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ORIGINAL STUDY

Matrix-induced adipose-derived mesenchymal stem cells implantation for knee articular cartilage repair. Two years follow-up

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The present study aims to evaluate the efficacy of matrix-induced adipose-derived mesenchymal stem cells (Ad-MSCs) for cartilage repair of focal chondral knee lesions.

Twenty patients were initially treated for symptomatic full-thickness chondral defects and then prospectively followed for two years. 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. Knee-related function was evaluated based on subjective scores given by two self-reported questionnaires (KOOS and IKDC).

Data analysis shows significant improvements ($p<0.001$) in all values. The mean preoperative scores in the subscales of KOOS, as well as in the IKDC subjective score were constantly increased during the follow-up period with statistically significant differences at 6, 12 and 24 months follow-up.

The findings of this study indicate that matrix-induced adipose-derived mesenchymal stem cells implantation is an effective and safe single-staged cell-based procedure to manage full-thickness focal chondral lesions of the knee.

Keywords : chondral lesion ; adipose-derived stem cells ; cartilage repair ; regenerative medicine.

INTRODUCTION

Articular cartilage defects occur frequently and represent common findings among knee arthroscopic procedures *(1,13,40,42)*. If left untreated they can lead to several morbidities, joint dysfunction and eventually to osteoarthritis *(5)*. Patients with symptomatic chondral lesions describe a variety of symptoms such as pain, swelling, stiffness,

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clicking or locking. The management of these lesions remains challenging due to the limited healing capacity of the tissue *(8,14)*. Hence, various repair strategies have been proposed in the literature including debridement, bone marrow-stimulation, cells-based, cells plus scaffold-based and whole tissue transplantation techniques *(43)*.

In the last two decades, the biological applications have become more and more commonplace as a treatment option for cartilage defects in order to enhance healing and promote regeneration. Indeed, regenerative medicine stands by the natural healing in the tissue reconstruction, by generating conditions that promote tissue rebuilding in order to restore impaired function *(25)*.

The first clinical application of cell therapy procedures, that provided cartilage regeneration with tissue resembling to the original, was the autologous chondrocyte implantation (ACI). Besides its successful clinical results *(37)*, it presented various disadvantages such as the double stage procedure, in vitro dedifferentiation of the chondrocytes to a fibroblast-like phenotype during cultivation, delamination, periosteal hypertrophy, donor site morbidity as well as the poor integration of the repair tissue *(23,27,29)*. To surpass some of the difficulties related to the technique, the periosteum was replaced by a membrane-scaffold (second generation of ACI) and then the chondrocytes embedded in a scaffold (matrix-induced autologous chondrocyte implantation/ MACI, ACI third generation) *(6,20)*.

Nowadays, the research field is oriented to different treatments and novel technologies in order to overcome the disadvantages of the previously used methods. Mesenchymal stem cells (MSCs) combined with biomaterials keep great promise in this procedure. MSCs are defined as multipotent cells derived from various human tissues, including bone marrow, adipose tissue, peripheral blood and synovium. By definition, stem cells are characterized by their ability to self-renew and due to their developmental plasticity are able to differentiate into specific therapeutic cell types *(2)*. The MSCs value for cartilage repair was recorded since the early years of their use, in bone marrow stimulation techniques such as the microfractures. The penetration of subchondral bone gave way for these cells to come to the defect's site and play key role in fibrocartilage formation *(3)*.

Meanwhile, numerous scaffolds exist for clinical use, produced by a variety of materials including natural and synthetic polymers or their composites. Special requirements are needed as well, such as biocompatibility, biodegrability and mechanical properties. Scaffolds operate as an artificial extracellular matrix, mimicking the structure and function of the native extracellular matrix to physically guide or chemically inform cell response and thus promote tissue growth *(30)*.

