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

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The investigation was performed at Orthopaedic Arthroscopy Surgery International, Milan Italy.
ABSTRACT

Objective: The purpose of our study was to determine the effectiveness of cartilage repair utilizing one-step surgery with Bone Marrow Aspirate Concentrate (BMAC) and a collagen I/III matrix (Chondro-Gide® Geistlich Wolhusen, CH).

Material and Methods: We prospectively followed up for 2 years 15 patients (mean age 48 years) operated for grade IV cartilage lesions of the knee. Six of the patients had multiple chondral lesions; the average size of the lesions was 9.2 cm². All patients underwent a mini arthroty and concomitant transplantation with BMAC covered with the collagen matrix. Co-existing pathologies were treated before or during the same surgery. X-rays and MRI were collected preoperatively and at 1 and 2 years follow up. VAS, IKDC, KOOS, Lysholm, Marx, SF36 (physical/mental) and Tegner scores were collected at pre-op and at 6, 12 and 24 months follow up. Four patients gave their consent for second look arthroscopy and three of them for a concomitant biopsy.

Results: Patients showed significant improvement in all scores at final follow-up (p < .005). Patients presenting single lesions and patients with small lesions showed higher improvement. MRI showed coverage of the lesion with hyaline-like tissue in all patients in accordance with clinical results. Hyaline-like histological findings were also reported for all the specimens analysed. No adverse reactions or post-operative complications were noted.

Conclusion: This study showed that one-step surgery with BMAC and collagen I/III matrix could be a viable technique in the treatment of grade IV knee chondral lesions.

Key Words: Knee, Cartilage, BMAC, Chondral Lesion, Chondro-Gide®, Plateltex®
INTRODUCTION

The limited intrinsic healing potential of articular cartilage is attributed to the presence of few and specialized cells with a 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).\textsuperscript{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 have troublesome knee pain and, of those, 25\% are severely disable.\textsuperscript{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 lesions healing and demonstrated variable results. Autologous chondrocyte implantation (ACI), which was first introduced by Peterson,\textsuperscript{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.\textsuperscript{5-7} However, the need of two surgical procedures, the sacrifice of periosteal tissue, the uncertain distribution of chondrocytes solution\textsuperscript{5,7-10} and complications such as periosteal patch hypertrophy and arthrofibrosis\textsuperscript{6,10-13}, prompted the scientific community to develop new techniques, namely second generation ACI. The use of a three-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.\textsuperscript{12,14-18} However, this technique is still a two-step procedure including an arthroscopic biopsy, in vitro cells cultivation and subsequent implantation, either using an arthroscopic technique or mini-arthrotomy.\textsuperscript{16,17,19,20} Apart from donor site morbidity and the risks of two surgical procedures; 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 one-step surgical procedure. In this regard, the use of bone marrow aspirate concentrated cells (BMAC), which contain pluripotent Mesenchymal Stem Cells (MSCs) and growth factors (GF), 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. The aim of this study was to validate a one 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’s 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 (ChondroGide®-Geistlich Wolhusen, CH). Inclusion criteria were: patients with knee cartilage injury grade ICRS 4, minimum follow up of 2 years, age between 30 and 60 years, BMI <30, knee stable or stabilized, normal alignment or corrected at the time of cartilage repair. Exclusion criteria included: tri-compartmental arthritis, osteonecrosis, untreated malalignment (varus / valgus > 5°), knee instability (no compliance to concomitant stabilization) and patients who have had multiple intra-articular injections with steroids in the 3 months preceding the study, hip disorders that lead to abnormal gait, general systemic
illnesses such as rheumatic diseases, Bechterew syndrome, chondrocalcinosis, gout and neurovascular diseases, non compliance to our rehabilitation protocol.

