Introduction

The limited intrinsic healing potential of articular cartilage is attributed to the presence of few and specialized cells with low mitotic activity, to the lack of vessels and of undifferentiated cells that can promote tissue repair. Therefore, once injury occurs, surgical intervention is necessary to achieve repair of the articular surface to obtain good functional outcome and to avoid subsequent cartilage degeneration, which could lead to the development of osteoarthritis (OA).\(^1\)\(^2\)

The incidence of chondral defects is frequent with sporting injuries, especially in patients over 40 years of age, usually resulting in persistent pain. Furthermore, community-based studies have shown that 10% of the population over the age of 55 years has troublesome knee pain, and of those, 25% are severely disabled.\(^3\) The social impact of bone and cartilage pathologies entails high costs in terms of therapeutic treatments and loss of income.
Many surgical techniques have been utilized to improve cartilage lesion healing and demonstrated variable results. Autologous chondrocyte implantation (ACI), which was first introduced by Peterson,4 is considered an effective procedure for cartilage defects of the knee restoring hyaline-like cartilage tissue, which is mechanically and functionally stable at long-term follow-up.5,7 However, the need of 2 surgical procedures, the sacrifice of periosteal tissue, the uncertain distribution of chondrocyte solution,5,7-10 and complications such as periosteal patch hypertrophy and arthrofibrosis6,10-13 prompted the scientific community to develop new techniques, namely second-generation ACI. The use of a 3-dimensional scaffold for autologous chondrocyte culture was developed with the aim to improve both the biological performance of chondrogenic autologous cells as well as render the surgical technique easier, and surgeons have been enabled to perform this procedure arthroscopically.12,14-18 However, this technique is still a 2-step procedure including an arthroscopic biopsy, in vitro cell cultivation, and subsequent implantation, either using an arthroscopic technique or mini-arthroscopy.16,17,19,20 Apart from donor site morbidity, the risks of 2 surgical procedures, and the limited quantity of cartilage that could be harvested, the total cost of surgeries, scaffold, and in vitro culture still represents the major limitation of this technique.

Therefore, research has been moving towards the possibility to perform a 1-step surgical procedure. In this regard, the use of bone marrow aspirate concentrate (BMAC) cells, which contain pluripotent mesenchymal stem cells (MSCs) and growth factors (GFs), can represent a possible alternative to regenerate cartilage tissue. In particular, it allows to avoid the first surgery for cartilage biopsy and the subsequent chondrocyte cell cultivation, with a significant reduction of the cost of the total procedure.21-30 The aim of this study was to validate a 1-step procedure for the treatment of large chondral defects of the knee based on BMAC covered with a commercially available collagen I/III matrix. The rationale of this procedure was to paste the BMAC into the cartilage defect and protect the in-growth of the neotissue with a user-friendly scaffold impermeable to cells; furthermore, our technique maximizes cell-to-cell contact and provides a strong chondrogenic environment utilizing a collagen I/III matrix promoting chondrogenic differentiation of MSC and cartilage regeneration. Our hypothesis was that this technique could provide satisfactory clinical results, avoiding biopsy and cell cultivation and reducing the cost of cartilage transplantation procedure.

**Materials and Methods**

From April 2007, we prospectively followed up 15 symptomatic patients, presenting chronic large full-thickness cartilage lesions, treated at our institution with BMAC pasted—after activation—into the lesions and covered with a collagen type I/III matrix (Chondro-Gide, Geistlich, Wolhusen, Switzerland). Inclusion criteria were patients with knee cartilage injury of International Cartilage Repair Society (ICRS) grade 4; minimum follow-up of 2 years; age between 30 and 60 years; body mass index (BMI) <30; and knee stable or stabilized, normal alignment, or corrected at the time of cartilage repair. Exclusion criteria included tricompartmental arthritis; osteonecrosis; untreated malalignment (varus-valgus >5°); knee instability (no compliance to concomitant stabilization); patients who have had multiple intra-articular injections with steroids in the 3 months preceding the study; hip disorders that led to abnormal gait; general systemic illnesses such as rheumatic diseases, Bechterew syndrome, chondrocalcinosis, gout, and neurovascular diseases; and noncompliance to our rehabilitation protocol.12,14,19