Up to date several clinical studies have been established in the clinical field of cartilage repair exploring the use of scaffolds impregnated with MSCs delivering some promising results. During the last decade, scaffolds were used after bone marrow stimulation techniques to cover the lesion's site and improve the possible chondrogenesis from the secreted MSCs (Autologous Matrix Induced Chondrogenesis/ AMIC). However, the number of cells may have not been enough. Therefore, more recent studies used bone marrow-derived MSCs mostly from iliac crest spine, either as culture-expanded bone marrow derived MSCs or bone marrow aspirate concentrates *(11,24,41)*. Nevertheless the latter encloses limited quantity of mesenchymal stem cells *(39)*.

Towards a less invasive procedure, the research is directed to alternative sources of MSCs, for instance, adipose derived mesenchymal cells. The latter, compared to bone marrow-derived, were found to have an equal potential to differentiate into cells and tissue. However, due to the easy and repeatable access to subcutaneous adipose tissue as well as its simple isolation procedure it does provide a clear advantage *(38)*.

The purpose of this study was to evaluate the efficacy of matrix-induced autologous adipose-derived mesenchymal stem cells in the cartilage defects of the knee, having as hypothesis that all patients would improve clinically.

MATERIALS AND METHODS

A total of twenty consecutive patients were treated and prospectively followed for two years in

this pilot study between April 2013 and December 2014. The diagnosis was based on clinical and radiological features. Patients reported clinical semiology of pain, swelling, stiffness, clicking or locking.

Inclusion criteria comprise subjects (male or female) between the age of 16 and 45; body mass index (BMI) ≤ 30 kg/m2; focal International Cartilage Repair Society (ICRS) grade IV cartilage defects of the knee diagnosed on preoperative magnetic resonance imaging (MRI); cartilage defect size >1cm2. The exclusion criteria consisted of any previous knee operation within the last 6 months before screening; varus or valgus malalignment exceeding 5°; inflammatory joint disease, rheumatoid or septic arthritis; osteochondritis dissecans (OCD); osteonecrosis; previous cartilage repair procedure; intra-articular injections (corticosteroid, Hyaluronic Acid, PRP, etc.) within 90 days before enrolment; contraindications to magnetic resonance imaging.

All the patients were followed-up at the same post-operative intervals (6 months, 12 months and 24 months). Functional evaluation was performed with two validated subjective knee questionnaires, the Injury & Osteoarthritis Outcome Score (KOOS) *(4,28)* and the International Knee Documentation Committee (IKDC) subjective score *(9,21)* while all included patients accepted to follow a specific rehabilitation protocol.

SURGICAL PROCEDURE : ISOLATION AND IMPLANTATION OF AD-MSCs

Approximately 1 mg of subcutaneous adipose tissue was harvested from the patient's hypogastric region by a small incision under local anaesthesia in the outpatient clinic. The tissue block was then placed in a sterile tube containing saline solution and it was stored in an isothermal kit at about 4° C. The adipose tissue was then transported to the laboratory where cells were isolated and cultivation lasted for approximately 40 days. Identification of the mesenchymal cells was established according to the criteria of the International Society for Cellular Therapy *(10)*. The characterization of adipose-derived mesenchymal cells was indicated by microscopic morphological check and specific surface antigen expression such as CD90, CD29, CD73 and CD105 markers and expression lack of CD3, CD14, CD19, CD31, CD34, HLA DR, CD62 and CD45 markers as measured by flow cytometry.

All the procedures were performed by a senior surgeon with extensive experience in cartilage repair. General anaesthesia with tourniquet application as well as the standard sterile preparation and draping were systematically used. Common antibiotic prophylaxis was also administrated. Patients were placed in supine position, giving the possibility to perform hyperflexion of the knee (120°). An initial arthroscopic evaluation was conducted to estimate the type, size and location of the defect and to cope with any concomitant intraarticular injury such as meniscal or ACL tears. The cartilage repair was performed either arthroscopically or by mini arthrotomy, depending on the characteristics of the defect. Accordingly, the chondral defect was prepared by removing all the damaged tissue using shaver and curettes. The debridement completed to a stable cartilage margin in order to fit better the membrane. The calcified layer was equally removed without penetrating the subchondral bone. Next, the prepared defect was templated and covered with autologous culture-expanded mesenchymal stem cells embedded in a trimmed-to-fit biodegradable matrix (Hyalofast® Anika Therapeutics, Inc.). Finally, the stability of the implanted matrix was tested intraoperatively during intense knee mobilization.