All patients (10 males and 5 females) reached a minimum follow up of 2 years (24-38 months) and were active in sports, but not professional. The mean age was 48 years, ranging from 32 to 58 years. The BMI of the patients was 24.5 (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 lesions size per patient was 9.2 cm² (SD 6.3), ranging from 1.5 to 22 cm². Twelve of our patients had co-existing pathologies such as tibio-femoral axial alignment, patello-femoral alignment and ligamentous insufficiency, which were treated before or during the same surgery. Detailed demographic data, size and location of lesions and surgical management of co-existing pathologies are reported in Table 1. All patients followed the same rehabilitation protocol for 8 months, which is similar to rehabilitation after 2nd generation autologous chondrocyte implantation, based on current knowledge of the graft healing biology and on functional criteria and therapy goals progression (Table 2).

X-rays and MRI were collected preoperatively, at 1 and 2 year follow up; the standard radiographic evaluation included a standing AP long-leg radiograph, including also hips and ankles, standing AP/lateral views of knees, skyline patellofemoral and standing 45° bent knee views.

Visual Analogue Score (VAS) for pain, International Knee Documentation Committee (IKDC), Knee Injury and Osteoarthritis Outcome score (KOOS), Short-Form Health Survey (SF36-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: a) Small-medium (1.5-5 cm²), b) Medium-large (5 to 10cm²) and c) Large-multiple (>10 cm²).

Four patients gave their consent for second look arthroscopy but only three for a concomitant biopsy.
<table>
<thead>
<tr>
<th>Patient / Side</th>
<th>Age / Sex</th>
<th>BMI</th>
<th>Sport / Activity</th>
<th>Location &amp; Size of lesions (mm x mm)</th>
<th>Size (cm²)</th>
<th>CFU MSC/mL</th>
<th>Associated procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Right</td>
<td>45 M</td>
<td>24</td>
<td>Motocross</td>
<td>MFC 50x20</td>
<td>10</td>
<td>4700</td>
<td>ACLR</td>
</tr>
<tr>
<td>2. Right</td>
<td>39 F</td>
<td>25</td>
<td>Gymnastics</td>
<td>Patella 40x20</td>
<td>8</td>
<td>2600</td>
<td>Patellar realignment (Fulkerson)</td>
</tr>
<tr>
<td>3. Left</td>
<td>47 M</td>
<td>24</td>
<td>Tennis</td>
<td>Trochlea 25x20</td>
<td>5</td>
<td>4600</td>
<td>Opening wedge osteotomy</td>
</tr>
<tr>
<td>4. Right</td>
<td>49 M</td>
<td>23</td>
<td>Running</td>
<td>Trochlea 20x12</td>
<td>2.4</td>
<td>4550</td>
<td>None</td>
</tr>
<tr>
<td>5. Right</td>
<td>48 M</td>
<td>24</td>
<td>Tennis</td>
<td>Patella 45x15</td>
<td>6.75</td>
<td>4600</td>
<td>Opening wedge osteotomy</td>
</tr>
<tr>
<td>6. Left</td>
<td>48 F</td>
<td>22</td>
<td>Trekking Cycling</td>
<td>MTP 20x10</td>
<td>3</td>
<td>4650</td>
<td>None</td>
</tr>
<tr>
<td>7. Left</td>
<td>58 M</td>
<td>30</td>
<td>Swimming Cycling</td>
<td>MFC 20x30 MTP 13x10</td>
<td>7.3</td>
<td>3650</td>
<td>Opening wedge osteotomy</td>
</tr>
<tr>
<td>8. Right</td>
<td>32 M</td>
<td>22</td>
<td>Soccer</td>
<td>Patella 40x20</td>
<td>8</td>
<td>5700</td>
<td>ACLR</td>
</tr>
<tr>
<td>9. Right</td>
<td>33 F</td>
<td>20</td>
<td>Alpine skiing</td>
<td>Trochlea 30x25 Patella 25x25 MFC 25x20</td>
<td>18.75</td>
<td>5700</td>
<td>Patellar realignment (Fulkerson)</td>
</tr>
<tr>
<td>10. Left</td>
<td>50 F</td>
<td>25</td>
<td>Gymnastics</td>
<td>Patella 12x8 Patella 20x15</td>
<td>3.95</td>
<td>2640</td>
<td>Lateral release</td>
</tr>
<tr>
<td>11. Left</td>
<td>41 M</td>
<td>28</td>
<td>Hockey</td>
<td>Trochlea 40x30 MCF 18x23</td>
<td>16.15</td>
<td>3100</td>
<td>None</td>
</tr>
<tr>
<td>12. Left</td>
<td>58 M</td>
<td>27</td>
<td>Skiing</td>
<td>MTP 20x30 MFC 40x30 Trochlea 20x20</td>
<td>22</td>
<td>2435</td>
<td>Opening wedge osteotomy</td>
</tr>
<tr>
<td>13. Left</td>
<td>55 M</td>
<td>26</td>
<td>Trekking Cycling</td>
<td>MTP 20x10 MFC 40x30 Trochlea 15x10</td>
<td>15.5</td>
<td>2808</td>
<td>Opening wedge osteotomy</td>
</tr>
<tr>
<td>14. Left</td>
<td>45 M</td>
<td>23</td>
<td>Skiing</td>
<td>Patella 40x25</td>
<td>10</td>
<td>4900</td>
<td>ACLR (Allograft)</td>
</tr>
<tr>
<td>15. Right</td>
<td>53 F</td>
<td>25</td>
<td>Skiing</td>
<td>LFC 11x11</td>
<td>1.5</td>
<td>2000</td>
<td>ACLR</td>
</tr>
</tbody>
</table>
Table 2. Rehabilitation phases, objectives and criteria to progress between phases.

