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One-Step Surgery With Multipotent Stem Cells for the Treatment of Large Full-Thickness Chondral Defects of the Knee

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Background: Chondral lesions in athletically active patients cause considerable morbidity, and treatment with existing cell-based therapies can be challenging. Bone marrow has been shown as a possible source of multipotent stem cells (MSCs) with chondrogenic potential and is easy to harvest during the same surgical procedure.

Purpose: To investigate the clinical outcome in a group of active patients with large full-thickness chondral defects of the knee treated with 1-step surgery using bone marrow–derived MSCs and a second-generation matrix.

Study Design: Case series; Level of evidence, 4.

Methods: From January 2007 to February 2010, 25 patients (average age, 46.5 years) with symptomatic large chondral defects of the knee (International Cartilage Repair Society grade 4) who underwent cartilage transplantation with MSCs and a collagen type I/III matrix were followed up for a minimum of 3 years. The average lesion size was 8.3 cm^2. Coexisting injuries were treated during the same surgical procedure in 18 patients. All patients underwent a standard postoperative rehabilitation program. Preoperative and postoperative evaluations at 1-year, 2-year, and final follow-up included radiographs, magnetic resonance imaging (MRI), and visual analog scale (VAS) for pain, International Knee Documentation Committee (IKDC), Knee injury and Osteoarthritis Outcome Score (KOOS), Lysholm, Marx, and Tegner scores. Seven patients underwent second-look arthroscopic surgery, with 4 consenting to a tissue biopsy.

Results: No patients were lost at final follow-up. The average preoperative values for the evaluated scores were significantly improved at final follow-up ($P < .001$): VAS, 5.4 ± 0.37 to 0.48 ± 0.19; IKDC subjective, 37.92 ± 4.52 to 81.73 ± 2.42; KOOS pain, 61.04 ± 3.95 to 93.32 ± 1.92; KOOS symptoms, 55.64 ± 3.23 to 89.32 ± 2.32; KOOS activities of daily living, 63.96 ± 4.48 to 91.20 ± 2.74; KOOS sports, 34.20 ± 5.04 to 80.00 ± 3.92; KOOS quality of life, 32.20 ± 4.43 to 83.04 ± 3.37; Lysholm, 46.36 ± 2.25 to 86.52 ± 2.73; Marx, 3.00 ± 0.79 to 9.04 ± 0.79; and Tegner, 2.12 ± 0.32 to 5.64 ± 0.26. Patients younger than 45 years of age and those with smaller or single lesions showed better outcomes. The MRI scans showed good stability of the implant and complete filling of the defect in 80% of patients, and hyaline-like cartilage was found in the histological analysis of the biopsied tissue. No adverse reactions or postoperative complications were noted.

Conclusion: The 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 1-step surgery, avoiding a previous surgical procedure to harvest cartilage and subsequent chondrocyte cultivation.

Keywords: cartilage; chondral defects; MSCs; BMAC; collagen I/III matrix

Articular cartilage is a highly specialized tissue responsible for load bearing as well as for providing a smooth gliding interface for a joint; however, it has a very limited healing potential, in particular because of the low mitotic activity of chondrocytes as well as its avascular nature. Therefore, once an injury occurs, surgical intervention may be necessary to achieve repair of the resulting focal chondral defects. Failure to obtain a good functional outcome can lead to cartilage degeneration, which could subsequently lead to the development of osteoarthritis (OA).[31]

Studies have found a 60% incidence of chondral lesions in all patients aged between 40 and 50 years[55] as well as an increase in symptoms and disability with age. In view of the high costs and the complexities involved in the treatment of OA,[27] the trend of research is now moving toward preventive interventions and therapeutic solutions that can lead to an enhancement of tissue regeneration and

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a reduction of degenerative mechanisms.[51] The idea of "biological solutions for biological problems" has led to the development of less invasive procedures and accelerated treatments that in general reduce morbidity while enhancing functional recovery.[2,13] Autologous chondrocyte implantation[4] (ACI) is considered an effective treatment for cartilage defects of the knee; the resulting tissue has hyaline-like characteristics along with mechanical and functional stability at long-term follow-up.[3,41] However, the treatment requires 2 surgical procedures, there is difficulty in obtaining an adequate distribution of chondrocytes,[3,34,41] and it is associated with donor site morbidity.[4,30,34] Although second-generation ACI addresses some of these concerns, it essentially remains a 2-surgery technique and is an expensive solution.[13,15,26,32,50]

Research has been moving toward the possibility of performing a single-step procedure to avoid the 2 surgical procedures and chondrocyte cultivation; in this regard, the use of bone marrow aspirate concentrate (BMAC), which contains multipotent stem cells (MSCs) and growth factors, can represent a viable alternative.[1,5,6] The easy availability, coupled with the self-renewal capacity and multilineage differentiation potential of MSCs, leading to the generation of chondrogenic tissue, offers a promising option in cartilage surgery.[7,13-15,20,36,52] These cells are characterized by their ability to adhere to plastic in standard culture conditions and to express CD105, CD73, and CD90 and lack the expression of CD45, CD34, CD14 or Cd11b, CD 79a or CD19, and HLA-DR surface molecules.[7,10,20] Furthermore, their isolation is devoid of the need to harvest healthy cartilage tissue and therefore bypasses the first surgical step required for cartilage biopsy and subsequent chondrocyte cell cultivation.[11]

Since 2006, we have been using autologous bone marrow–derived MSCs combined with various scaffolds for the treatment of full-thickness cartilage defects of different joints. The purpose of this study was to prospectively investigate the clinical outcome in a group of athletically active patients treated with 1-step surgery with MSCs and a second-generation matrix for full-thickness large chondral defects of the knee. Our hypothesis was that this single-step procedure would restore articular cartilage defects and provide good functional outcomes at medium-term follow-up.