All patients (10 males and 5 females) reached a minimum follow-up of 2 years (range, 24-38 months) and were active in sports but were not professional. The mean age was 48 years, ranging from 32 to 58 years. The BMI of the patients was 24.5 (standard deviation [SD], 2.53). Cartilage lesions were diagnosed by MRI and arthroscopy as grade 4 of ICRS classification. Six patients had multiple chondral lesions; the location of the lesions was 7 patella, 6 trochlea, 4 medial tibial plateau, 6 medial, and 1 lateral femoral condyle. The average cartilage lesion size per patient was 9.2 cm² (SD, 6.3), ranging from 1.5 to 22 cm². Twelve of our patients had coexisting pathologies such as tibiofemoral axial alignment, patellofemoral alignment, and ligamentous insufficiency, which were treated before or during the same surgery.31 Detailed demographic data, size and location of lesions, and surgical management of coexisting pathologies are reported in **Table 1**. All patients followed the same rehabilitation protocol for 8 months, which is similar to rehabilitation after second-generation ACI, based on current knowledge of the graft healing biology and on functional criteria and therapy goal progression (**Table 2**).

X-rays and MRI were collected preoperatively and at 1- and 2-year follow-up; the standard radiographic evaluation included a standing anteroposterior long-leg radiograph, including also the hips and ankles, standing anteroposterior/lateral views of knees, skyline patellofemoral, and standing 45° bent-knee views. Visual analog scale (VAS) for pain, International Knee Documentation Committee (IKDC), Knee injury and Osteoarthritis Outcome Score (KOOS), Short-Form Health Survey (SF-36 Physical/Mental), Lysholm, Tegner, and Marx scores were collected preoperatively and at 6-, 12-, and 24-month follow-up. We also studied the difference in improvement between patients with single or multiple lesions as well as between subgroups of our patients according to the size of the lesion: 1) small-medium (1.5-5 cm²), 2) medium-large
Table 1. Demographic Data, Lesion Size, Colony-Forming Unit (CFU/mL), and Associated Procedures

| Patient/Side | Age/Sex | BMI | Sport / Activity | Location and Size of Lesions (mm × mm) | Size (cm²) | CFU MSC/mL | Associated Procedures |
|--------------|---------|-----|------------------|----------------------------------------|-----------|-----------|----------------------|
| 1 / Right    | 45 / M  | 24  | Motocross        | MFC 50 × 20                             | 10        | 4,700     | ACLR                 |
| 2 / Right    | 39 / F  | 25  | Gymnastics       | Patella 40 × 20                         | 8         | 2,600     | Patellar realignment (Fulkerson) |
| 3 / Left     | 47 / M  | 24  | Tennis           | Trochlea 25 × 20                        | 5         | 4,600     | Opening wedge osteotomy |
| 4 / Right    | 49 / M  | 23  | Running          | Trochlea 20 × 20                        | 2.4       | 4,550     | None                 |
| 5 / Left     | 48 / M  | 24  | Tennis           | Patella 45 × 15                         | 6.75      | 4,600     | Opening wedge osteotomy |
| 6 / Left     | 48 / F  | 22  | Trekking, cycling | MTP 20 × 10                             | 3         | 4,650     | None                 |
| 7 / Left     | 58 / M  | 30  | Swimming, cycling | MFC 20 × 30, MTP 13 × 10                | 7.3       | 3,650     | Opening wedge osteotomy |
| 8 / Right    | 32 / M  | 22  | Soccer           | Patella 40 × 20                         | 8         | 5,700     | ACLR                 |
| 9 / Right    | 33 / F  | 20  | Alpine skiing, trekking | Trochlea 30 × 25, patella 25 × 25, MFC 25 × 20 | 18.75     | 5,700     | Patellar realignment (Fulkerson) |
| 10 / Left    | 50 / F  | 25  | Gymnastics       | Patella 12 × 8, patella 20 × 15         | 3.95      | 2,640     | Lateral release      |
| 11 / Left    | 41 / M  | 28  | Hockey           | Trochlea 40 × 30, MFC 18 × 23           | 16.15     | 3,100     | None                 |
| 12 / Left    | 58 / M  | 27  | Skiing, hunting  | MTP 20 × 30, MFC 40 × 30, trochlea 20 × 20 | 22        | 2,435     | Opening wedge osteotomy |
| 13 / Left    | 55 / M  | 26  | Trekking, cycling | MTP 20 × 10, MFC 40 × 30, trochlea 15 × 10 | 15.5      | 2,808     | Opening wedge osteotomy |
| 14 / Left    | 45 / M  | 23  | Skiing           | Patella 40 × 25                         | 10        | 4,900     | ACLR (allograft)     |
| 15 / Right   | 53 / F  | 25  | Skiing           | LFC 11 × 11                             | 1.5       | 2,000     | ACLR                 |