STATISTICAL ANALYSIS

Data were analyzed by an independent statistician. Power analysis was performed using STATA 13 (StataCorp LP, College Station, TX) for sample size estimation to demonstrate a significant difference in KOOS assessments of 10 points with an expected standard deviation of 10. To satisfy, a power of 90% for detecting this difference at the 5% level of significance a sample size of 13 was found adequate. Generalized estimating equations (GEE) models for linear regression with Fisher's Least Significant Difference (LSD) post-hoc tests were used for the analysis of the questionnaires KOOS and IKDC to examine the difference in scores at

pre-operation and during the three follow-up time points. P-values less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS 24.0 (IBM Corp, Armonk, NY).

RESULTS

In total 20 patients were included (10 males and 10 females) in this study with a minimum follow-up of 2 years. The mean age of the study population was 31.10 years (range 16-43) with a mean BMI of 24.53 ± 3.34 kg/m2. Cartilage defects were classified as ICRS grade 4 lesions according to preoperative MRI and verified during arthroscopy. Eleven lesions were located on the right knee whilst nine on the left. The majority of the lesions involved the medial femoral condyle (55%), followed by the lateral femoral condyle (30%), the patella (10%) and the trochlea (5%). Nine patients presented a concomitant anterior cruciate ligament tear with or without associate meniscal injury and they were treated at the same surgical time. The detailed population's demographic characteristics, lesion location, as well as associated procedures are provided in Table I. The data analysis recorded a significant improvement ($p < 0.001$) in all the values as shown in Table II. Overall, the subjective IKDC score obtained preoperatively increased constantly during all the follow-up period. More precisely as shown in Figure 1, the mean preoperative score of 44.33 ± 2.97 increased to 54.57 ± 3.49 at 6 months, before improving to 63.30 ± 3.39 at 12 months and then to 70.05 ± 3.12 at 24 months after the procedure. Similarly, the subscales of KOOS score at the final follow-up presented significant differences compared to their preoperative values ($p<0.001$). In particular, the average preoperative KOOS-Pain of

Table I. — Patients Demographic Characteristics, Location of the lesions and associated procedures

Patients	Sex	Age	Side	BMI	Location	Associated Procedures
1	Female	40	Right	29	Patella	None
2	Female	32	Right	23	Medial Femoral Condyle	None
3	Female	42	Right	26	Medial Femoral Condyle	None
4	Female	40	Right	20	Lateral Femoral Condyle	None
5	Male	26	Left	23	Lateral Femoral Condyle	ACLR
6	Male	27	Left	30	Lateral Femoral Condyle	ACLR
7	Female	16	Right	23	Lateral Femoral Condyle	ACLR + Meniscectomy
8	Male	22	Right	29	Medial Femoral Condyle	ACLR + Meniscectomy
9	Female	28	Left	22	Lateral Femoral Condyle	ACLR
10	Male	25	Right	24	Medial Femoral Condyle	None
11	Male	20	Left	20	Medial Femoral Condyle	ACLR
12	Female	43	Right	26	Medial Femoral Condyle	None
13	Male	40	Right	25	Lateral Femoral Condyle	ACLR + Meniscectomy
14	Male	34	Right	22	Medial Femoral Condyle	ACLR
15	Male	20	Left	21	Patella	None
16	Female	38	Right	25	Medial Femoral Condyle	None
17	Male	36	Left	30	Trochlea	None
18	Female	39	Left	23	Medial Femoral Condyle	None
19	Female	31	Left	21	Medial Femoral Condyle	ACLR
20	Male	23	Right	27	Medial Femoral Condyle	None