MATERIALS AND METHODS

The study was approved by our institutional ethics committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All patients provided their informed consent before inclusion in the study.

Between January 2007 and February 2010, a total of 33 patients were treated with bone marrow–derived MSCs and a collagen I/III matrix for large full-thickness chondral lesions of the knee; among them, 25 patients met the below-mentioned inclusion criteria and were prospectively followed up for a minimum of 3 years, and preliminary results on 15 patients have been previously reported.[14] We included patients between 30 to 60 years of age, with a body mass index (BMI) of less than 30 kg/m^2, who actively engaged in recreational sport activities with grade 4 cartilage lesions (per the International Cartilage Repair Society [ICRS] classification [Brittberg M, Aglietti P, Gambardella R, et al. "The ICRS Clinical Cartilage Injury Evaluation System." Presented at the International Knee Society Meeting, 2000]). Patients had a stable knee with normal alignment and patellofemoral tracking or had these corrected at the time of surgery (see Appendix 1, available in the online version of this article at http://ajsm.sagepub.com/supplemental; all appendices are available online). We excluded patients with tricompartmental arthritis, osteonecrosis of the knee, multiple intra-articular injections with steroids, general systemic illnesses, and neurovascular diseases and those who did not consent to follow the strict rehabilitation protocol.

The preoperative evaluation included a detailed history and physical examination of the patient; the functional evaluation was performed using various scoring systems. Severity of pain, range of motion (ROM), and visual analog scale (VAS) for pain (0 = no pain, 10 = worst pain), International Knee Documentation Committee (IKDC) (International Knee Documentation Committee. "IKDC Form." Presented at the International Knee Society Meeting, 1991),[22,57] Knee injury and Osteoarthritis Outcome Score (KOOS),[46] Lysholm,[29] Tegner,[53] and Marx[33] scores were documented preoperatively and repeated at 1 year and 2 years postoperatively and at final follow-up.

Radiographic and magnetic resonance imaging (MRI) results were collected preoperatively and at 1-year, 2-year, and final follow-up. The standard radiographic evaluation included standing anteroposterior (AP) long-leg views, including the hips and ankles, standing AP/lateral views of the knee, skyline patellofemoral views, and standing views with the knee bent at 45°. The MRI evaluation was performed with a 1.5-T system (Quad Knee/8-CH SENSE-Knee, Philips, Amsterdam, the Netherlands), using the T1-weighted, T2-weighted, and intermediate-weighted contrast mapping protocol for MRI of the knee recommended by the Hospital for Special Surgery[42] (see Appendix 2). The MRI scans were evaluated by 2 independent musculoskeletal radiologists who were blinded to the clinical history of the patients; the preliminary results have been previously reported.[14] Features of the graft that were assessed included the extent of filling of the defect by repair tissue, integration of the graft with native cartilage and subchondral bone, and appearance of the graft surface along with the underlying tissue and underlying bone. The extent of underfilling or hypertrophy of the graft in the defect and changes in the articular surface were compared between the preoperative and final MRI results.

All patients followed the same 4-phase rehabilitation protocol based on current knowledge of graft healing biology, functional criteria, and therapeutic goal progression, similar to the rehabilitation used after second-generation ACI[15,26,32] (see Appendix 3).

Surgical Technique

All the procedures were performed by the senior author (A.G.). The patient was placed supine on the table after

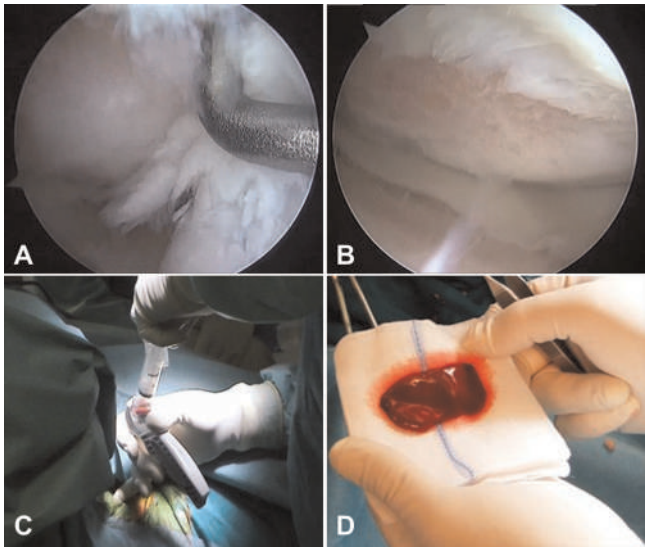

Figure 1. Diagnostic arthroscopic surgery was initially performed to evaluate the condition of the joint and confirm magnetic resonance imaging findings: grade 4 cartilage lesion on the medial femoral condyle (A) before and (B) after debridement of the lesion of patient 9 (also shown in Figure 2). (C) Bone marrow aspiration and (D) a bone marrow aspirate concentrate clot after activation.

undergoing spinal anesthesia. An arthroscopic evaluation was initially performed to evaluate the condition of the joint and confirm the MRI findings with regard to the size and location of the cartilage defects (Figure 1, A and B). Subsequently, approximately 60 mL of bone marrow was harvested from the ipsilateral iliac crest using a dedicated aspiration kit (Figure 1C) and centrifuged using a commercially available system (BMAC Harvest Smart PreP2 System, Harvest Technologies, Plymouth, Massachusetts, USA) to obtain a concentration of bone marrow cells 4 to 6 times the baseline value. With the use of batroxobin enzyme (Plateltex Act, Plateltex SRO, Bratislava, Slovakia), the bone marrow concentrate was activated to produce a sticky clot material (Figure 1D).

