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ORIGINAL ARTICLE

One-step arthroscopic technique for the treatment of osteochondral lesions of the knee with bone-marrow-derived cells: three years results

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Abstract Osteochondral lesions of the knee (OLK) are a common cause of knee pain and associated diseases. A new bone-marrow-derived mesenchymal stem cells technique has been developed for the treatment of OLK. 30 patients with OLK underwent arthroscopic one-step procedure. The bone marrow was harvested from the patients' posterior iliac crest and arthroscopically implanted with a scaffold into the lesion site. Clinical inspection and MRI were performed. Mean International Knee Documentation Committee (IKDC) score before surgery was 29.9 ± 13.2 and 85.4 ± 4.2 at 29 ± 4.1 months ($p < 0.0005$), while Knee injury and Osteoarthritis Outcome Score (KOOS) before surgery was 35.1 ± 11.9 and 87.3 ± 7.3 at 29 ± 4.1 months ($p < 0.0005$). Control MRI and bioptic samples showed an osteochondral regeneration of the lesion site. The one-step technique appears to be a good and reliable option for treatment of OLK at three years of follow-up.
Level of evidence Case series, Level IV.

Keywords Bone marrow · Osteochondral lesion · Knee · Arthroscopic · Mesenchymal · Stem cells

Introduction

Osteochondral lesions of the knee (OLK) are lesions of the cartilaginous layer and the subchondral bone below, most frequently traumatic in origin [1].

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In most cases, these defects can be found at the level of medial femoral condyle, and associated ligamentous or meniscal pathology is reported in 40 % of cases [2, 3].

It was found through careful biomechanical studies that intense action of localized stress on surface of the osteochondral defect may have significant implications for cartilage health [4].

It is well recognized that the cartilage has limited regenerative capacity so the surgeon must face the problem of treating cartilage OLK lesions before they can lead to osteoarthritis [5–8].

Various surgical options have been proposed for osteochondral repair [6–9] but among them, only few have shown the ability to provide repair of the lesion site with hyaline cartilage [5, 10, 11, 46–49].

Traditionally, hyaline cartilage repair may be reached by cartilage replacement (osteoarticular transfer system OATS, mosaicplasty) [13] or cartilage regeneration through autologous chondrocyte implantation (ACI) [6, 14]. Cartilage replacement procedures have the advantage of repairing cartilage defects by already mature autologous cartilage cells but donor site pathology, difficulties in the orienting of the cartilage plugs, and fibrocartilage in the gaps are limits of these techniques [13]. On the other hand, cartilage regeneration by ACI provides continuous cartilage repair with minimal donor site pathology. ACI has been used for the past 16 years, and several studies have reported successful treatment of lesions, with stable and satisfactory results over time [6, 7, 15–18].

The limit of ACI treatment is that the surgeon needs to run two surgical procedures to complete it properly and requires high costs, and that is the main reason why new methods of cartilage regeneration have been searched.

In the last years, a new bone-marrow-derived mesenchymal stem cells (BMDC) technique has been developed

for the treatment of osteochondral defects, and a new one-step technique for BMDC transplantation was proposed.

Following the excellent results achieved in the ankle [19], the same technique has been transferred to the knee joint.

The aim of this study was to investigate the validity of the "one-step" technique in OLK repair and to present the results of a series of 30 patients consecutively treated.

Materials and methods

From March 2006 to February 2007, 20 patients (12 males, 8 females) with OLK underwent the one-step procedure. In 18 cases the lesions were post-traumatic in origin, 2 were due to osteochondritis dissecans, and 4 were secondary to malalignments overload. The treatment was indicated in patients with grade III and IV osteochondral lesions (International Cartilage Repair Society ICRS classification) [20] of the femoral condyle, with clinical symptoms of pain, swelling, locking, or giving way.

In 14 cases, the lesions involved the medial femoral condyle, and in 4 cases, the lateral femoral condyle was involved. In 2 cases, both the medial and the lateral femoral condyles had cartilage defects.