Table II. — Summary of Clinical Outcomes

Outcomes	Preoperative	6-months Follow-Up	1-year Follow-Up	2-years Follow-Up	P- value		
					Preoperative versus 2y FU	6m FU versus 1y FU	1y FU versus 2y FU
KOOS pain	58.75 ± 3.99	76.66 ± 3.00	86.52 ± 1.96	92.50 ± 1.62	p<0.001	p<0.001	p<0.001
KOOS symptoms	62.67 ± 3.53	76.07 ± 2.20	84.64 ± 2.11	91.42 ± 1.62	p<0.001	p<0.001	p<0.001
KOOS ADL	60.51 ± 5.17	78.23 ± 3.25	84.63 ± 2.41	91.25 ± 1.95	p<0.001	p<0.001	p<0.001
KOOS Sports/Rec	33.50 ± 4.24	48.75 ± 4.12	57.50 ± 4.20	64.75 ± 3.88	p<0.001	p<0.001	p<0.001
KOOS QOL	27.50 ± 3.19	45.62 ± 4.05	58.75 ± 4.15	69.68 ± 3.83	p<0.001	p<0.001	p<0.001
IKDC Subjective	44.33 ± 2.97	54.57 ± 3.49	63.30 ± 3.39	70.05 ± 3.12	p<0.001	p<0.001	p<0.001

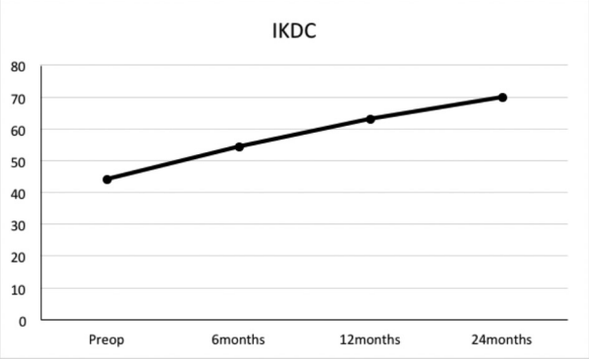

Fig. 1. — Evolution of the IKDC score from preoperative to 6, 12, and 24 months

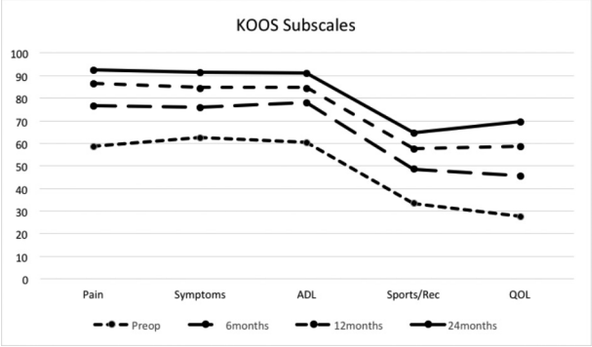

Fig. 2. — Evolution of the KOOS subgroups from preoperative to 6, 12, and 24 months

58.75 ± 3.99 increased to 76.66 ± 3.00 at 6 months, to 86.52 ± 1.96 and to 92.50 ± 1.62 at 1 year and 2 years after the surgery respectively. The final mean score of KOOS-Symptoms was significantly higher as well. The initial value of 62.67 ± 3.53 increased to 76.07 ± 2.20 at 6 months, to 84.64 ± 2.11 at 12 months, before achieving the final score of 91.42 ± 1.62 at 24 months review. With respect to the KOOS-ADL, again a statistically significant difference was found with an increase of the preoperative score of 60.51 ± 5.17 to 91.25 ± 1.95 after 24 months while the scores of 78.23 ± 3.25 and 84.63 ± 2.41 were found at 6 and 12 months of follow-up respectively. Additionally, the KOOS Sports/Recreation of 33.50 ± 4.24 was improved to 48.75 ± 4.12 at 6 months, while further increased to 57.50 ± 4.20 at 12 months and finally to 64.75 ± 3.88 at 2 years review. Lastly, the KOOS QOL of 27.50 ± 3.19 was found significantly higher after 24 months, reaching the mean of 69.68 ± 3.83. The mean values at 6 and 12 months rise to 45.62 ± 4.05 and 58.75 ± 4.15 respectively as illustrated in Figure 2. Neither treatment-related adverse events nor complications were observed.

