ACL reconstruction) and biopsies of the repair tissue were performed. Their results revealed the coverage of the defect by a hyaline-like cartilage tissue. No infection was recorded. Summary of all outcomes is demonstrated in Table 5.

Table 3 Evolution of the IKDC objective evaluation form

	Pre-operatively	6 months FU	12 months FU	24 months FU	36 months FU
A		6/25 (24%)	12/25 (48%)	17/25 (68%)	18/25 (72%)
B		6/25 (24%)	13/25 (52%)	8/25 (32%)	7/25 (28%)
C	13/25 (52%)	12/25 (48%)			
D	12/25 (48%)	1/25 (4%)			

Table 4 MRI evaluation (MOCART Score) of the repair tissue at 12 and 24 months after implantation

Variables	Characteristics	12 months	24 months
Degree of defect repair and filling of the defect	Complete	12/20 (60%)	13/20 (65%)
	Hypertrophy	4/20 (20%)	4/20 (20%)
	Incomplete > 50% of adjacent cartilage	4/20 (20%)	3/20 (15%)
	Incomplete < 50% of adjacent cartilage	0/20 (0%)	0/20 (0%)
	Subchondral bone exposed	0/20 (0%)	0/20 (0%)
Integration to border zone	Complete	13/20 (65%)	13/20 (65%)
	Demarcating border	5/20 (25%)	5/20 (25%)
	Defect visible		
	< 50% of the length of the repair tissue	2/20 (10%)	2/20 (10%)
	> 50% of the length of the repair tissue	0/20 (0%)	0/20 (0%)
Surface of repair tissue	Intact	10/20 (50%)	10/20 (50%)
	Damaged		
	< 50% of the repair tissue	10/20 (50%)	10/20 (50%)
	> 50% of the repair tissue	0/20 (0%)	0/20 (0%)
Adhesions	Yes	20/20 (100%)	20/20 (100%)
	No	0/20 (0%)	0/20 (0%)
Structure of the repair tissue	Homogeneous	10/20 (50%)	11/20 (55%)
	Heterogeneous	10/20 (50%)	9/20 (45%)
Signal Intensity of the repair tissue	Isointense	10/20 (50%)	10/20 (50%)
	Moderately hyper/hypo-intense	7/20 (35%)	8/20 (40%)
	Markedly hyper/hypo-intense	3/20 (15%)	2/20 (10%)
Subchondral lamina	Intact	0/20 (0%)	0/20 (0%)
	Not intact	20/20 (100%)	20/20 (100%)
Subchondral bone	Intact	2/20 (10%)	2/20 (10%)
	Not intact	18/20 (90%)	18/20 (90%)
Effusion	Yes	5/20 (25%)	2/20 (10%)
	No	15/20 (75%)	18/20 (90%)

Values presented as numbers of subjects and percentage

Discussion

The most important findings of this study were that implantation of culture-expanded AD-MSCs was found to be a safe and efficient single-stage cell-based procedure for symptomatic, full-thickness knee chondral lesions, with significant mid-term improvement of clinical, functional and radiological outcomes.

The results verified the hypothesis of the study and indicate that this approach can be considered among the alternative treatment strategies in cartilage repair procedures. To our knowledge, this is the first study analysing clinical, functional and radiological results after culture-expanded AD-MSCs implantation. Only, Jo et al. [12], reported so far outcomes from culture-expanded AD-MSCs but after intraarticular injection for osteoarthritic knees.

Mesenchymal stem cells were widely used in the last years to manage symptomatic cartilage defects of the knee. This approach aims to promote tissue regeneration, restoration of impaired function and viability of the clinical results. Furthermore, important points in the clinical use of the MSCs are the phenotypic stability [24] and the