Associated comorbidities were as follows: meniscal injury in 7 (4 medial and 3 lateral meniscus), anterior cruciate ligament injuries in 2, femoro-tibial malalignments in 3, and osteophytosis in 3.

A partial meniscectomy was performed in 6 cases, while in 1 case, the meniscus was repaired. In the patients with ACL injuries, ACL reconstruction was performed with semitendinosus and gracilis tendon grafts and "over the top" technique. In the 3 femoro-tibial malalignments cases, a high tibial osteotomy was performed, while the osteophytes were tangentially resected.

Two patients had previous surgical procedures for cartilage repair by microfractures.

Exclusion criteria were as follows: age <15 years and >50 years, diffuse osteoarthritis, and comorbidities with general medical conditions (diabetes, rheumatoid arthritis), hematological disorders, and infections.

The study protocol was approved by an independent Ethical Committee, and signed informed consent was obtained from all patients participating in this study.

Surgical technique

Step 1: Platelet gel production

About 120 mL of the patient's venous blood was taken and treated the day before surgery with the Vivostat System® (Vivolution, Denmark) in order to provide 6 mL of platelet-rich fibrin gel (PRF) [19, 21, 22].

Step2: Bone marrow aspiration

A total of 60 mL bone marrow aspirate was taken from the posterior iliac crest (Fig. 1), with the patient prone under spinal or general anesthesia. The bone marrow harvesting was performed with a marrow needle (size 11 G × 100 mm) inserted 3 cm deep into the iliac crest marrow. 5 mL of bone marrow was aspirated into a 20-mL plastic syringe internally coated with calcium-heparin solution, repeating the procedure with several perforations into the iliac crest through the same skin opening, until a total of 60 mL of bone marrow aspirate was collected. The marrow was aspirated in small fractions from different points to maximize the harvesting of the marrow stromal cells and to reduce dilution by peripheral blood.

Step 3: Bone marrow concentration

The harvested bone marrow was treated directly in the operating room, by removing most of the erythrocytes and plasma. A cell separator (Smart PReP®, Harvest Technologies Corp., USA or IOR-G1, Novagenit, Mezzolombardo, TN, Italy), (Figs. 2, 3) provided 6 mL of concentrate containing nucleated cells after 15 min of multiple centrifugation cycles.