DISCUSSION

The main finding of the present study was that culture-expanded adipose derived MSCs embedded in a trimmed-to-fit biodegradable matrix is an effective and safe procedure to manage full-thickness focal chondral lesions of the knee, while it also improves function outcomes at two years follow-up.

The application of evidence-based medicine to manage focal cartilage defects of the knee can be complex not only due to the variety in the conditions and pathologies included, but also to

the numerous treatment options. The increasing interest and the development of new biologic repair techniques is moving towards to more effective procedures with better results. However, extended research is still required to validate the collected data and to propose a decisional algorithm.

Several studies have explored the efficacy of cell-based tissue engineering approaches. These procedures target the rebuilding of hyaline-like tissue, in order to restore impaired function and durable clinical results. It has been documented that autologous chondrocyte implantation (ACI) seems to be more efficient than microfractures, especially in large cartilage defects with good to excellent long-term outcomes *(20,22,31,36)*. However, it is a demanding two stage procedure, hence not cost-effective, associated with donor site morbidity and limited number of harvested chondrocytes. To improve the outcomes of this procedure, the matrix assisted ACI was introduced. The application of scaffolds has a clear benefit to surpass some of the technical difficulties related to the first generation of ACI and in the meantime, it shows adequate clinical results. Nevertheless, the associated disadvantages as the dedifferentiation to fibroblast-like phenotype as well as the inadequate distribution of the chondrocytes *(3,37)* raise some doubts concerning its results. These technical difficulties shifted the treatment towards other cells-based procedures and especially to one stage surgery.

Mesenchymal stem cells hold to fulfil this process. Several studies suggested that the repair of osteochondral defects may be enhanced by implanting cultured MSCs and demonstrated that MSCs embedded in a matrix and then placed into a full-thickness cartilage defect show a hyaline cartilage-like histology *(16,17,35)*.

In the clinical application of MSCs for cartilage repair, the guarantee of phenotypic stability and functional suitability is a critical point *(35)*. Indeed, scaffolds are crucial as they act as a biodegradable support for the cells.

Wakitani et al. *(41)*, for instance, investigated the efficacy of autologous culture-expanded bone marrow mesenchymal cell transplantation for repairing articular cartilage defects. Six months after transplantation, the patients' clinical symptoms improved while the improvements were further maintained through the follow-up periods (17-27 months). They concluded that autologous bone marrow mesenchymal cells transplantation may be an effective approach to promote the repair of articular cartilage defects.

Kuroda et al. *(24)* studied the effectiveness of autologous bone-marrow stromal cells, which were embedded within a collagen scaffold to repair a full-thickness articular cartilage defect in the medial femoral condyle of an athlete. One year after surgery, the clinical symptoms had improved significantly and the patient retained his previous activity level without pain or other complications. Therefore, the study concluded that autologous bone-marrow stromal cells can promote the repair of large focal articular cartilage defects in young and active patients.

Gobbi et al. *(12)* explored the results of cartilage repair utilizing one step surgery with bone marrow aspirate concentrate (BMAC) and a collagen matrix. Fifteen patients operated for grade IV cartilage lesions of the knee were prospectively followed up for 2 years. All the patients showed significant improvement in all the scores at the final follow-up ($p < 0.005$). Later, the same authors investigated *(11)*, the clinical outcome in a group of active patients with large full-thickness chondral defects of the knee who were also treated with one step surgery using bone marrow-derived MSCs and a second-generation matrix. The average preoperative values for the evaluated scores were significantly improved at final follow-up ($p < 0.001$). Considering their findings, concluded that treatment of large chondral defects with MSCs is an effective procedure and can be performed routinely in clinical practice. Moreover, it can be achieved with one-step surgery, avoiding a previous surgical procedure to harvest cartilage and subsequent chondrocyte cultivation.