Then, the knee was approached during mini-arthrotomy, and the chondral defects were prepared and debrided with the use of curettes (Figure 2A). Specific attention was paid to remove the calcified layer, if present, without penetrating the subchondral bone and thereby reduce bleeding from the bottom of the lesion. Damaged cartilage was removed until a contained, shouldered defect remained, which was templated, and the collagen membrane fashioned according to the defect size (Figure 2B). Finally, the prepared clot was implanted into the prepared cartilage defect and covered with a collagen-based membrane scaffold (ChondroGide, Geistlich, Wolhusen, Switzerland) (Figure 2, C and D), which was anchored to the surrounding cartilage using a polydioxanone suture (PDS II 6-0, Ethicon, Somerville, New Jersey, USA) and sealed with fibrin glue (Tissucol, Baxter Spa, Rome, Italy) (Figure 2, E and F). The knee was then subjected to flexion and extension movements to check the stability of the implanted membrane. Coexisting knee pathological conditions such as tibiofemoral axial alignment, patellofemoral alignment, and ligamentous insufficiency were treated during the same surgical procedure.

At the end of the surgical procedure, a sample of the patient's bone marrow concentrate was sent to an independent laboratory to quantify the number of colony-forming units (CFUs) of MSCs per patient.

Second-Look Arthroscopic Surgery and Histochemistry

Second-look arthroscopic surgery was performed in 7 knees; furthermore, 4 patients consented for a concomitant biopsy, and tissue was taken from regenerated tissue at the site of

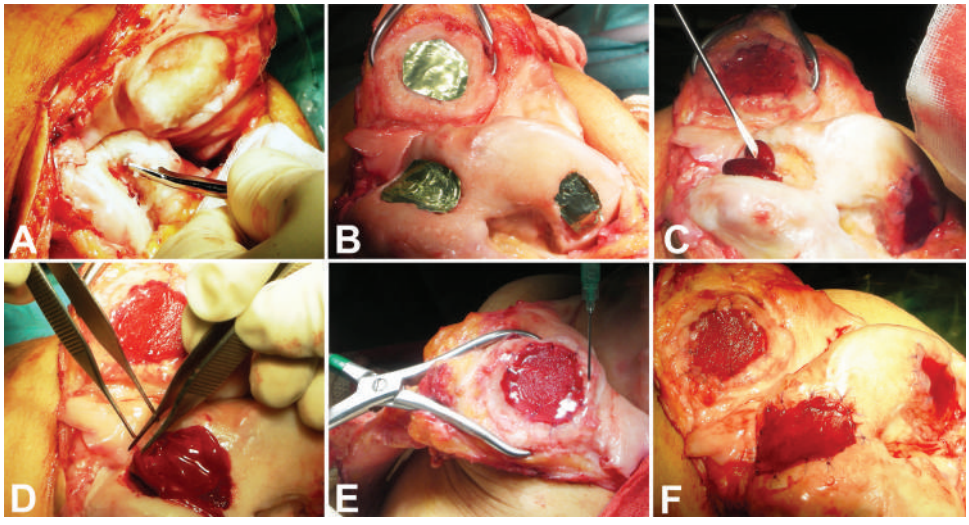

Figure 2. Patient 9, evaluated with grade 4 cartilage lesions on the medial femoral condyle, patella, and trochlea: lesions were debrided (A) and templated and the collagen membrane fashioned according to the defect size (B); a bone marrow aspirate concentrate clot was pasted into the lesions (C) and covered with a collagen-based matrix (D), which was anchored to the surrounding cartilage and sealed with fibrin glue (E, F).

the treated chondral lesion. Data of the 4 second-look arthroscopic surgeries and 3 concomitant biopsies and methodology have been reported previously[14]; however, because of the small number of available patients, we provide records for all the examined biopsy specimens.

Statistical Analysis

Statistical analysis was performed by an independent statistician using SPSS software (SPSS 17.0, SPSS Inc, Chicago, Illinois, USA). The general linear model for repeated-measure tests was used to investigate within-time variations for continuous variables (Marx, Lysholm, VAS, and IKDC subjective scores) for all patients and each evaluated subgroup. The evaluated factors were lesion size, number, and location; patient age; and presence of concomitant procedures. When sphericity was not verified, the Greenhouse-Geisser P value was reported. A post hoc test with a Bonferroni adjustment for pairwise comparisons within times and between subgroups was used to investigate the improvement and deterioration for each variable and between subgroups.

The nonparametric Friedman test was used to detect within-time significant differences in ordinal variables (Tegner and IKDC objective scores), and the post hoc nonparametric Wilcoxon rank test was used with a Bonferroni adjustment of the significance level. Multivariate analysis was performed to assess whether the size and number of lesions and patient age were relevant and whether the presence of concomitant surgical procedures affected outcomes. The Kruskal-Wallis nonparametric test was used for comparisons of more than 2 subgroups, with the post hoc nonparametric Mann-Whitney U test with a Bonferroni adjustment of the significance level; only the Mann-Whitney U test with a Bonferroni adjustment was used when there were 2 evaluated subgroups.

Continuous data are described as the average ± standard error of the mean. Reported P values are 2-tailed, with an α level of .05, indicating significance.