<table>
<thead>
<tr>
<th>PHASE</th>
<th>OBJECTIVES</th>
<th>CRITERIA TO PROGRESS</th>
</tr>
</thead>
</table>
| **Phase 1:**           | Protect the transplant from excessive loads and shearing forces  
• Decrease pain and effusion  
• Gain full extension and gradual recovery of knee flexion  
• Retard muscle atrophy | • Full active knee extension  
• Knee flexion > 120°  
• No or minimum pain and swelling  
• No pain during weight-bearing  
• Adequate muscle recruitment (quadriceps) |
| Protection of the implant |                                          |                                                          |
| **Phase 2:**           | Return to normal gait pattern                          | Normal gait                                             |
| Transition and recovery of gait | - Progressive recovery in daily functional activities  
- Increase the strength of the quadriceps and flexors  
- Recovery of full range of motion | • Recovery of nearly full ROM (full extension, flexion>135°)  
• Adequate muscle tone and neuromuscular control  
• No pain or swelling |
| **Phase 3:**           | Return to a correct running pathway                    | Running without pain/swelling at 8 km/h for 10’  
• Adequate recovery of coordination and neuro-muscular control.  
• Recovery of strength  
• > 80% contralateral limb.  
• Single leg hop test: > 80% contralateral limb | Maturation and recovery of running  
- Further increase in strength of quadriceps and flexors muscles  
- Further increase in functional activities level |
| **Phase 4:**           | Sustain high loads and impact activities  
• Recovery sport specific skills  
• Prepare athlete for a return to team and competition with good recovery of the aerobic endurance  
- Maintain a good quality of life, avoiding excess of body fat and preventing risk of re-injury | Running without pain/effusion at 10 km/h for 15’  
• Recovery of strength > 90% contralateral limb.  
• Single leg hop test: > 90% contralateral limb  
• Recovery of sport specific skills |
| Turnover and sport specific recovery |                                          |                                                          |
MRI protocol