Table 5 Summary of outcomes

Variables	Pre-operative	6 months FU	12 months FU	24 months FU	36 months FU	*p* value			
						6 months FU versus pre-operative	1 year FU versus 6 months FU	2 years FU versus 1 year FU	3 years FU versus 2 years FU
KOOS pain	64 (22–83)	78 (44–94)	86 (61–97)	94 (72–100)	97 (72–100)	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
KOOS symptoms	61.0 (29–89)	71 (61–94)	82 (64–100)	93 (71–100)	95 (71–100)	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
KOOS ADL	60 (15–90)	78 (46–97)	84 (62–100)	91 (65–100)	93 (65–100)	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
KOOS sports/rec	35 (0–60)	45 (15–85)	55 (25–85)	65 (35–90)	70 (40–95)	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
KOOS QOL	31 (0–69)	50 (0–81)	69 (13–88)	75 (31–94)	88 (44–100)	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
IKDC subjective	40.9 (20.7–65.6)	50.55 (33.3–89.7)	65.2 (34.5–89.7)	71.7 (36.8–89.2)	76.9 (42–90.3)	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Tegner activity	3 (2–4)	3 (2–4)	3 (2–4)	4 (3–4)	4 (3–4)	$p < 0.05$	$p < 0.05$	$p < 0.05$	NS
VAS pain	6 (4–8)	3 (2–5)	3 (1–4)	2 (1–4)	1 (0–3)	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
IKDC objective	13D/12C	12C/6A/6B/1D	12A/13B	17A/8B	18A/7B	$p < 0.05$	$p < 0.05$	$p < 0.05$	NS
MOCART			67.5 (50–85)	70 (55–85)				NS	

All outcome values are described as median and range

application of a biodegradable scaffold providing support for the cells and a chondroprotective coverage.

Several studies have been conducted using MSCs embedded in a scaffold and reported filling of the defect with hyaline cartilage-like tissue [13, 14, 24]. The majority of them used autologous bone marrow mesenchymal cells either in form of bone marrow aspirate concentrate or as culture expanded cells and reported good clinical, radiological and histological results [9, 10, 29].

However, the demanding harvesting procedure and the donor site morbidity shifted the research field to less invasive procedures and alternative cell sources. To this end, AD-MSCs were studied especially in the clinical field of osteoarthritis. Previous experience has demonstrated that the use of AD-MSCs has good clinical and arthroscopic results [4, 14–16, 22, 23]. So far, there is a lack in the existing literature to evaluate the efficacy of AD-MSCs in focal cartilage defects. Therefore, this study was conducted to explore this challenging topic.

A significant improvement in all the analysed clinical assessment tools from baseline to the latest follow-up was observed ($p < 0.05$). In detail, all the subscales of KOOS score as well as IKDC subjective score improved significantly.

Although the Tegner Activity Score improved statistically significant, it remained relatively low. Specifically, the initial level of three was unchangeable in the first and second evaluations at 6 and 12 months of follow-up, respectively. This finding could possibly be explained by the demanding and long-term rehabilitation program needed for cartilage repair procedures. Afterwards, an improvement of the score was detected reaching a final value of 4 at the last evaluation. Gobbi et al. [10] described different result relatively concerning the degree of improvement. More precisely, in their series, they detected a more obvious improvement of three levels more than baseline which could be a result of the different activity at the pre-operative level of the two cohorts. In fact, whereas Gobbi et al. included in their study only patients active in the athletic setting, and this study's cohort patients with limited sports interest were also included.

A high prevalence of ACL deficient knees was observed as well. Indeed, it is well documented that ligamentous insufficiency is often associated with cartilage defects [19]. All the concomitant procedures such as ACL reconstruction or partial meniscectomy was performed in the same surgery.

The MOCART system was used twice to evaluate the quality of the repair tissue after procedure. The median score increased from 67.5 at 12 months to 70 in 24 months after surgery but the difference was not statistically significant. Numerous other studies used the same evaluation system but the scores were expressed as a percentage of the maximum scores with higher percentage signifying better results. Regarding this evaluation method, complete filling of the repair tissue was observed in 65% of the patients which is slightly inferior to the results reported by Kon et al. [17] and Delcogliano et al. [6] who reported 70% and 80%, respectively. On the contrary, Verdonk et al. [28] reported complete filling of the defect in only 16%

of their patients. Another interesting finding concerns the subchondral bone evaluation. Indeed, in the present series, intact subchondral bone was observed in only 10% of the patients. This result is in accordance with Verdonk et al. [28] who reported 8.3% but in discordance with Kon et al. [17] who reported approximately 50% in their series. Moreover, complete integration was found in 13 patients (65%) which seems quite similar with the results reported by Kon et al. (70%) [17] and Delcogliano et al. (80%) [6]. In contrast, Gobbi et al. [10] reported 93.3% of complete integration with the adjacent cartilage but Verdonk et al. [28] only in 25% of their series. It is obvious that more studies are needed to clarify these conflicting results and help understanding the reliability and the reproducibility of the evaluation system.