RESULTS

All 25 patients (16 male and 9 female) were available at final follow-up (minimum, 3 years; average, 41.3 ± 7 months). The average age was 46.5 ± 1.71 years (range, 32-58 years). The average BMI was 24.4 ± 0.6 kg/m^2. There were 13 lesions located on the left knee and 12 on the right. The average lesion size was 8.3 cm^2 (range, 2.5-22 cm^2). The medial femoral condyle was the most frequently (40.5%) involved site, followed by the patella (24.5%) and trochlea (21.5%). Eighteen patients had coexisting knee pathological abnormalities including tibiofemoral or patellofemoral malalignment and ligamentous insufficiency, which were treated at the same time as MSC transplantation. The average number of colony-forming units of MSCs per patient was 4041 ± 284 CFU/mL, ranging from 2500 to 5700 CFU/mL per patient. No adverse reactions or postoperative complications were noted. The demographic data of patients, lesion characteristics, and associated pathological abnormalities are provided in Appendix 1; absolute values of the evaluated scores preoperatively and at final follow-up are provided in Appendix 4.

The analysis of data revealed significant improvement in Tegner, Marx, Lysholm, VAS, IKDC subjective, and KOOS scores at final follow-up when compared with their respective preoperative scores ($P < .001$) (Table 1).

Twenty-three patients (92%) had moderate or severe pain (VAS score, 4-10) preoperatively, while at final follow-up, only 8 patients complained of mild pain (VAS score, 1-2). The average VAS score significantly improved from 5.4 ± 0.37 preoperatively to 0.48 ± 0.19 at final follow-up ($P < .001$) (Figure 3A). Average preoperative KOOS pain (61.04 ± 3.95) and KOOS symptoms (55.64 ± 3.23) scores significantly increased to 93.32 ± 1.92 and 89.32 ± 2.32, respectively, at final follow-up ($P < .001$) (Figure 3B).

Preoperative IKDC objective scores were 0 A, 0 B, 13 C, and 12 D, while at final follow-up, 18 patients were classified as normal (A) and 7 as nearly normal (B) ($P < .001$). The average ROM preoperatively was 4.3° to 113.0° (±2.9°), while at final follow-up, it was 0° to 128.2° (±1.2°). The average preoperative IKDC subjective score was 37.92 ± 4.52 and significantly improved to 74.15 ± 3.38, 78.19 ± 3.16, and 81.73 ± 2.42 at 1-year, 2-year, and final follow-up, respectively ($P < .001$).

All patients returned to their previous daily and specific sport activities; however, only 32% were able to perform at their preinjury level at final follow-up (average preinjury Tegner score, 6.60 ± 0.32). The average Lysholm score significantly improved from 46.36 ± 2.25 preoperatively to 86.52 ± 2.73 at final follow-up ($P < .001$) (Figure 4A). The average preoperative Tegner score (2.12 ± 0.32) increased to 4.44 ± 0.37 at 1-year follow-up and to 5.64 ± 0.26 at final follow-up ($P < .001$) (Figure 4B). Similarly, the average Marx score significantly improved from 3.00 ± 0.79 preoperatively to 9.04 ± 0.79 at final follow-up ($P < .001$). The average preoperative KOOS sports score (34.20 ± 5.04) significantly increased to 80.00 ± 3.92 at final follow-up ($P < .001$).

We also studied the difference in improvement in subgroups according to lesion size, number, and location; patient age; and presence of associated procedures (see Appendix 5). Multivariate analysis in homogeneous subgroups with similar baseline characteristics revealed that, although patients younger than 45 years of age showed better improvement than older patients, there was no significant difference in improvement between subgroups at final follow-up ($P > .05$). Furthermore, patients with medium-sized lesions had better outcomes than those with larger lesions; however, the difference was significant only for patients evaluated with medium lesions ($P < .05$), while there was no significant difference in improvement between patients with single or multiple (and kissing) chondral lesions ($P > .05$). Patients with patellofemoral joint lesions showed significantly better results than patients with medial femoral condyle lesions at final follow-up ($P < .005$). In addition, there was not any significant difference in improvement in clinical outcomes between patients who had undergone or not undergone

TABLE 1
Clinical Outcomes[a]

Variable	Preoperative	Follow-up			*P* Value[b]		
		1-Year	2-Year	Final	Preoperative vs Final	1-Year vs 2-Year	2-Year vs Final
VAS	5.40 ± 0.37	1.16 ± 1.14	0.84 ± 1.02	0.48 ± 0.19	.001	.345	.807
KOOS pain	61.04 ± 3.95	88.16 ± 2.40	92.00 ± 2.21	93.32 ± 1.92	.001	.072	.563
KOOS symptoms	55.64 ± 3.23	84.80 ± 3.26	85.48 ± 3.24	89.32 ± 2.32	.001	.999	.435
KOOS ADL	63.96 ± 4.48	88.12 ± 3.00	90.24 ± 2.61	91.20 ± 2.74	.001	.999	.999
KOOS sports	34.20 ± 5.04	69.40 ± 5.03	73.20 ± 4.99	80.00 ± 3.92	.001	.634	.019
KOOS QOL	32.20 ± 4.43	75.52 ± 5.44	77.00 ± 4.44	83.04 ± 3.37	.001	.999	.126
IKDC subjective	37.92 ± 4.52	74.15 ± 3.38	78.19 ± 3.16	81.73 ± 2.42	.001	.213	.020
IKDC objective,[c] n	13 C, 12 D	12 A, 11 B, 2 C	16 A, 9 B	18 A, 7 B	.001	.014	.157
Tegner[d]	2.12 ± 0.32	4.44 ± 0.37	5.40 ± 0.35	5.64 ± 0.26	.001	.004	.999
Marx	3.00 ± 0.79	7.12 ± 0.74	8.36 ± 0.74	9.04 ± 0.79	.001	.043	.423
Lysholm	46.36 ± 2.25	82.80 ± 3.38	85.96 ± 3.44	86.52 ± 2.73	.001	.118	.999

[a]All scores showed significant improvement from preoperatively to 1-year, 2-year, and final follow-up. Values are expressed as the average ± standard error of the mean unless otherwise indicated. ADL, activities of daily living; IKDC, International Knee Documentation Committee; KOOS, Knee injury and Osteoarthritis Outcome Score; QOL, quality of life; VAS, visual analog scale.
[b]$P < .001$ for all nonreported time interval improvements (post hoc test with Bonferroni adjustment).
[c]Nonparametric Wilcoxon test with Bonferroni adjustment.
[d]Preinjury Tegner score of 6.60 ± 0.32.