Note: MSC = mesenchymal stem cell; MFC = medial femoral condyle; MTP = medial tibial plateau; LFC = lateral femoral condyle; ACLR = anterior cruciate ligament reconstruction.

Table 2. Rehabilitation Phases, Objectives, and Criteria to Progress between Phases

| Phase 1: Protection of the implant | Objectives | Criteria to Progress |
|-----------------------------------|------------|----------------------|
| Protect the transplant from excessive loads and shearing forces | Full active knee extension | Knee flexion >120° |
| Decrease pain and effusion | No or minimum pain and swelling | No pain during weightbearing |
| Gain full extension and gradual recovery of knee flexion | Adequate muscle recruitment (quadriceps) | |
| Retard muscle atrophy | Normal gait | Recovery of nearly full range of motion (full extension, flexion >15°) |
| | Adequate muscle tone and neuromuscular control | No pain or swelling |
| | Running without pain/swelling at 8 km/h for 10 minutes | |

Phase 2: Transition and recovery of gait

| Objectives | Criteria to Progress |
|------------|----------------------|
| Progressive recovery in daily functional activities | Adequate recovery of coordination and neuromuscular control |
| Increase the strength of the quadriceps and flexors | Recovery of strength >80% contralateral limb |
| Recovery of full range of motion | Single-leg hop test >80% contralateral limb |
| | Running without pain/effusion at 10 km/h for 15 minutes |

Phase 3: Maturation and recovery of running

| Objectives | Criteria to Progress |
|------------|----------------------|
| Further increase in strength of quadriceps and flexors muscles | Recovery of strength >90% contralateral limb |
| Further increase in functional activities level | Single-leg hop test >90% contralateral limb |
| | Recovery of sport-specific skills |

Phase 4: Turnover and sport-specific recovery

| Objectives | Criteria to Progress |
|------------|----------------------|
| Sustain high loads and impact activities | Running without pain/effusion at 8 km/h for 10 minutes |
| Recover sport-specific skills | Recovery of strength >90% contralateral limb |
| Prepare athlete for a return to team and competition with good recovery of the aerobic endurance | Single-leg hop test >90% contralateral limb |
| Maintain a good quality of life, avoiding excess of body fat and preventing risk of reinjury | Recovery of sport-specific skills |
(5-10 cm²), and 3) large-multiple (>10 cm²). Four patients gave their consent for second-look arthroscopy but only 3 for a concomitant biopsy.

**MRI Protocol**

MRI assessment was carried out by a 1.5-T system (Quad Knee/8-CH SENSE Knee, Philips, Amsterdam, the Netherlands), and the recommended T1-weighted, T2-weighted, and intermediate-weighted contrast mapping protocol for MRI of the knee by the Hospital for Special Surgery was considered. Series I were performed using T2*-weighted 2-dimensional gradient recalled echo (FFE) sequences in an axial plane, with a TR of 33 milliseconds, TE of 13 milliseconds, flip angle (FA) of 30°, field of view (FOV) of 24 × 24 cm, thickness of 5 mm, and matrix of 256 × 128 (frequency × phase). Series II were carried out using proton density (PD)–weighted 2-dimensional fast/turbo spin echo (TSE) sequences in a coronal plane with TR of 4,000 to 4,500 milliseconds, TE of 34 milliseconds, FOV of 11 to 13 × 11 to 13 cm, thickness of 3.0 mm, intersection gap of 0.0 mm, and matrix of 512 × 288; receiver bandwidth was 125 Hz/pixel (water-fat shift 0.58 pixel at 1.5 T). Series III were performed using PD-weighted 2-dimensional TSE sequences with frequency-selective fat-signal suppression in a sagittal plane with TR of 3,500 to 4,000 milliseconds, TE of 40 milliseconds, FOV of 16 × 16 cm, thickness of 3.5 to 4.0 mm, intersection gap of 0.0 mm, and matrix of 256 × 224. T1-weighted 2-dimensional TSE sequences were performed in a sagittal plane with TR of 620 to 640 milliseconds, TE of 10 to 12 milliseconds, FOV of 16 × 16 cm, thickness of 4.0 mm, intersection gap of 0.4 mm, and matrix of 256 × 192. In MRI, we evaluated the filling of the defects, the restoration of the cartilage layer, the remodeling of the subchondral bone, and presence of hypertrophy of the neotissue.

