



One-step cartilage repair in the knee: Collagen-covered microfracture and autologous bone marrow concentrate. A pilot study



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ARTICLE INFO

Article history:

Received 27 August 2013

Received in revised form 21 October 2014

Accepted 28 October 2014

Keywords:

Cartilage

Bone marrow concentrate

Knee

Arthroscopy

Tissue engineering

Scaffold

ABSTRACT

Background: Different single-stage surgical approaches are currently under evaluation to repair cartilage focal lesions. To date, only little is known on even short-term clinical follow-up and almost no knowledge exists on histological results of such treatments. The present paper aims to analyze the clinical and histological results of the collagen-covered microfracture and bone marrow concentrate (C-CMBMC) technique in the treatment of focal condylar lesions of knee articular cartilage.

Methods: Nine patients with focal lesions of the condylar articular cartilage were consecutively treated with arthroscopic microfractures (MFX) covered with a collagen membrane immersed in autologous bone marrow concentrate (BMC) from the iliac crest. Patients were retrospectively assessed using several standardized outcome assessment tools and MRI scans. Four patients consented to undergo second look arthroscopy and biopsy harvest.

Results: Every patient was arthroscopically treated for a focal condylar lesion (mean area 2.5 SD(0.4) cm²). All the patients (mean age 43 SD(9) years) but one experienced a significant clinical improvement from the pre-operative condition to the latest follow-up (mean 29 SD(11) months). Cartilage macroscopic assessment at 12 months revealed that all the repairs appeared almost normal. Histological analysis showed a hyaline-like cartilage repair in one lesion, a fibrocartilaginous repair in two lesions and a mixture of both in one lesion.

Conclusions: The first clinical experience with single-stage C-CMBMC for focal cartilage defects in the knee suggests that it is safe, it improves the short-term knee function and that it has the potential to recreate hyaline-like cartilage.

Level of evidence: IV, case series.

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1. Introduction

Focal cartilage defects are a common cause of knee symptoms and disability and may progress to osteoarthritis (OA) [1,2]. To be effective a cartilage repair procedure should recreate hyaline-like cartilage and ultimately prevent OA [3].

The limits of the microfracture (MFX) treatment with respect to lesion size and to long term functional improvements [3,4] and the high cost and the need for two operations of the autologous chondrocyte implantation (ACI) and ACI-related procedures [5] have prompted the search for new one-step cartilage repair methods. Autologous matrix-induced chondrogenesis (AMIC) has emerged as a new technique adopting a collagenic scaffold combined with microfractures [6]. Similar procedures have been developed, adopting synthetic polymers like polyglycolic acid (PGA)/hyaluronan in combination with microfracture and have shown the potential to restore hyaline-like cartilage [7–9].

In all these techniques microfracture should permit the migration of mesenchymal stem cells (MSCs) from the subchondral bone, and the scaffold should keep cells in situ and serve as support for tissue differentiation.

The intra-articular delivery of bone marrow concentrate (BMC) and marrow aspirate improved the outcomes of microfracture in full thickness cartilage defect in the horse model [10] and in the goat model [11] respectively. This observation may be possibly explained by the fact that the BMC from the iliac crest contains higher concentration of MSCs with respect to tibial or femoral bone marrow blood, and with greater doubling potential [12]. This observation has led to modifications to the original single-stage technique involving the addition of BMC to treat talar osteochondral lesions [13].

In the present study cartilage lesions have been treated with the association of MFX, BMC from the iliac crest and a collagenic coverage scaffold. The aim of this study was to analyze clinical and histological outcomes of collagen-covered microfracture and bone marrow concentrate (C-CMBMC) for the treatment of patients with full-thickness, focal, condylar knee cartilage defect.

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2. Materials and methods

2.1. Study design

From February 2008 to March 2011, nine consecutive patients with symptomatic chondral lesions of the knee underwent all-arthroscopic C-CMBMC. After ethical committee approval, full informed consent was obtained from each patient. Inclusion criteria were: lesion size ≥ 1.5 cm², age ≤ 60 , chondral defect Outerbridge type III or IV, adherence to the rehabilitation protocol, full anagraphics available, signed consent, and full surgeon report available. Exclusion criteria were tibiofemoral or patellofemoral malalignment, knee instability, kissing lesions, advanced OA, rheumatic, metabolic or neoplastic diseases. Every patient, after informed consent, was asked to undergo a second look arthroscopy with biopsy for assessing the state of the repair at 12 month follow-up. Failure was defined as the need of a new surgical procedure to treat persisting pain or effusion in the previously operated knee. Patients were retrospectively analyzed with standardized assessment tools such as the International Knee Documentation Committee (IKDC) score, the Lysholm score the VAS and the Tegner activity scale. Patients were also evaluated with MRI scans at variable follow-up.