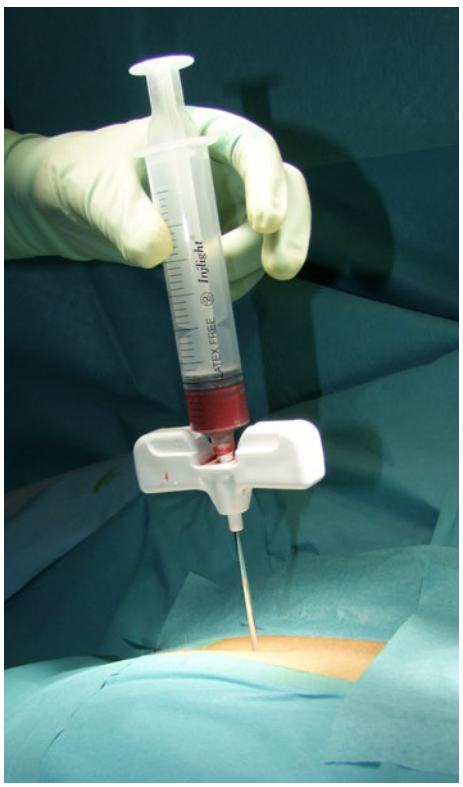

Fig. 1 The bone marrow is aspirated from the posterior iliac crest

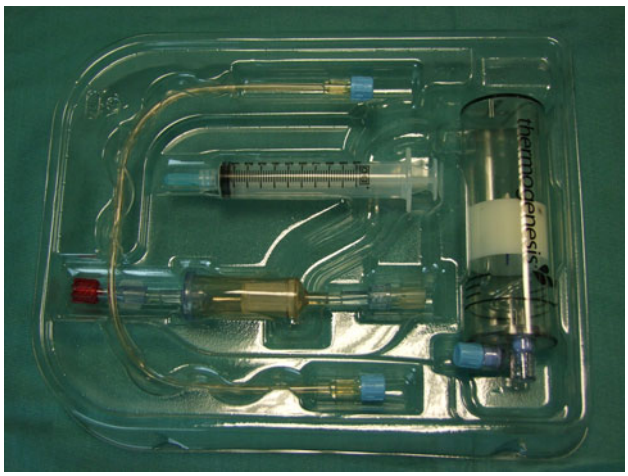

Fig. 2 The IOR-G1 disposable kit is used for the cell concentration directly in OR

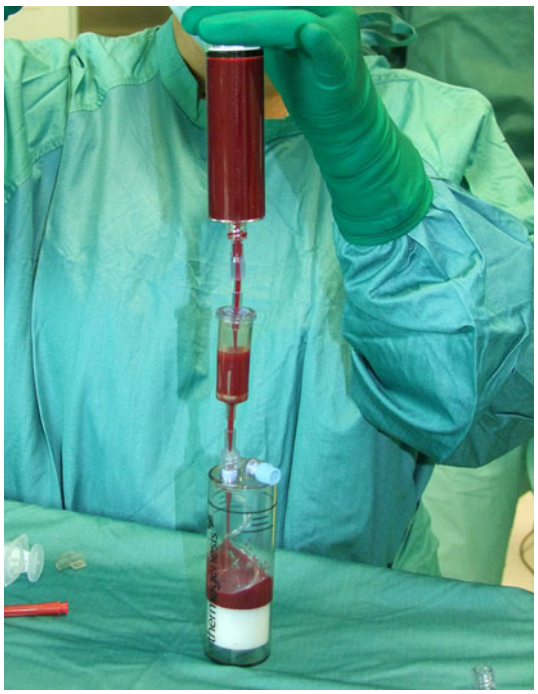

Fig. 3 The aspirated 60 mL of bone marrow are then transferred to the specific disposable device for the nucleated cells concentration

Step 4: Arthroscopic BMDC transplantation

After the bone marrow harvesting phase, a standard knee arthroscopy was performed, with the patient in the supine position.

The cartilage lesion was identified (Fig. 4), and a flipped cannula was inserted into the portal ipsilateral to the lesion to enable insertion of the surgical instrumentations and to retract the fat pad from the operative field [14].

A specifically designed low profile drill (Fig. 5) was used to debride the lesion, resulting in a circular area with regular healthy cartilage margins for biomaterial implantation (Fig. 6).

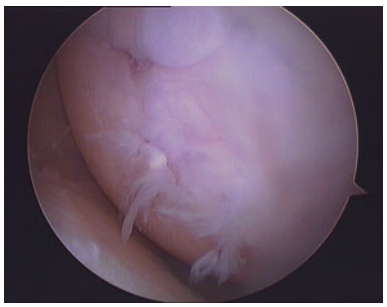

Fig. 4 Arthroscopic view showing osteochondral lesion

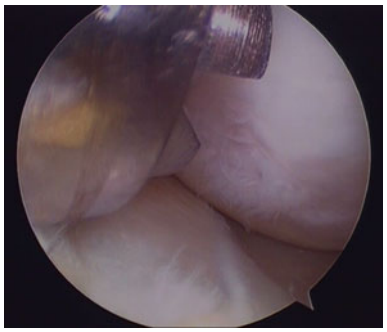

Fig. 5 Arthroscopic view showing a low profile drill used to debride the osteochondral lesion