**Surgical Technique**

All the procedures were performed under spinal anesthesia and routine sterile preparation and draping; 60 mL of bone marrow aspirate were harvested from the ipsilateral iliac crest using a dedicated aspiration kit and centrifuged using a commercially available system (BMAC Harvest Smart PreP2 System, Harvest Technologies, Plymouth, MA). In order to concentrate the baseline value of the bone marrow cells 4 to 6 times, we followed the method recommended by the manufacturer. Using a batroxobin enzyme (Plateltex Act, Plateltex S.R.O., Bratislava, Slovakia), the bone marrow concentrate was activated in order to produce a sticky clot material (Fig. 1A), which was implanted into the prepared cartilage defect.

After arthroscopic evaluation, the knee was approached with a mini-arthrotomy, and the chondral defect was prepared and debrided with the use of curettes (Fig. 1B). Specific attention was paid to remove the calcified layer if present, while avoiding penetration of the subchondral bone and reducing the bleeding, as much as possible, from the bottom of the lesion. Damaged cartilage was removed until a contained, shouldered defect remained, which is necessary in order to facilitate suturing the scaffold. The defect was templated and the collagen membrane fashioned according to the defect size. Finally, the prepared clot was pasted into the lesion. In order to protect MSC, the defect was covered with a collagen-based membrane scaffold (Fig. 1C).

The membrane was anchored to the surrounding cartilage using PDS 6-0 and sealed with fibrin glue (Tissucol, Baxter, Rome, Italy); the knee was then ranged through flexion and extension in order to check the stability of the implanted membrane. Coexisting knee pathologies such as tibiofemoral axial alignment, patellofemoral alignment, and ligamentous insufficiency were treated during the same surgery in 12 patients.

**Second-Look Arthroscopy**

Second-look arthroscopy and biopsy were done in 4 knees after an average of 13.5 months of follow-up, but only 3 patients gave their consent for a biopsy (Fig. 1D). The first knee had a second look after the patient started complaining of mid–joint-line pain after 6 months. Knees 2 and 3 had a second-look arthroscopy in concomitance with hardware removal for a previous medial opening wedge osteotomy at 12 and 24 months. The fourth knee had a second-look arthroscopy in concomitance with an arthroscopy to the opposite knee for a partial meniscectomy at 12 months.

**Histochemistry**

Biopsies for histological analysis were fixed in 10% buffered formalin and washed and decalcified with a 4% HCl, 5% formic acid decalcificant solution until required. The samples were then dehydrated through a graded series of alcohol and embedded in paraffin. Sections, 4 μm thick, were obtained from the specimens and stored at room temperature. The slides were stained with 0.001% Fast Green and 0.1% Safranin-O (Sigma, St. Louis, MO) to evaluate the cellular morphology, visualize the proteoglycan content of the extracellular matrix, and highlight the presence of hyaline-like tissue. An independent, experienced histologist examined 4 distinct regions within the specimens: a global area, a superficial zone, an intermediate zone, and a deep zone, and calcified layer/bone transition. The knees were assessed using the ICRS visual scoring system.
Immunohistochemistry: Type I and II Collagens