MRI assessment was carried out by a 1.5 Tesla system (Philips, Quad. Knee/8-CH SENSE-Knee) and the recommended T1 weighted, T2 – weighted and intermediate – weighted contrast mapping protocol for MRI of the knee by Hospital for Special Surgery was considered. Series I were performed using T2*-weighted 2D gradient recalled - echo (FFE) sequences in an axial plane, with a repetition time (TR): 33 msec, time to echo (TE): 13 msec, flip angle (FA): 30°, field of view (FOV): 24 x 24 cm, thickness (THK): 5 mm and matrix: 256 x 128 (frequency x phase). Series II were carried out using Proton Density (PD) - weighted 2D fast/turbo spin echo (TSE) sequences in a coronal plane with: TR: 4000-4500 msec, TE: 34 msec, FOV: 11-13 x 11-13 cm, THK: 3.0 mm, intersection gap 0.0 mm and matrix 512 x 288; receiver bandwidth was 125 Hz/pixel (water-fat shift 0.58 pixel at 1.5 Tesla). Series III were performed using Proton Density (PD) – weighted 2D fast/turbo spin echo (TSE) sequences with frequency - selective fat-signal suppression (Fat Sat) in a sagittal plane with: TR: 3500-4000 msec, TE: 40 msec, FOV: 16 x 16 cm, THK: 3.5-4.0 mm, intersection gap 0.0 mm and matrix 256 x 224. T1 - weighted 2D fast/turbo spin echo (TSE) sequences in a sagittal plane with: TR: 620 - 640 msec, TE: 10 - 12 msec, FOV: 16 x cm, THK: 4.0 mm, intersection gap 0.4 mm and matrix 256 x 192. In MRI we evaluated the filling of the defects, the restoration of the cartilage layer, the remodelling of the subchondral bone and presence of hypertrophy of the neotissue.

Surgical technique

All the procedures were performed under spinal anaesthesia 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’s cells four to six times, we followed the method recommended by the manufacturer. Using Batroxobin enzyme (Plateltex® act-
Plateltex S.R.O. Bratislava, SK) the bone marrow concentrate was activated in order to produce a sticky clot material (Figure 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 (Figure 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 (Figure 1c).

The membrane was anchored to the surrounding cartilage using PDS 6-0 and sealed with fibrin glue (Tissuecol®, Baxter. Spa, Rome, Italy) the knee was then ranged through flexion and extension in order to check the stability of the implanted membrane.

Co-existing knee pathologies such as tibio-femoral axial alignment, patello-femoral alignment and ligamentous insufficiency were treated during the same surgery in 12 patients.

Figure 1. a) BMAC clot after activation b) grade IV lesion of the patella c) covering the lesion with a collagen type I/III matrix after pasting the clot into the lesion and d) second look arthroscopy at 2 year follow up.
Second-look Arthroscopy

Second look arthroscopy and biopsy was done in 4 knees after an average of 13.5 months of follow up, but only three patients gave their consent for a biopsy (Figure 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 2nd look arthroscopy in concomitance with hardware removal for a previous medial opening wedge osteotomy at 12 and 24 months. The fourth knee had a 2nd 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, 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, 4µm 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, USA) to evaluate the cellular morphology, visualise the proteoglycan content of the extracellular matrix and to highlight the presence of hyaline-like tissue. An independent, experienced histologist examined four 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 International Cartilage Repair Society (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, USA) and anti human-collagen type II mouse monoclonal antibody (Chemicon International). Paraffin sections were deparaffinised 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, USA) to
prevent non-specific bindings. The slides were incubated with the anti-human- type I and II collagen primary antibodies diluted 1:20 in 0.04M 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 three times with 0.04M TBS pH 7.6 and incubated with goat anti-mouse and anti-rabbit immunoglobulins labelled with dextran molecules-alkaline phosphatase (Envision, Dako, Carpinteria, CA, USA) at room temperature for 30 minutes. After three washes with 0.04M TBS pH 7.6 the reactions were developed using the new fucsin kit (Kit New Fucsin Substrate System, Dako, Carpinteria, CA, USA) 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 haematoxylin and mounted in glycerol gel. All the samples were visualized with a Zeiss Axioscope Microscope (Carl Zeiss, Oberkochen, Germany).

Statistical analysis

For statistical analysis the SPSS software was used (SPSS 17.0, SPSS, Chicago, Illinois, US). Non-parametric analysis was performed with Wilcoxon Signed Ranks Tests in order to analyse the clinical outcome between pre-operative and post-operative at 6, 12 and 24 months evaluation. Continuous data are described as average means ± standard error of the mean (SEM). Z-Score and p-values are provided for all the parameters evaluated. Reported p-values are one-tailed with an alpha 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) x 100. In particular, for Tegner score the pre-injury value was considered as the best score while for 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) (Figures 2, 3). No adverse reactions or postoperative complication were noted. Results are summarized in Table 3.