There are also some limitations to be considered in the current study. This study is a case series without comparison group and lack of randomisation that predisposes to selection bias. However, there are significant contributions to the discourse of using matrix-induced autologous mesenchymal stem cells for the repair of knee cartilage defects in a single-stage procedure. First, adipose tissue appears to be the preferable source of MSCs due to the easy harvesting procedure, and the large number of stem cells per gram. Second, the culture expansion of the mesenchymal stem cells offers higher number of cells to achieve better chondrogenesis. Another limitation of the study is that there is a variety of cartilage defects site and size (e.g. femoral condyles, patellofemoral, both III and IV grades); however, this is usual in knee cartilage cohorts' series. Moreover, post-operative MRI was performed in 20 out of 25 patients in the first 2 years after procedure. Additionally, the evaluation of the repair tissue was performed only by one observer. Finally, histological evaluation was obtained only in two cases, during operations for unrelated to the initial procedure pathology, as second look arthroscopy only for biopsies is not obviously a regular procedure.

Conclusion

The hypothesis of the present study that autologous AD-MSCs after culture expansion, embedded to a biodegradable scaffold will improve chondrogenesis in a mid-term follow-up period was confirmed. There was significant clinical, functional and radiological improvement of all patients. AD-MSCs implantation was shown to be an efficient and stable over time single-stage cell-based procedure for symptomatic, full-thickness knee chondral lesions.

Acknowledgements The authors are grateful to the radiologist Afarine Madani MD, PhD for her contribution in MR images evaluation.

Author contributions TK: data collection, writing and critical review of the paper; IM: performed surgeries, data collection, writing and critical review of the paper; EM: performed culture isolation; IM: data collection, writing the paper; CSG: performed culture isolation; RV: critical review of the paper; TK and MI have contributed equally to this article and share first authorship.

Funding The authors received no financial support for the research, authorship and/or publication of this article.

Compliance with ethical standards

Conflict of interest The authors declared no potential conflict of interest with respect to the research, authorship and/or publication of this article.

Ethical approval Ethical approval for this study was approved by the Bioethics Committee of the Aristotle University of Thessaloniki (1.4.2013/34).

Informed consent Written informed consent was obtained from all subjects before the study.

References

1. Barry FP, Murphy JM (2004) Mesenchymal stem cells: clinical applications and biological characterization. Int J Biochem Cell Biol 36:568–584
2. Bekkers JE, de Windt TS, Raijmakers NJ, Dhert WJ, Saris DB (2009) Validation of the Knee Injury and Osteoarthritis Outcome Score (KOOS) for the treatment of focal cartilage lesions. Osteoarthr Cartil 17:1434–1439
3. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L (1994) Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 331:889–895
4. Bui K, Duong T, Nguyen N, Nguyen T, Le V, Mai V, Phan N, Le D, Phan N, Pham P (2014) Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet- rich plasma: a clinical study. Biomed Res Ther 1:2–8
5. Collins NJ, Misra D, Felson DT, Crossley KM, Roos EM (2011) Measures of knee function: International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Form, Knee Injury and Osteoarthritis Outcome Score (KOOS), Knee Injury and Osteoarthritis Outcome Score Physical Function Short Form (KOOS-PS), Knee Outcome Survey Activities of Daily Living Scale (KOS-ADL), Lysholm Knee Scoring Scale, Oxford Knee Score (OKS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Activity Rating Scale (ARS), and Tegner Activity Score (TAS). Arthritis Care Res (Hoboken) 63(Suppl 11):S208–S228
6. Delcogliano M, de Caro F, Scaravella E, Ziveri G, De Biase CF, Marotta D et al (2014) Use of innovative biomimetic scaffold in the treatment for large osteochondral lesions of the knee. Knee Surg Sports Traumatol Arthrosc 22:1260–1269
7. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D et al (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315–317
8. Freitag J, Bates D, Wickham J, Shah K, Huguenin L, Tenen A et al (2019) Adipose-derived mesenchymal stem cell therapy in