For immunohistochemical analyses, the following primary antibodies were used: mouse monoclonal anti-human type I collagen (Chemicon International, Temecula, CA) and anti-human collagen type II mouse monoclonal antibody (Chemicon International). Paraffin sections were deparaffinized and rehydrated. For epitope unmasking, the samples were treated with 0.1% hyaluronidase (Sigma) in phosphate buffered saline (PBS) at 37 °C for 5 minutes. After washing, the slides for the detection of type I and II collagens were incubated at room temperature for 30 minutes in 1x PBS containing 5% of normal goat serum (NGS) (Dako, Carpinteria, CA) to prevent nonspecific bindings. The slides were incubated with the anti-human type I and II collagen primary antibodies diluted 1:20 in 0.04 M Trizma base saline (TBS), pH 7.6, containing 1% bovine serum albumin (BSA) and 0.1% Triton X-100 for 1 hour at room temperature. The slides were washed 3 times with 0.04 M TBS, pH 7.6, and incubated with goat anti-mouse and anti-rabbit immunoglobulins labeled with dextran molecules/alkaline phosphatase (Envision, Dako) at room temperature for 30 minutes. After 3 washes with 0.04 M TBS, pH 7.6, the reactions were developed using the new fuchsin kit (Kit New Fuchsin Substrate System, Dako) in the presence of 5 mM levamisole (Sigma) to block endogenous alkaline phosphatase. Negative staining controls were performed either by omitting the primary antibody or using a control isotype-matched antibody. Slides were counterstained with hematoxylin and mounted in glycerol gel. All the samples were visualized with a Zeiss axioskope microscope (Carl Zeiss, Oberkochen, Germany).

Statistical Analysis

For statistical analysis, the SPSS software was used (SPSS 17.0, SPSS, Chicago, IL). Nonparametric analysis was performed with Wilcoxon signed-rank tests in order to
analyze the clinical outcome between preoperative and postoperative at 6, 12, and 24 months’ evaluation. Continuous data are described as average mean ± standard error of the mean (SEM). Z score and P values are provided for all the parameters evaluated. Reported P values are 1-tailed with an α level of 0.05 indicating significance.

We also studied the difference in improvement between patients with single or multiple lesions as well as between subgroups of our patients according to the size of the lesion; however, statistical analysis was not performed because of the small size of our subgroups. Therefore, we calculated the percentage of the maximum possible improvement for each score as follows: (score at final follow-up – preoperative score) / (best score – preoperative score) × 100. In particular, for the Tegner score, the preinjury value was considered as the best score, while for the VAS score, the best score was zero.

### Results

Patients showed significant improvement in all scores at 6, 12, and 24 months’ follow-up (P < 0.05) (Figs. 2 and 3). No adverse reactions or postoperative complication were noted. Results are summarized in Table 3.

At the final follow-up, patients with single lesions showed higher improvement than patients with multiple lesions in all scores (Fig. 4 and Table 4), except for KOOS pain and symptom subgroups. However, the average KOOS values for these subgroups were comparable at final follow-up. Patients with smaller lesion sizes showed higher improvement at final follow-up (Fig. 5).

After harvesting the bone marrow, we sent a sample to an independent laboratory in order to quantify the colony-forming unit (CFU/mL) of MSC per patient, which is a measurement of the viability of the bone marrow. The average CFU/mL of MSC per patient was 3,904 CFU/mL (SD, 1,232), ranging from 2,000 to 5,700 CFU/mL per patient (Table 1). However, we were unable to standardize patients according to the provided volume of the clot and the size of the lesion (CFU/cm²) because it was not possible to quantify the exact volume of the BMAC clot used to fill each lesion.