In light of new potentials, this study investigated an alternative source of MSCs. Here, we used culture-expanded adipose derived MSCs loaded in a sterile, non-woven, biodegradable hyaluronic acid-based scaffold (Hyalofast® Anika Therapeutics, Inc.) which was used for the entrapment of the mesenchymal stem cells (MSCs) and we observed a significant improvement in all the analyzed clinical

assessment tools from baseline to the latest follow-up ($p < 0.001$).

Specifically, the IKDC subjective score and the subscales of KOOS score were constantly increased through the time at 6, 12 and 24 months with statistically significant differences in all the reviews. These promising clinical outcomes were obtained in a very demanding group of still young and active patients. Additionally, the data confirm that patients with knee laxity present often associate cartilage defects as already described in previous studies *(26)*.

To our knowledge, this study is novel since it is the first prospective in vivo report of a single stage procedure using cultured adipose-derived mesenchymal stem cells impregnated in a 3-dimensional matrix to manage symptomatic focal knee defects.

Up to date, there is no available data focusing on the same source of MSCs to treat focal lesions. On the contrary, several studies were conducted in the field of osteoarthritis and concluded that adipose derived mesenchymal stem cells are safe, with good clinical and arthroscopic results *(7,17,18,19,32-34)*. The majority of these studies used isolate direct intra-articular injection of adipose derived MSCs in form of stromal vascular fraction (SVF) or combined with PRP, and several were performed under arthroscopic guidance. As far as we aware there is only one study that used injections of culture-expanded adipose derived MSCs via arthroscopy. Jo et al. *(15)*, in a Phase I and II clinical trial, reported that intra-articular injection of culture-expanded adipose derived MSCs into the osteoarthritic knee improved function and pain of the knee joint without causing adverse events, while it also reduces cartilage defects by regeneration of hyaline-like articular cartilage.

The strongest point of this study was probably the harvesting of autologous adipose mesenchymal stem cells. Nowadays, adipose tissue appears to be the preferable source of MSCs due to the easy and repeatable access to the subcutaneous tissue, in addition to the large number of stem cells per gram. Another strong point of the study was the culture expansion of the mesenchymal stem cells in order to achieve a higher number of cells. In addition, the prospective character of the study and the collection of the data as well as the single surgeon were also considered as strengths of the study.

This study has also a number of limitations. It was a case series study without randomization, subject to selection bias. Consequently, its results cannot be generalized to the wider population. However, it could be assumed that cartilage lesions cannot be easily collected in large cohorts for prospective studies, due to their large variety in anatomical site, size and degree as already noted previously. The repair tissue was not evaluated with specific histological criteria. However, ethical and practical reasons do not allow a systematic second look arthroscopy and biopsies of the treated lesions. Finally, the study follow-up period is, limited hence the results need to be confirmed by long-term follow-up studies.

CONCLUSION

In conclusion, this study confirmed the hypothesis that adipose-derived mesenchymal stem cells combined with a biodegradable scaffold is an efficient and safe single-staged cell-based procedure to achieve good clinical outcomes in young patients with symptomatic focal defects of the knee.