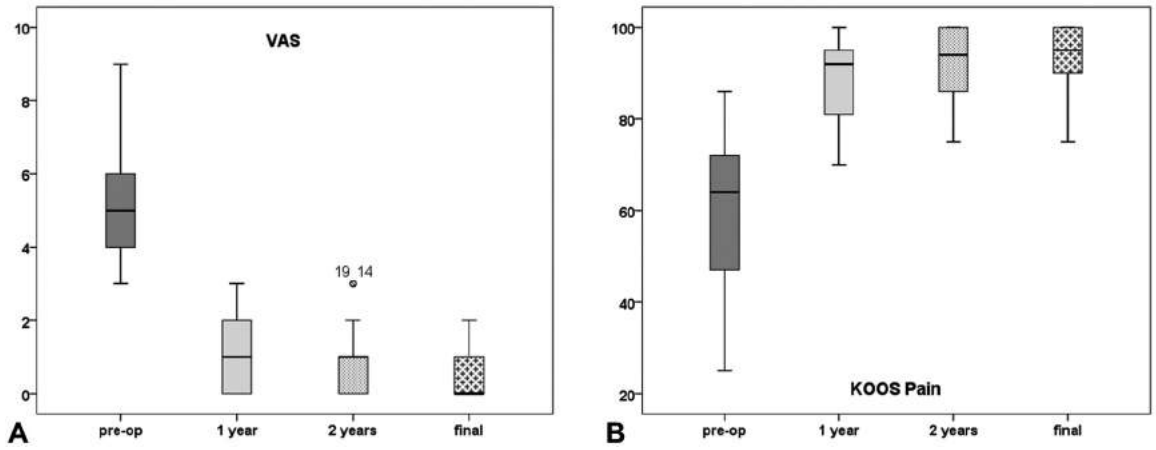

Figure 3. Box plots showing within-time improvement in visual analog scale (A) and Knee injury and Osteoarthritis Outcome Score (KOOS) pain (B) scores.

a concomitant procedure or between patients who underwent different concomitant procedures ($P > .05$).

The MRI analysis at final follow-up showed stable implantation and complete filling of the defect in 20 of 25 patients (80%) and incomplete filling (<50% of the adjacent cartilage) in 5 of 25 patients (20%), while no signs of hypertrophy were identified. Integration with the adjacent cartilage was complete in 22 of 25 patients (88%) with restoration of the cartilage layer and subchondral bone (Figure 5). We did not identify cysts or sclerosis of the subchondral bone, while edema was identified in 6 patients. These results, compared with the results at 1-year follow-up, showed that new hyaline-like tissue is still maturing and the tissue stabilized at final follow-up with no documented deterioration in the new tissue formed (Figure 6).

Second-look arthroscopic surgery was performed in 7 knees (average time after surgery, 13.1 months); 4 patients who underwent a previous high tibial osteotomy (HTO) sought hardware removal, and consented to a second-look procedure. One patient underwent second-look arthroscopic surgery for partial medial meniscectomy at 6 months. Additionally, 2 patients consented to second-look arthroscopy while they were undergoing surgery on the opposite knee for another reason. Second-look arthroscopic surgery revealed smooth, newly formed intact tissue continuous with the healthy cartilage in all the patients; no hypertrophy was identified (Figure 7). The stability of the implant

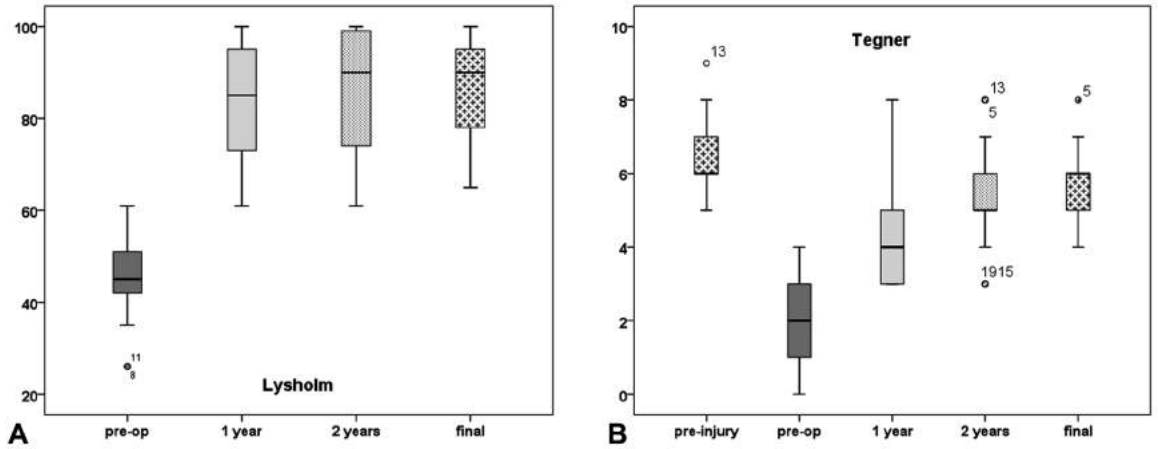

Figure 4. Box plots showing within-time improvement in Lysholm (A) and Tegner (B) scores.