### Table 3. Summary of Clinical Outcome

| Variable | Preoperative | 6-Month Follow-up | 12-Month Follow-up | 24-Month Follow-up |
|----------|--------------|------------------|--------------------|--------------------|
|          | Mean ± SEM   | Mean ± SEM       | P Value / Z        | Mean ± SEM         |
| VAS      | 5.1 ± 0.04   | 0.8 ± 0.3        | 0.001 / −3.307°    | 0.7 ± 0.3          |
| KOOS pain| 66.2 ± 5.6   | 88.7 ± 2.7       | 0.001 / −2.858°    | 94.0 ± 2.7         |
| KOOS sym.| 68.2 ± 4.6   | 83.2 ± 2.5       | 0.001 / −2.906°    | 90.0 ± 2.7         |
| KOOS ADL | 70.0 ± 6.1   | 91.6 ± 2.1       | 0.001 / −2.587°    | 95.1 ± 2.0         |
| KOOS sport| 41.6 ± 7.9  | 59.0 ± 6.2       | 0.001 / −3.234°    | 71.3 ± 6.7         |
| KOOS QOL | 37.2 ± 5.4   | 59.6 ± 6.0       | 0.001 / −2.482°    | 77.5 ± 4.4         |
| IKDC subj.| 43.6 ± 5.6   | 66.9 ± 3.1       | 0.001 / −2.920°    | 80.7 ± 3.7         |
| IKDC obj. | 39.0 ± 1.3 | 52.6 ± 1.1       | 0.001 / −3.233°    | 56.0 ± 0.4         |
| SF-36 phys. | 46.9 ± 1.7 | 54.1 ± 1.6       | 0.004 / −1.789°    | 55.8 ± 1.0         |
| SF-36 mental | 2.07 ± 0.3 | 3.2 ± 0.4        | 0.050 / −1.912°    | 4.7 ± 0.4          |
| Marx     | 91.1 ± 3.9   | 92.7 ± 2.8       | 0.001 / −2.272°    | 71.3 ± 6.7         |
| IKDC subj. | 41.6 ± 7.9  | 59.0 ± 6.2       | 0.001 / −3.234°    | 71.3 ± 6.7         |
| Lysholm  | 56.0 ± 0.4   | 59.0 ± 5.6       | 0.001 / −3.297°    | 92.9 ± 2.4         |

Note: The variables are described as mean ± SEM (standard error of the mean). All the scores showed significant improvement from preoperative evaluation to 6-, 12-, and 24-month follow-up. VAS = visual analog scale; KOOS = Knee injury and Osteoarthritis Outcome Score; ADL = activities of daily living; QOL = quality of life; IKDC = International Knee Documentation Committee.

| Variable | Multiple Lesions | Single Lesions |
|----------|------------------|---------------|
|          | Mean ± SEM       | Mean ± SEM    |
| VAS      | 79.8% ± 8.9      | 84.4% ± 9.5   |
| KOOS pain| 78.8% ± 8.6      | 74.2% ± 8.7   |
| KOOS symptoms | 74.9% ± 6.1 | 67.4% ± 8.6 |
| KOOS ADL | 75.6% ± 10.2     | 86.7% ± 7.6   |
| KOOS sport | 56.3% ± 9.3 | 62.9% ± 7.6   |
| KOOS QOL | 69.3% ± 8.2      | 73.9% ± 6.8   |
| Tegner   | 79.3% ± 17.8     | 87.0% ± 16    |
| Marx     | 48.9% ± 6.1      | 66.8% ± 8.2   |
| IKDC subj.| 64.9% ± 8.3     | 71.6% ± 7.5   |
| Lysholm  | 75.1% ± 8.7      | 85.1% ± 7.6   |

Note: SEM = standard error of the mean; VAS = visual analog scale; KOOS = Knee injury and Osteoarthritis Outcome Score; ADL = activities of daily living; QOL = quality of life; IKDC = International Knee Documentation Committee.

### Table 4. Comparison of the Outcome between Multiple and Single Lesion Patients

|            | Multiple Lesions | Single Lesions |
|------------|------------------|---------------|
| VAS        | 79.8% ± 8.9      | 84.4% ± 9.5   |
| KOOS pain  | 78.8% ± 8.6      | 74.2% ± 8.7   |
| KOOS symptoms | 74.9% ± 6.1 | 67.4% ± 8.6 |
| KOOS ADL   | 75.6% ± 10.2     | 86.7% ± 7.6   |
| KOOS sport | 56.3% ± 9.3      | 62.9% ± 7.6   |
| KOOS QOL   | 69.3% ± 8.2      | 73.9% ± 6.8   |
| Tegner     | 79.3% ± 17.8     | 87.0% ± 16    |
| Marx       | 48.9% ± 6.1      | 66.8% ± 8.2   |
| IKDC subj. | 64.9% ± 8.3      | 71.6% ± 7.5   |
| Lysholm    | 75.1% ± 8.7      | 85.1% ± 7.6   |

Note: SEM = standard error of the mean; VAS = visual analog scale; KOOS = Knee injury and Osteoarthritis Outcome Score; ADL = activities of daily living; QOL = quality of life; IKDC = International Knee Documentation Committee.
Figure 2. (A) Boxplots showing the significant improvement in Tegner score from preoperative evaluation to 6, 12, and 24 months ($P < 0.005$); however, the patients did not reach the preinjury value. (B) Diagram showing the significant improvement in Knee injury and Osteoarthritis Outcome Score (KOOS) subgroups from preoperative to 6, 12, and 24 months ($P < 0.005$).