REFERENCES

1. **Aroen A, Loken S, Heir S** *et al.* Articular cartilage lesions in 993 consecutive knee arthroscopies. *Am J Sports Med.* 2004 ; 32 : 211-5.
2. **Barry P, Murphy M.** Mesenchymal stem cells: clinical applications and biological characterization. *Int J Biochem Cell Biol.* 2004 ; 36 : 568-84.
3. **Behrens P, Bitter T, Kurz B, Russlies M.** Matrix-associated autologous chondrocyte transplantation/implantation (MACT/MACI)--5-year follow-up. *Knee.* 2006 ; 13 : 194-202.
4. **Bekkers E, de Windt S, Raijmakers N** *et al.* Validation of the Knee Injury and Osteoarthritis Outcome Score (KOOS) for the treatment of focal cartilage lesions. *Osteoarthritis Cartilage.* 2009 ; 17 : 1434-9.
5. **Bhosale A, Richardson J.** Articular cartilage: structure, injuries and review of management. *Br Med Bull.* 2008 ; 87 : 77-95.
6. **Brittberg M.** Cell carriers as the next generation of cell therapy for cartilage repair: a review of the matrix-induced

autologous chondrocyte implantation procedure. *Am J Sports Med.* 2010 ; 38 : 1259-71.

7. **Bui K, Duong, T., Nguyen, N** *et al.* Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet- rich plasma: a clinical study. *Biomedical Research And Therapy.* 2014 ; 1 : 2-8.
8. **Chen F, Frenkel S, Di Cesare P.** Repair of articular cartilage defects: part I. Basic Science of cartilage healing. *Am J Orthop* (Belle Mead NJ). 1999 ; 28 : 31-3.
9. **Collins NJ, Misra D, Felson D** *et al.* 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). 2011 ; 63 : S208-28.
10. **Dominici M, Le Blanc K, Mueller I** *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* 2006 ; 8 : 315-7.
11. **Gobbi A, Karnatzikos G, Sankineani S.** One-step surgery with multipotent stem cells for the treatment of large full-thickness chondral defects of the knee. *Am J Sports Med.* 2014 ; 42 : 648-57.
12. **Gobbi A, Karnatzikos G, Scotti C** *et al.* 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.* 2011 ; 2 : 286-99.
13. **Hjelle K, Solheim E, Strand T** *et al*. Articular cartilage defects in 1,000 knee arthroscopies. *Arthroscopy.* 2002 ; 18 : 730-4.
14. **Hunter W.** Of the structure and disease of articulating cartilages. 1743. *Clin Orthop Relat Res.* 1995 : 3-6.
15. **Jo C, Lee Y, Shin W** *et al.* Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. *Stem Cells.* 2014 ; 32 : 1254-66.
16. **Kim Y, Choi Y, Suhet** *et al.* Mesenchymal stem cell implantation in osteoarthritic knees: is fibrin glue effective as a scaffold? *Am J Sports Med.* 2015 ; 43 : 176-85.
17. **Koh Y, Choi Y, Kwon O, Kim Y.** Second-Look Arthroscopic Evaluation of Cartilage Lesions After Mesenchymal Stem Cell Implantation in Osteoarthritic Knees. *Am J Sports Med.* 2014 ; 42 : 1628-37.
18. **Koh Y, Choi Y, Kwon S** *et al.* Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis. *Knee Surg Sports Traumatol Arthrosc.* 2015 ; 23 : 1308-16.
19. **Koh YG, Kwon OR, Kim YS, Choi YJ.** 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.* 2014 ; 30 : 1453-60.
20. **Kon E, Filardo G, Di Martino A, Marcacci M.** ACI and MACI. *J Knee Surg.* 2012 ; 25 : 17-22.
21. **Koumantakis G, Tsoligkas K, Papoutsidakis A** *et al.* Cross-cultural adaptation and validation of the International Knee Documentation Committee Subjective Knee Form in Greek. *J Orthop Traumatol.* 2016 ; 17 : 123-9.
22. **Kraeutler M, Belk J, Purcell J, McCarty E.** Microfracture Versus Autologous Chondrocyte Implantation for Articular Cartilage Lesions in the Knee: A Systematic Review of 5-Year Outcomes. *Am J Sports Med.* 2017 : 46 ; 995-999.
23. **Kreuz P, Steinwachs M, Erggelet C** *et al.* Classification of graft hypertrophy after autologous chondrocyte implantation of full-thickness chondral defects in the knee. *Osteoarthritis Cartilage.* 2007 ; 15 : 1339-47.
24. **Kuroda R, Ishida K, Matsumoto T** *et al.* Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. *Osteoarthritis Cartilage.* 2007 ; 15 : 226-31.
25. **Kyriakidis T, Verdonk R, Verdonk P.** Current Concepts in Natural History of Meniscal Injury and Future Options in Meniscus Healing: Orthobiologics. In: Gobbi A, Espregueira-Mendes J, Lane J, Karahan M, editors. *Bio-orthopaedics - A New Approach*: Springer. 2017 ; 339-53.
26. **Lohmander L, Englund P, Dahl L, Roos E.** The long-term consequence of anterior cruciate ligament and meniscus injuries: osteoarthritis. *Am J Sports Med.* 2007 ; 35 : 1756-69.
27. **Marquass B, Schulz R, Hepp P** *et al.* Matrix-associated implantation of predifferentiated mesenchymal stem cells versus articular chondrocytes: in vivo results of cartilage repair after 1 year. *Am J Sports Med.* 2011 ; 39 : 1401-12.
28. **Moutzouri M, Tsoumpos P, Billis E** *et al.* Cross-cultural translation and validation of the Greek version of the Knee Injury and Osteoarthritis Outcome Score (KOOS) in patients with total knee replacement. *Disabil Rehabil.* 2015 ; 37 : 1477-83.
29. **Nejadnik H, Hui JH, Feng Choong E** *et al.* Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. *Am J Sports Med.* 2010 ; 38 : 1110-6.
30. **O'Shea TM, Miao X.** Bilayered scaffolds for osteochondral tissue engineering. *Tissue Eng Part B Rev.* 2008 ; 14 : 447-64.
31. **Oussedik S, Tsitskaris K, Parker D.** Treatment of articular cartilage lesions of the knee by microfracture or autologous chondrocyte implantation: a systematic review. *Arthroscopy.* 2015 ; 31 : 732-44.
32. **Pak J, Chang J, Lee J, Lee S.** Safety reporting on implantation of autologous adipose tissue-derived stem cells with platelet-rich plasma into human articular joints. *BMC Musculoskelet Disord.* 2013 ; 14 : 337.
33. **Pak J, Lee J, Park K, Jeong B, Lee S.** Regeneration of Cartilage in Human Knee Osteoarthritis with Autologous Adipose Tissue-Derived Stem Cells and Autologous