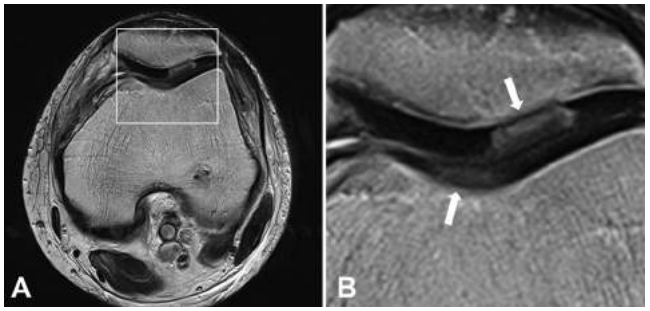

Figure 5. A magnetic resonance imaging scan of a 44-year-old male patient with a patellofemoral chondral defect at 3 months after surgery shows stability of the implant in the patella and trochlea (A, B).

appeared similar to the adjacent tissue when checked with a probe, and macroscopic evaluation showed normal to nearly normal cartilage according to the ICRS visual scoring system. Four patients consented to undergo a concomitant biopsy, while 3 patients did not give their consent because they were fearful of damage to the graft. Good histological findings were reported for the 4 biopsy specimens analyzed, which had many hyaline-like cartilage features. Results of the ICRS histological evaluation score are reported in Appendix 6. Histochemical and immunohistochemical evaluations of the 4 biopsy specimens are described in Appendix 7.

DISCUSSION

The treatment of chondral lesions is an area of active research necessitated by their high incidence in athletically active patients and the demand for near normal function by patients. The prevailing therapeutic options partly address these concerns, albeit with some drawbacks. The objective of this study was to investigate the effectiveness of bone marrow–derived MSCs for the treatment of large chondral defects of the knee with 1-step surgery. To our knowledge, this is the first study at medium-term follow-up showing the results of a single-step approach for the treatment of large full-thickness knee chondral defects in athletically active patients.

All patients showed significant improvement at final follow-up ($P < .001$) (Table 1). No adverse reactions or postoperative complications were noted. Moreover, the clinical outcomes obtained correlated with MRI, arthroscopic surgery, and tissue biopsy findings. Lesion size and location were important predictors of outcome, as patients with medium-sized lesions and those with patellofemoral joint lesions showed better outcomes. On the contrary, no significant role of the multiplicity of lesions or the presence of concomitant limb alignment surgical procedures on the influence of outcomes was noted (see Appendix 5).

Preliminary results of MSC cartilage repair on 15 patients at 2-year minimum follow-up have been previously reported[14]; however, medium-term studies are needed to evaluate a new technique for cartilage repair as the deterioration of outcomes has been demonstrated with other techniques.[12,26] The present study confirmed preliminary findings in a bigger sample of patients and revealed that the described technique provided durable clinical outcomes that correlated with MRI findings at medium-term follow-up (average, 41.3 ± 7 months).

It is widely recognized that associated pathological conditions such as tibiofemoral axis malalignment, patellofemoral maltracking, and ligamentous insufficiency should be addressed in a previous or concomitant cartilage repair procedure.[51] In this study, associated knee pathological conditions were addressed concomitantly in 18 patients to create the essential mechanical environment to protect the implanted cells and provide long-term stability of the outcome. Also, HTO has been established as an effective treatment of the varus osteoarthritic knee to decrease the stress on the load-bearing cartilage in the medial compartment; however, only partial remodeling of the articular cartilage has been reported. Moreover, a poor clinical improvement should be expected after HTO or patellofemoral realignment when large osteochondral defects are present. In a recently published review, significantly