**Figure 2.** a) Box plots showing the significant improvement in Tegner score from preoperative evaluation to 6, 12, 24 months (p< .005); however, the patients did not reach the pre-injury value b) diagram showing the significant improvement in KOOS subgroups from preoperative to 6, 12, 24 months (p< .005).

**Figure 3.** IKDC objective score showed significant improvement in A and B subgroups from preoperative to 6, 12, 24 months (p< .005).

At the final follow up patients with single lesions showed higher improvement than patients with multiple lesions in all scores (Figure 4 and Table 4), except for KOOS pain and symptoms 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 (Figure 5).
Table 3. The variables are described as mean ±SEM (standard error of the mean); reported p-values are one-tailed with a alpha level of 0.05 indicating significance. All the scores showed significant improvement from preoperative evaluation to 6, 12, and 24 month follow up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preoperative</th>
<th>6 month follow up</th>
<th>12 month follow up</th>
<th>24 month follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SEM</td>
<td>Mean ±SEM</td>
<td>P value / Z</td>
<td>Mean ±SEM</td>
</tr>
<tr>
<td>VAS</td>
<td>5.1 ± 0.04</td>
<td>0.8 ± 0.3</td>
<td>.001 / -3.307a</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>KOOS pain</td>
<td>66.2 ± 5.6</td>
<td>88.7 ± 2.7</td>
<td>.004 / -2.858a</td>
<td>87.7 ± 3.8</td>
</tr>
<tr>
<td>KOOS sym.</td>
<td>68.2 ± 4.6</td>
<td>83.2 ± 2.5</td>
<td>.004 / -2.906a</td>
<td>86.5 ± 3.7</td>
</tr>
<tr>
<td>KOOS ADL</td>
<td>70.0 ± 6.1</td>
<td>91.6 ± 2.1</td>
<td>.010 / -2.587a</td>
<td>91.1 ± 3.9</td>
</tr>
<tr>
<td>KOOS sport</td>
<td>41.6 ± 7.9</td>
<td>59.0 ± 6.2</td>
<td>.001 / -3.234a</td>
<td>71.0 ± 5.2</td>
</tr>
<tr>
<td>KOOS QOL</td>
<td>37.2 ± 5.4</td>
<td>59.6 ± 6.0</td>
<td>.013 / -2.482a</td>
<td>76.1 ± 4.9</td>
</tr>
<tr>
<td>IKDC subj.</td>
<td>43.6 ± 5.6</td>
<td>66.9 ± 3.1</td>
<td>.004 / -2.920a</td>
<td>78.8 ± 3.3</td>
</tr>
<tr>
<td>IKDC obj.</td>
<td>8C / 7D</td>
<td>5A / 8B / 2C</td>
<td>.002 / -3.145a</td>
<td>6A / 9B</td>
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<td>SF36 phys.</td>
<td>39.0 ± 1.3</td>
<td>52.6 ± 1.1</td>
<td>.001 / -3.233a</td>
<td>56.0 ± 0.4</td>
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<td>SF36 mental</td>
<td>46.9 ± 1.7</td>
<td>54.1 ± 1.6</td>
<td>.074 / -1.789a</td>
<td>55.8 ± 1.0</td>
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<td>Tegner</td>
<td>2.07 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>.050 / -1.912b</td>
<td>4.7 ± 0.4</td>
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<td>Marx</td>
<td>4.2 ± 1.0</td>
<td>5.4 ± 1.1</td>
<td>.406 / -0.831b</td>
<td>7.8 ± 0.9</td>
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<tr>
<td>Lysholm</td>
<td>60.4 ± 5.5</td>
<td>88.3 ± 2.5</td>
<td>.002 / -3.111b</td>
<td>93.0 ± 2.5</td>
</tr>
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</table>

Table 4. Comparison of the outcome between multiple and single lesion patients.