the treatment of knee osteoarthritis: a randomized controlled trial. Regen Med 14:213–230
9. Gobbi A, Karnatzikos G, Sankineani SR (2014) One-step surgery with multipotent stem cells for the treatment of large full-thickness chondral defects of the knee. Am J Sports Med 42:648–657
10. Gobbi A, Karnatzikos G, Scotti C, Mahajan V, Mazzucco L, Grigolo B (2011) One-step cartilage repair with bone marrow aspirate concentrated cells and collagen matrix in full-Thickness Knee cartilage lesions: results at 2-year follow-up. Cartilage 2:286–299
11. Hurley ET, Yasui Y, Gianakos AL, Seow D, Shimozono Y, Kerkhoffs G et al (2018) Limited evidence for adipose-derived stem cell therapy on the treatment of osteoarthritis. Knee Surg Sports Traumatol Arthrosc 26:3499–3507
12. Jo CH, Lee YG, Shin WH, Kim H, Chai JW, Jeong EC et al (2014) Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. Stem Cells 32:1254–1266
13. Kim YS, Choi YJ, Suh DS, Heo DB, Kim YI, Ryu JS et al (2015) Mesenchymal stem cell implantation in osteoarthritic knees: is fibrin glue effective as a scaffold? Am J Sports Med 43:176–185
14. 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 42:1628–1637
15. Koh YG, Choi YJ, Kwon SK, Kim YS, Yeo JE (2015) Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis. Knee Surg Sports Traumatol Arthrosc 23:1308–1316
16. 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 30:1453–1460
17. Kon E, Delcogliano M, Filardo G, Busacca M, Di Martino A, Marcacci M (2011) Novel nano-composite multilayered biomaterial for osteochondral regeneration: a pilot clinical trial. Am J Sports Med 39:1180–1190
18. Kraeutler MJ, Belk JW, Purcell JM, McCarty EC (2018) Microfracture versus autologous chondrocyte implantation for articular cartilage lesions in the knee: a systematic review of 5-year outcomes. Am J Sports Med 46:995–999
19. Maffulli N, Binfield PM, King JB (2003) Articular cartilage lesions in the symptomatic anterior cruciate ligament-deficient knee. Arthroscopy 19:685–690
20. 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 57:16–23
21. Oussedik S, Tsitskaris K, Parker D (2015) Treatment of articular cartilage lesions of the knee by microfracture or autologous chondrocyte implantation: a systematic review. Arthroscopy 31:732–744
22. Pak J, Chang JJ, Lee JH, Lee SH (2013) Safety reporting on implantation of autologous adipose tissue-derived stem cells with platelet-rich plasma into human articular joints. BMC Musculoskelet Disord 14:337
23. Pak J, Lee JH, Park KS, Jeong BC, Lee SH (2016) Regeneration of cartilage in human knee osteoarthritis with autologous adipose tissue-derived stem cells and autologous extracellular matrix. Biores Open Access 5:192–200
24. Pelttari K, Steck E, Richter W (2008) The use of mesenchymal stem cells for chondrogenesis. Injury 39(Suppl 1):S58–S65
25. Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A (2000) Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. Clin Orthop Relat Res 374:212–234
26. Peterson L, Vasiliadis HS, Brittberg M, Lindahl A (2010) Autologous chondrocyte implantation: a long-term follow-up. Am J Sports Med 38:1117–1124
27. Schaffler A, Buchler C (2007) Concise review: adipose tissue-derived stromal cells—basic and clinical implications for novel cell-based therapies. Stem Cells 25:818–827
28. Verdonk P, Dhollander A, Almqvist KF, Verdonk R, Victor J (2015) Treatment of osteochondral lesions in the knee using a cell-free scaffold. Bone Jt J 97:318–323
29. Wakitani S, Nawata M, Tensho K, Okabe T, Machida H, Ohgushi H (2007) Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees. J Tissue Eng Regen Med 1:74–79