Extracellular Matrix. *Biores Open Access.* 2016 ; 5 : 192-200.

34. Pak J. 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. 2011;5:296.

35. Pelttari K, Steck E, Richter W. The use of mesenchymal stem cells for chondrogenesis. *Injury.* 2008 ; 39 : S58-65.

36. Peterson L, Minas T, Brittberg M *et al.* Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Relat Res.* 2000 : 212-34.

37. Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous chondrocyte implantation: a long-term follow-up. *Am J Sports Med.* 2010 ; 38 : 1117-24.

38. Schaffler A, Buchler C. Concise review: adipose tissue-derived stromal cells--basic and clinical implications for novel cell-based therapies. *Stem Cells.* 2007 ; 25 : 818-27.

39. Smith J, van Wijnen A.J. Understanding Regenerative Medicine Terminology. Regenerative treatments in sports and orthopedic medicine: *Demols Medical Publishing* ; 2018.

40. Solheim E, Krokeide AM, Melteig P *et al.* Symptoms and function in patients with articular cartilage lesions in 1,000 knee arthroscopies. *Knee Surg Sports Traumatol Arthrosc.* 2016 ; 24 : 1610-6.

41. Wakitani S, Nawata M, Tensho K *et al.* 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.* 2007 ; 1 : 74-9.

42. Widuchowski W, Widuchowski J, Trzaska T. Articular cartilage defects: study of 25,124 knee arthroscopies. *Knee.* 2007 ; 14 : 177-82.

43. Williams RJ, 3rd. Cartilage repair strategies. Totowa, *NJ: Humana Press* ; 2007.