Figure 3. International Knee Documentation Committee (IKDC) objective score showed significant improvement in A and B subgroups from preoperative to 6, 12, and 24 months ($P < 0.005$).
Figure 4. Visual analog scale (VAS) and Tegner score showing higher improvement in single versus multiple lesion patients from preoperative evaluation to final follow-up.

Figure 5. Visual analog scale (VAS) and Knee injury and Osteoarthritis Outcome Score (KOOS) scores showing higher improvement in patients with smaller lesions from preoperative to 6, 12, and 24 months.
cartilage) in 3 of 15 patients (20%), while no signs of hypertrophy were identified. Integration with adjacent cartilage was complete in 14 of 15 patients (93.3%) with restoration of the cartilage layer and subchondral bone (Fig. 6). We also did not identify edema, cysts, or sclerosis of subchondral bone either.

Second-look arthroscopies in 4 knees revealed smooth, newly formed tissue with continuous intact to the healthy surrounding cartilage in all 3 patients; no hypertrophy was identified. The stability of the implant appeared similar to the adjacent tissue as checked with a probe. Macroscopic evaluation showed normal to nearly normal as classified by the ICRS visual scoring system.

Good histological findings were reported for the 3 specimens analyzed, which presented many hyaline-like features. Results of the ICRS histological evaluation score are reported in Table 5. Histochemical and immunohistochemical evaluations of the 3 biopsies are described in Figures 7, 8, and 9.

**Discussion**

The purpose of our study was to determine the effectiveness of cartilage repair utilizing 1-step surgery with BMAC, which represents a cell source of MSCs and GFs, covered after activation with a commercially available collagen type I/III matrix. Our group of patients showed significant improvement in all the scores (<0.005); furthermore, these good outcomes were correlated with MRI, arthroscopy, and available biopsy findings. Although only 15 patients were included in this nonrandomized prospective study, there are no corresponding studies in the literature analyzing a similar
1-step surgery procedure and providing clinical outcome and MRI evaluation. Despite the high number of experimental studies performed, only one study reported the use of BMAC in a single-step surgical procedure in the talus.\textsuperscript{32} To our knowledge, this is the first report of this 1-step approach for the treatment of large full-thickness cartilage lesions of the knee. Another unique feature of this study is the large average size of lesions (9.2 cm\textsuperscript{2}). Considering that microfractures are usually used to treat lesions smaller than 3 cm\textsuperscript{2},\textsuperscript{33} and that the average size of lesions treated with
ACI is also smaller (5.3 cm$^2$ in the last report by Peterson et al. on 224 patients), our data pave the way to the treatment of large articular cartilage lesions. Another interesting result is that 80% of patients required concomitant procedures, which implies that coexisting pathologies such as tibiofemoral axial alignment, patellofemoral alignment, and ligamentous insufficiency are common in patients with cartilage lesions; in these patients, a concomitant procedure is recommended in order to protect the newly formed tissue.

This study presents some limitations: the study design neither included a control group treated with an established procedure such as microfracture nor an untreated group for ethical reasons; furthermore, we did not find a control group with a comparable lesion size that could be treated with microfracture. Furthermore, there are possible confounding factors like tibiofemoral axial alignment, patellofemoral alignment, and ligamentous insufficiency, which may affect the outcome of treatment. The present study is a prospective nonrandomized one with a 2-year follow-up period, and only a limited number of patients gave written consent for second-look arthroscopy and biopsy.