<table>
<thead>
<tr>
<th></th>
<th>Multiple lesions</th>
<th>Single lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEAN</td>
<td>SEM</td>
</tr>
<tr>
<td>VAS</td>
<td>79.8 %</td>
<td>± 8.9</td>
</tr>
<tr>
<td>KOOS pain</td>
<td>78.8 %</td>
<td>± 8.6</td>
</tr>
<tr>
<td>KOOS symptoms</td>
<td>74.9 %</td>
<td>± 7.9</td>
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<td>KOOS ADL</td>
<td>75.6 %</td>
<td>± 10.2</td>
</tr>
<tr>
<td>KOOS sport</td>
<td>56.3 %</td>
<td>± 9.3</td>
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<tr>
<td>KOOS QOL</td>
<td>69.3 %</td>
<td>± 8.2</td>
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<tr>
<td>Tegner</td>
<td>79.3 %</td>
<td>± 17.8</td>
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<tr>
<td>Marx</td>
<td>48.9 %</td>
<td>± 6.1</td>
</tr>
<tr>
<td>IKDC (subj.)</td>
<td>64.9 %</td>
<td>± 8.3</td>
</tr>
<tr>
<td>Lysholm</td>
<td>75.1 %</td>
<td>± 8.7</td>
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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 3904 CFU/ml (SD 1232), ranging from 2000-5700 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.

**Figure 4.** VAS and Tegner score showing higher improvement in single vs. multiple lesion patients from preoperative evaluation to final follow up.

**Figure 5.** VAS and KOOS scores showing higher improvement in patients with smaller lesions from preoperative to 6, 12, 24 months.
MRI showed complete filling of the defect in 12 out of 15 patients (80%) and incomplete (<50% of the adjacent cartilage) in 3 out of 15 patients (20%) while no signs of hypertrophy were identified. Integration with adjacent cartilage was complete in 14 out of 15 patients (93.3%) with restoration of the cartilage layer and subchondral bone (Figure 6). We did not also identify oedema, cysts or sclerosis of subchondral bone either.

**Figure 6.** MRI in 33 years old amateur soccer player: a) preoperative T1 sequence in sagittal plane showing a grade IV patellar lesion b) T1 sequence in sagittal plane at 2 years follow up showing good coverage of the lesion

Second-look arthroscopies in 4 knees revealed a smooth newly formed tissue with continuous intact to the healthy surrounding cartilage in all the three 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 ICRS visual scoring system.

Good histological findings were reported for the three specimens analysed which presented many hyaline-like features. Results of the ICRS histological evaluation score are reported in Table 5. Histochemical and immunohistochemical evaluations of the three biopsies are described in figures 7, 8 and 9.
Figure 7. Biopsy of patient No 6 obtained after 6 months (Original Magnification 40x).

a) Safranin-O staining shows a structure, which is not well organized yet and with many fibrous features. The superficial layer is regular. Proteoglycans and cellular components are not represented. b) Collagen type I immunostaining shows the positivity of the extracellular matrix in line with the presence of a fibrous tissue. c) The presence of type-I collagen does not necessarily imply a negative outcome, since positive intracellular staining for type-II collagen in this case indicates ongoing remodelling.

Figure 8. Biopsy of patient No 3 obtained after 12 months (Original Magnification 40x).

a) Safranin-O staining shows a hyaline-like repair tissue. The superficial layer is regular. The subchondral bone shows some signs of ongoing remodelling. Extracellular matrix shows high levels of proteoglycans.
Columnar cellular organisation of the repair tissue is observed. b) Immunohistochemical analysis of collagen type I is completely negative. c) Collagen Type II immunostaining is positive at extracellular level.

**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 remodelling process. b) Immunohistochemical analysis of collagen type I is 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 cellular level.