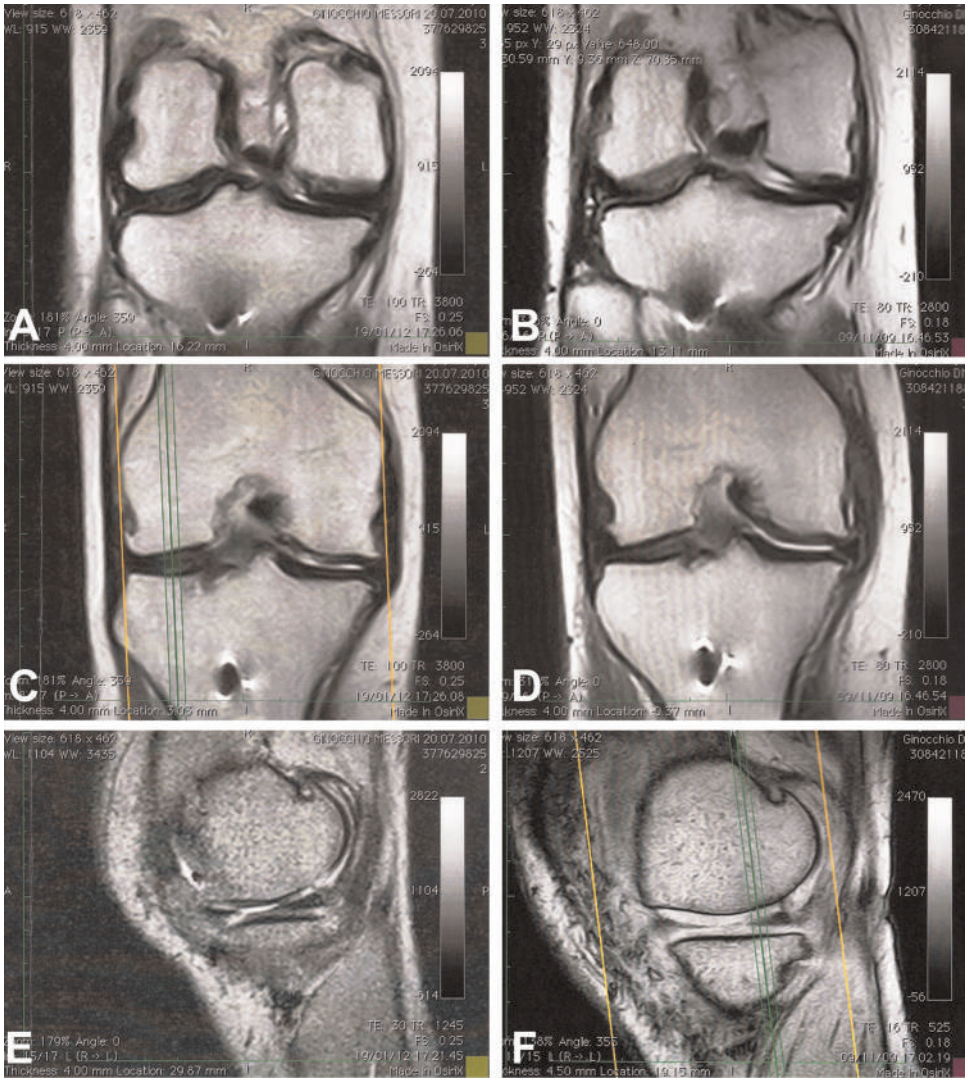

Figure 6. A magnetic resonance imaging scan of patient 9, showing the progression of cartilage restoration after implantation of multipotent stem cells in a medial femoral condyle defect at 6 months (A, C, E) and at 24 months (B, D, F).

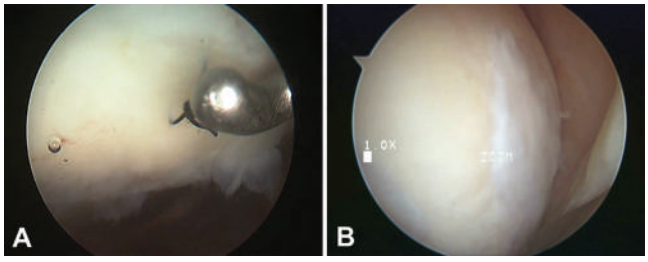

Figure 7. Second-look arthroscopic surgery, showing stability and good consistency of the newly formed tissue and its contiguity with the surrounding healthy cartilage (A, B).

greater survival at 5 years' follow-up was seen after HTO with articular cartilage surgery than after isolated HTO.[17] Therefore, correction of tibiofemoral axis malalignment is indicated when articular cartilage restoration techniques are applied.[35] Similarly, patellofemoral maltracking, when present, should be addressed to reduce overloading of the lateral patellofemoral joint and reduce the risk of future cartilage injuries.[28]

The multilineage potential of MSCs derived from bone marrow and their chondrogenic potential, in particular, have drawn the attention of researchers for various reasons.[11,23,44] The widespread availability of these cells and the possibility to isolate them from various sites make them easy targets for harvesting.[5,6] Furthermore, the finding that bone marrow–derived MSCs have a better proliferation rate than chondrocytes and that MSC transplantation has comparable outcomes to ACI portends positive trends for the use of these cells in cartilage surgery.[37] First- and second-generation ACI and various other cell- or scaffold-based therapies have shown good clinical results.[3,15,26,32,41] However, their widespread utilization

has been hampered by the fact that 2 surgical procedures are required, the high costs that they impose on the patient, and the extensive interdisciplinary support that they need, eventually limiting the use of these procedures to a few centers.[21,24,27]

An analysis of the literature shows that several authors investigated the role of MSCs in cartilage repair. Ochi et al[38] observed, in a rat model, that the injection of cultured MSCs combined with microfracture could accelerate the regeneration of cartilage and concluded that this approach could represent an effective and less invasive strategy for the regeneration of articular surfaces. In another experimental study, the same authors developed a cell delivery system based on stem cells bound to magnetic beads and used an electromagnetic field to direct them into the chondral defect, thus improving neocartilage synthesis and reducing the risk of ectopic cartilage formation.[25,58] Another equine study showed enhanced chondrogenesis and improved cartilage healing after arthroscopic implantation with MSCs.[56] However, a rapid loss of implanted cells and deterioration in cartilage quality were observed. Grigolo et al[16] transplanted a hyaluronan scaffold seeded with in vitro expanded bone marrow–derived MSCs in chondral lesions of the knee in a rabbit model and reported better quality of the regenerated tissue between the implants with scaffolds carrying MSCs compared with the scaffold alone or nontreated lesions in the control group at 6 months. Hui et al[21] compared MSC transplants with cultured chondrocytes, osteochondral autografts, and periosteal grafts in animal models of osteochondritis dissecans. Based on histological and biomechanical evaluations, the authors found the stem cell transplants to be comparable to cultured chondrocytes and superior to the periosteum and osteochondral autografts in their ability to repair chondral defects. Wakitani et al[54] first described the use of expanded bone marrow–derived stem cells to repair cartilage defects in osteoarthritic knees in a clinical study: they concluded that MSCs were capable of regenerating repair tissue. Nejadnik et al[37] compared the clinical outcomes of patients treated with first-generation ACI with those treated by MSC injections in a prospective study: at the end of 2 years, patients from both groups had comparable results with better results in patients in the MSC group.