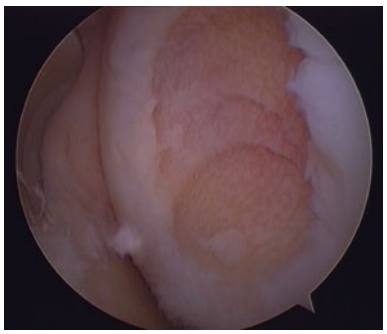

Fig. 6 Arthroscopic view showing the lesion area after the debridement with healthy cartilage margins and subchondral bone

A hyaluronic acid membrane (Hyalofast®, Fidia Advanced Biopolymers, Italy) or collagen membrane (IOR-G1, Novagenit, Mezzolombardo, TN, Italy) was used for cell support. The scaffold was filled with 2 mL of bone marrow concentrate and loaded onto the delivery device (Fig. 7), which was used to position the biomaterial within the defect (Fig. 8). Multiple stamp-sized pieces of membrane can be overlapped in order to cover the whole area.

A layer of PRF was placed onto the implanted material in order to provide growth factors (Fig. 9). Under arthroscopic control, the stability of implanted stamps was evaluated with knee flexions and extensions (Fig. 10).

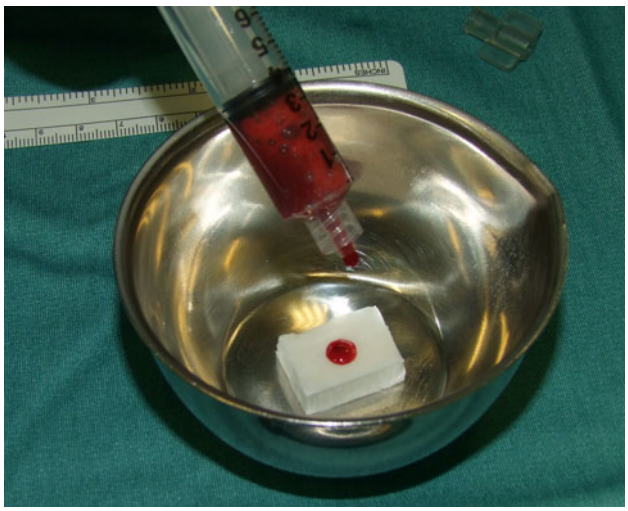

Fig. 7 The collagen scaffold is loaded with 2 mL of bone marrow concentrate

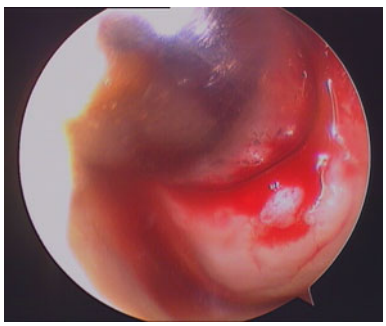

Fig. 8 Arthroscopic view of the biomaterial positioned in the lesion site

Postoperative treatment

The rehabilitation was articulated in different stages:

1. The day after surgery: gradual passive and active mobilization of the knee with no weight-bearing.
2. 4 weeks after the surgery: muscular reinforcement exercises, closed kinetic chain proprioceptive rehabilitation, static and walking exercises with partial and gradual weight-bearing, swimming.
3. 10 weeks after the surgery: open kinetic chain rehabilitation exercises recovery of muscular function, and walking with full weight-bearing, cycling.
4. 6 months after the surgery: light running.
5. 12 months after the surgery: high impact sports.

Follow-up evaluation

Clinical

Assessment was performed before surgery and at 6, 12, 18, and 24 months and after 3 years postoperatively, following the International Knee Documentation Committee (IKDC) score (subjective) and Knee injury and Osteoarthritis Outcome Score (KOOS) questionaries [23, 24].