Table 5: ICRS Visual Histological Assessment Scale

<table>
<thead>
<tr>
<th>Knee/Patient</th>
<th>I. Surface</th>
<th>II. Matrix</th>
<th>III. Cell distribution</th>
<th>IV. Cell population</th>
<th>V. Subchondral Bone</th>
<th>VI. Cartilage mineralization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. / Pt n° 6</td>
<td>1 (smooth)</td>
<td>1 (fibrocartilage)</td>
<td>0 (individual cells /disorganized)</td>
<td>2 (viable)</td>
<td>1 (active remodelling)</td>
<td>1 (normal)</td>
</tr>
<tr>
<td>2. / Pt n° 3</td>
<td>1 (smooth)</td>
<td>3 (hyaline /fibrocartilage)</td>
<td>2 (mixed: columnar /clusters)</td>
<td>2 (viable)</td>
<td>2 (active remodelling)</td>
<td>1 (normal)</td>
</tr>
<tr>
<td>3. / Pt n° 5</td>
<td>1 (smooth)</td>
<td>4 (hyaline)</td>
<td>3 (columnar)</td>
<td>2 (viable)</td>
<td>2 (active remodelling)</td>
<td>1 (normal)</td>
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DISCUSSION

The purpose of our study was to determine the effectiveness of cartilage repair utilizing one-step surgery with BMAC, which represents a cell source of MSCs and GF, covered after activation with a commercial available collagen type I/III matrix. Our group of patients showed significant improvement in all the scores (< .005) furthermore these good outcomes were correlated with MRI, arthroscopy and available biopsies findings. Although only 15 patients were included in this non-randomized prospective study, there are no corresponding studies in the literature analysing a similar one 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 one-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 microfracture are usually used to treat lesion smaller than 3 cm\textsuperscript{2},\textsuperscript{33} and that the average size of lesion treated with ACI is also smaller (5.3 cm\textsuperscript{2} in the last report by Peterson et al on 224 patients),\textsuperscript{34} 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 co-existing pathologies such as tibio-femoral axial alignment, patello-femoral 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.\textsuperscript{31}

This study presents some limitations: study design neither include 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 tibio-femoral axial alignment, patello-femoral alignment and ligamentous insufficiency, which may affect the outcome of treatment. The present study is prospective non-randomised 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 MSC for regenerative medicine, recent studies demonstrated that MSCs secrete bioactive molecules that stimulate angiogenesis and mitosis of tissue-specific and intrinsic progenitors, reduce T cells surveillance and inflammation and some authors have also recognized that the presence of other nucleated cells are 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 one 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 neo-cartilage synthesis and reducing the risk of ectopic cartilage formation. Enhanced chondrogenesis and improved cartilage healing has been demonstrated also in equine models after arthroscopic implantation with MSC. However, a rapid loss of implanted cells and deterioration in cartilage quality were observed. The authors concluded that the development of a system for intra-operative stem cell isolation, purification for immediate grafting, and cells 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 marrow-derived MSCs. They performed histological, histomorphometric and immunohistological evaluation 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.
Another crucial issue in the clinical application of MSCs for cartilage repair is their phenotype stability. In fact, MSCs-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. In this regard, our strategy, based on the use of ChondroGide® may provide both the suitable environment to maintain cell phenotype stable and cell stabilization into the defect.

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-defined tidemark, suggesting that maturation of the neotissue is still undergoing. Specimens also showed a mild positivity for type I collagen, suggesting the presence of some fibrous features. Histological features of the 6-month biopsy demonstrated low cartilaginous quality of the tissue suggesting that the repair tissue was still undergoing remodelling. Overall, even if biopsy specimens were obtained only from three 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.

Regarding previous experiences with a similar one step procedure, Giannini et al recently showed successful results of bone marrow-derived cell transplantation in talar osteochondral lesions by a one-step procedure based on concentrated bone marrow-derived cells and collagen powder or hyaluronic acid membrane as scaffolds. Despite the differences between this study and ours, these results are in accordance to 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 one-step nature, the use of collagen I/III based matrix (ChondroGide®), which favours cell concentration in the defect area and also allows early mobilization of the operated knee, and its lower cost if compared to standard 2 steps 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 randomised studies are needed to confirm these preliminary results.

REFERENCES