In our study, the MRI evaluation showed complete filling of the defect in 80% of the patients, while no signs of hypertrophy were identified, consistent with our preliminary results on 15 patients.[14] Additionally, consecutive MRI revealed that there is a constant growth of thickness in newly formed tissue up to 2 years after surgery, while no deterioration of the newly formed tissue has been identified at final follow-up (average follow-up, 41.3 ± 7 months) (Figure 6). Even though MRI has a low sensitivity for analyzing chondral lesions, the good functional outcomes in our patients imply the efficacy of the procedure.

Histological examination of the performed biopsies revealed the regeneration of new tissues with many hyaline-like cartilage features, including the presence of a noticeable proteoglycan component around the chondrons (see Appendix 7). The biopsies showed good organization of proteoglycans and collagen in the extracellular matrix, an intact superficial zone, and a not well-defined tidemark, suggesting that maturation of the neotissue is still undergoing. Histological features of biopsy specimens taken 6 and 8 months from implantation demonstrated immature cartilage tissue, suggesting that the repair tissue was still undergoing remodeling. Overall, even if only 4 patients gave their consent for biopsy, the observed level of maturity seems higher than that obtained with cell-suspension autologous chondrocyte transplantation techniques at a similar time point.[12,19,24]

Bone marrow aspirate contains both hematopoietic and mesenchymal stem cells in addition to other cell types that may play a role in promoting tissue regeneration.[9] The use of a point-of-care device that yields a sufficient concentration of MSCs, rapidly coupled with a single surgical step, is a major advantage in our technique. The device that we utilized provides a concentration of total nucleated cells (TNCs) and platelets included in the aspirate. Hermann et al,[18] in their study, reported that the resulting concentration in the total number of TNCs (utilizing the BMAC Harvest Smart PreP2 System) was 2.4-fold higher in comparison to the Ficoll isolation procedure for bone marrow mononucleated cells, while the colony-forming capacity was similar for both products. Interestingly, the migratory capacity was significantly higher for the harvested TNCs. In addition, the platelet content was approximately 75% of that in the original bone marrow aspirate in contrast to only 0.5% in the Ficoll preparation. Platelets contribute to the stimulation of progenitor cells and act as a chemoattractant, owing to the release of growth factors by α granules, including platelet-derived growth factor, transforming growth factor (TGF)–β1, TGF-β2, platelet-derived epidermal growth factor, vascular endothelial growth factor, and insulin growth factor–1.[2] Furthermore, platelet-rich plasma in combination with thrombin also increases the handling properties of the final grafts for osteogenic applications, owing to its clotting effect. In addition, in a study conducted at the Hospital for Special Surgery to evaluate the efficacy of 2 bone marrow concentration systems approved by the United States Food and Drug Administration, Hegde et al (unpublished data, 2012) reported that the Harvest system yielded a significantly greater number and concentration of progenitor cells both before and after concentration. An important issue in the clinical application of MSCs for cartilage repair is phenotype stability.[40] The MSCs derived chondrogenic cells still possess a degree of plasticity and a tendency to proceed along the endochondral ossification route that can lead to calcification of the implant.[40,48] In this regard, the use of a collagen I/III matrix in our series provided a suitable environment to maintain cell phenotype stability as well as cell stabilization in the defect.[49]

Several authors have reported a decrease in MSCs with increased age and some changes in differentiation, proliferation, attachment, senescence, or self-renewal.[8,45] Cavallo et al,[7] in their study, reported that there is a negative effect of donor age on differentiation, particularly

toward the osteogenic lineage, and confirmed the combination of intrinsic changes in the niche microenvironment and aging. Age-related atrophy of MSCs has been also suggested as a cause of the decreased number of osteoprogenitor cells and decreased bone formation.[43] However, other authors reported that patients with primary OA show no change with aging in the number of osteogenic precursors.[39] In our study, multivariate analysis of the outcome did not reveal any significant difference between young versus older patients. However, given the small number of patients, we cannot tell if the value of CFUs has influenced the outcome; future studies with a larger number of patients might give insights as to whether a higher number of CFUs may influence the outcome.

Our study presents some limitations: the absence of a control group treated with an established procedure and the nonrandomized design of the study might introduce bias in interpreting the results. The underlying reasons were the selective patient pool, the novelty of the procedure, and the lack of approval for conducting a randomized study by our ethics committee. Another limitation was the small number of patients available for second-look arthroscopic surgery and biopsy. A possible confounding factor of concomitant procedures was investigated, and multivariate analysis did not reveal any significant difference in clinical outcomes between patients who had undergone a concomitant procedure and those who had not; similarly, there was no significant difference between patients who underwent various concomitant procedures. Although addressing associated knee pathological abnormalities might positively affect the postoperative outcome, still, it is not documented that these procedures, when performed alone without a concomitant cartilage surgical procedure, can result in cartilage repair of large chondral defects.

The uniqueness of this study is that we treated large chondral lesions in an athletically active population with MSCs with 1-step surgery and that no patient had been lost at final follow-up. The average size of the lesions is too large to find an established procedure with which to compare. It is well documented that microfracture is usually performed to treat lesions smaller than 3 cm^2 in size[47] and that the average size of a lesion treated with ACI is about 5.3 cm^2.[41] The described technique would be of value in the treatment of large chondral defects as well as represent a feasible treatment modality for salvage procedures.

In conclusion, this study has shown that the use of bone marrow–derived MSCs can be a promising option in the treatment of large full-thickness articular cartilage lesions; however, a larger sample size and long-term prospective randomized studies are needed to confirm our findings.

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