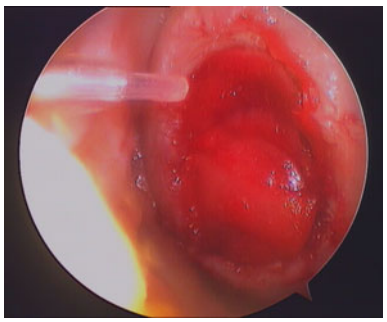

Fig. 9 Arthroscopic view: a layer of PRF was sprayed over the biomaterial in order to provide the growth factors

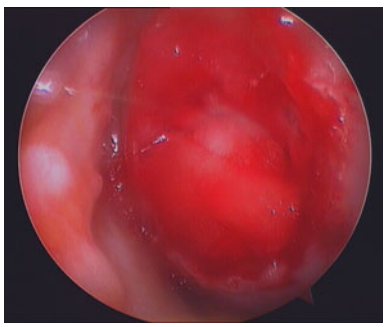

Fig. 10 Arthroscopic view of the final result

MRI

A magnetic resonance imaging scan was performed on all of the patients in the study preoperatively (Fig. 11) and at 6 months, 12 months, and at the final follow-up. Imaging sequences were carried out following the cartilage repair tissue grading scale (Mocart) [25].

Biopsy

After obtaining approval from the local ethics committee and informed consent from the patients, 4 patients underwent a second-look arthroscopy with a biopsy 12 months after surgery. Cartilage specimens were evaluated by hematoxylin/eosin and Safranin-O stainings and collagen type I and II immunohistochemical analyses.

Statistical analysis

The Wilcoxon test, the Mann–Whitney test, and the Student's paired t test were used to test for significant differences between baseline and various follow-up measurements. A p value of <0.05 was considered significant.

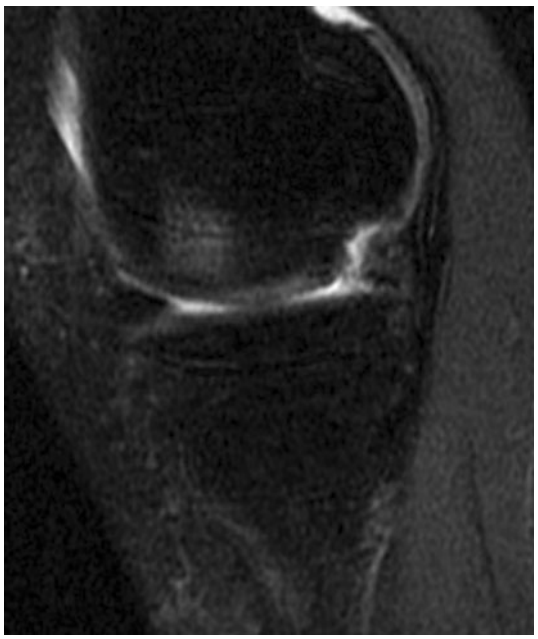

Fig. 11 Preoperative magnetic resonance imaging. Coronal view of a patient with osteochondritis dissecans of the medial femoral condyle

Results

Clinical

Mean IKDC score before surgery was 29.9 ± 13.2 and 85.4 ± 4.2 at 29 ± 4.1 months ($p < 0.005$), while KOOS score before surgery was 29.9 ± 13.2 and 85.4 ± 4.2 at 29 ± 4.1 months ($p < 0.0005$).

Patients' age at the time of surgery, gender, and size of the cartilage defect did not affect the results.

The clinical improvement was statistically significant both for IKDC and KOOS scores at each follow-up, with increased clinical improvement over time.

Associated procedures significantly affected IKDC score at 12 months ($p < 0.005$), while no differences were found at final follow-up.

Imaging

The control MRI at 12 and 24 months follow-up showed regeneration of the subchondral bone and the cartilaginous tissue in different parameters of the MOCART score (Fig. 12).

A significative relationship was found between KOOS score at 24 months and signal intensity ($p < 0.05$). Patients with a hyperintense signal (11 patients) had a KOOS score of 87 ± 7 at 24 months follow-up, while patients with an Isointense signal (19 patients) had a KOOS score of 95 ± 5.

No other significative relationships were found between clinical score and MOCART parameters.