Regarding the potential of MSCs for regenerative medicine, recent studies demonstrated that MSCs secrete bioactive molecules that stimulate angiogenesis and mitosis of tissue-specific and intrinsic progenitors and reduce T cell surveillance and inflammation, and some authors have also recognized that the presence of other nucleated cells is able to restore the damaged tissue. This recently revealed capacity of MSCs to secrete bioactive factors that are both immunomodulatory and regenerative paves the way to strategies that mimic natural tissue repair.

According to this paradigm, cell selection and cultivation in the laboratory may not be necessary with a significant reduction to the cost of the total procedure, allowing the development of 1-step surgical procedures.

Ochi et al. 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 animal study on rats and rabbits, the same authors developed a cell delivery system based on MSCs bound to magnetic beads and on the use of an electromagnetic field, demonstrating the feasibility of the MSCs injected into the joint accumulating in the chondral defect, thus improving neocartilage synthesis and reducing the risk of ectopic cartilage formation. Enhanced chondrogenesis and improved cartilage healing have been demonstrated also in equine models after arthroscopic implantation withMSC. However, a rapid loss of implanted cells and deterioration in cartilage quality were observed. The authors concluded that the development of a system for intraoperative stem cell isolation, purification for immediate grafting, and cell stabilization into the defect could have significant advantages in time-saving and immediate application of a cell-based approach for cartilage repair. Grigolo et al. transplanted in a rabbit model of an OA knee a hyaluronan-based scaffold seeded with in vitro expanded bone

Figure 9. Biopsy of patient no. 5 after 24 months (original magnification, 40x). (A) Safranin-O staining reveals a well-organized cartilage tissue with the typical features of normal articular cartilage. The superficial layer is regular. The tidemark is well evident. The proteoglycan component is well represented, and the cells show regular distribution along the extracellular matrix. The subchondral bone tissue is in a remodeling process. (B) Immunohistochemical analysis results of collagen type I are almost negative with only a few positive cells at the superficial layer. (C) Type II collagen is slightly positive in the extracellular matrix and at the cellular level.
marrow–derived MSCs. They performed histological, histomorphometric, and immunohistological evaluations showing better quality of the regenerated tissue between the implants with scaffolds carrying MSCs compared to the scaffold alone or controls in particular at 6 months.45

Another crucial issue in the clinical application of MSCs for cartilage repair is their phenotype stability.46 In fact, MSC-derived chondrogenic cells still possess a degree of plasticity and the tendency to proceed along the endochondral ossification route that can lead to calcification of the implant.46,47 In this regard, our strategy, based on the use of Chondro-Gide (Geistlich) may provide both the suitable environment to maintain stable cell phenotype and cell stabilization into the defect.48

Histological examination of the biopsies evaluated showed the regeneration of new tissues with many hyaline-like cartilage features such as the presence of a noticeable proteoglycan component around the chondrons and also collagen type II content. The biopsies showed a good organization of proteoglycans and collagens in the extracellular matrix, an intact superficial zone, and a not well-organized proteoglycan component around the chondrons and also collagen type II content. Histological features of the 6-month biopsy demonstrated low cartilaginous quality of the tissue, suggesting that the repair tissue was still undergoing remodeling. Overall, even if biopsy specimens were obtained only from 3 knees, the observed level of maturity, at the latest time point, seems higher than that obtained by other authors with cell suspension autologous chondrocyte transplantation techniques at a similar time point.49,50

Regarding previous experiences with a similar 1-step procedure, Giannini et al. recently showed successful results of bone marrow–derived cell transplantation in talar osteochondral lesions by a 1-step procedure based on concentrated bone marrow–derived cells and collagen powder or hyaluronic acid membrane as scaffolds.32 Despite the differences between this study and ours, these results are in accordance with the data obtained by our present work and suggest a potential for this approach in the treatment of articular cartilage lesions.

This approach presents several positive features: its 1-step nature, the use of a collagen I/III–based matrix (Chondro-Gide, Geistlich), which favors cell concentration in the defect area and also allows early mobilization of the operated knee, and its lower cost if compared to standard 2-step ACI procedures. The good clinical outcome showed that the use of BMAC in full-thickness articular cartilage lesion repair can be a promising option for the treatment of knee cartilage defects; however, an increased sample size and longer term prospective randomized studies are needed to confirm these preliminary results.

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The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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