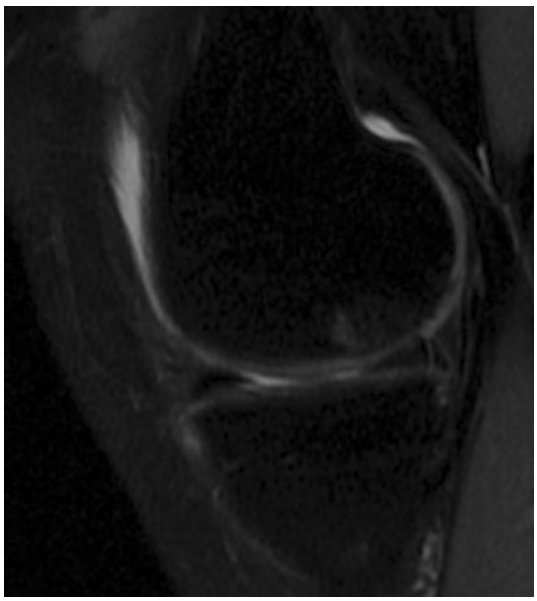

Fig. 12 Postoperative magnetic resonance imaging. Coronal view of the same patient at 24 months of follow-up

Histological analysis

Safranin-O staining showed a biopsy of the regenerated tissue obtained at 12 months showed in the two biopsies a cartilaginous tissue characterized by the presence of a proteoglycan rich matrix particularly in the middle and deep zone and high collagen layer in the superficial zone. The superficial layer was almost regular. The subchondral bones and the tide marks were well evident. Hematoxylin and eosin staining showed the presence of cells homogenously distributed throughout the tissue, regularly columnarized in the deep one.

Immunohistochemical analysis showed in both specimens a positivity for collagen type II throughout the entire thickness of the biopsies, while collagen type I was negative (data not shown).

Complications

Neither intraoperative nor postoperative complications were observed in this series.

Discussion and conclusions

ACI technique, introduced in 1994 by Brittberg et al. [26–33], has been showed to regenerate cartilage tissue with similar biomechanical properties to the surrounding healthy cartilage, and biomechanically superior to regenerated cartilage induced by other techniques.

In various studies, reliable and durable clinical results have been reported both with the use of the open field surgery

and more recently matrix-based techniques permitted an entirely arthroscopic technique [6–18]. The necessity of performing two surgical procedures and high cost, associated with cell expansion, are the weaknesses of ACI [34, 35].

Mesenchymal stem cells represent 2–3 % of the total mononuclear cells in bone marrow and have the ability to differentiate into various lineages, including osteoblasts and chondroblasts [36–41]. The rationale of the "one-step technique" is based on the idea to transfer into the lesion site not only mesenchymal stem cells, but the entire bone marrow cellular pool [19]. This allows not to loose "regenerative potential" present in the bone marrow and cells to be processed directly in the operating room without the need for a laboratory phase and allows BMDC transplantation to be performed in "one step" instead of the two required for ACI [42–44].

The results obtained in this study with the one-step technique in OLK repair are comparable to those achieved with treatment ACI for similar lesions in OLK disease [6, 7, 13, 45].

A significant improvement in both IKDC and KOOS score from preoperative to each follow-up considered was found ($p < 0.005$). Patients with associated procedures experienced a delay in their clinical improvement between 6 and 12 months, though the findings at final follow-up were not affected.

Magnetic resonance imaging examination showed promising growth of bone and cartilage, nearly complete defect filling and satisfactory integration of the graft at follow-up in 80 % of cases. Among all the considered MRI parameters, only signal intensity was significantly correlated with the KOOS score at 24 months follow-up.

Both biopsy specimens showed a regenerated cartilage tissue in an advanced remodeling phase. Immunohistochemical analysis confirmed the presence of proteoglycans and type-II collagen, well-recognized markers of hyaline cartilage. The number of cases treated is still limited, but the results to date are promising in a high percentage of patients from the clinical, imaging, and histological perspectives, making the one-step technique a concrete part of the "cartilage repair paradigm".

Conflict of interest None.

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