2.2. Surgical technique

The CMBMC surgical technique has been described in detail by Gigante et al. [14]. Briefly, a small area over the iliac crest donor site was draped. After diagnostic arthroscopy to confirm the indication for the procedure a 2.5 mm Jamshidi needle was inserted percutaneously into the iliac crest. Sixty milliliters of bone marrow blood were aspirated and processed with the MarrowStim Concentration kit (Biomet, Warsaw, IN) according to the manufacturer's instructions, obtaining 3–4 mL of BMC.

The chondral lesion was debrided and microfractures were performed using appropriate awls. The lesion main dimensions were measured and reported on a rubber template that was then adjusted to the exact shape of the defect. A Biocollagen MeRG® collagen membrane (Bioteck, Vicenza, Italy) was cut to match the defect shape and immersed in BMC until implantation.

The water flow was stopped and water was aspirated from the joint cavity. A 10:one mixture of 1–2 mL fibrin glue and BMC was laid on the lesion bed using a long needle. The membrane was inserted through the appropriate portal with a grasper and fitted into place with a probe. An additional 2–3 mL of the fibrin glue-BMC mixture was injected over the membrane and left to solidify for 2–3 min. Finally, the excess of the fibrin glue-BMC mixture was removed and the knee repeatedly flexed and extended to check membrane stability.

The patients started continuous passive motion on days 4–5 and partial weight-bearing at 3 weeks, progressing to full weight-bearing at 6 weeks. Isometric quadriceps and hamstrings training and straight leg raising were advised during the non-weight-bearing period. Light sports activities such as swimming, cycling or jogging on even soft

ground were allowed at 6 months. Permission to participate in unrestricted sports activity was given after 12 months.

2.3. Second-look arthroscopy

Four patients consented to second-look arthroscopy and biopsy harvest. Biopsies were performed with a standard 2.5 mm diameter Jamshidi needle (Fig. 1a). The specimens were placed in 10% formalin and sent for histology processing. The quality of the implanted tissue was evaluated by the surgeon using the criteria of the International Cartilage Repair Society [15] Cartilage Repair Assessment (CRA) [15].

2.4. Histology

Histological and histochemical characteristics of the repair tissue were evaluated. Specimens were decalcified, paraffin-embedded and stained with Safranin-O to detect the presence of glycosaminoglycans. Polarized microscopy was used to discriminate between hyaline-like cartilage and fibrocartilage. The ICRS II Histology Scoring System [16] was used to evaluate the quality of the repair tissue. Histological evaluation was performed blindly by two different investigators and scores were averaged.

2.5. Statistical analysis

The Student *t*-test was performed for the IKDC score, the Lysholm score and the VAS to compare pre- and postoperative values. Data are expressed as means with standard deviations. The nonparametric Wilcoxon-signed rank test was performed for the Tegner activity scale to compare pre- and postoperative values. Data are expressed as medians and interquartile ranges. For all tests, $p < 0.05$ was considered significant. The statistical software SPSS (Version 17.0) was used for biometric analysis.

3. Results

3.1. Clinical outcome

No patient-related complications nor device-related complications were encountered. None of the patients was lost at follow-up. All patients followed the standardized rehabilitation protocol. Patients' characteristics and patient-reported outcomes are shown in Table 1. Associated intervention at the time of surgery was two (one partial meniscectomy and one synovectomy). A patient with an unshouldered cartilage defect required a mini-arthrotomy to fix the membrane with polar stitches.

A statistically significant improvement in the mean IKDC subjective score from 49 SD(11) to 82 SD(12), mean Lysholm score from 58 SD(13) to 88 SD(11) and mean VAS from 7.6 SD(1) to 2.3 SD(2.2) from preoperative values to the latest follow-up were obtained ($p < 0.05$) (see Table 1 for single values). The median Tegner activity scale showed no significant difference from pre-injury level of 4 (interquartile range 4–7) to post-operative level of 4 (interquartile range 3.5–6.5) at latest follow-up ($p > 0.05$) (see Table 1 for single values). On the other hand, a significant increase in the activity level from post-injury 2 (interquartile range 2–3) to post-operative was observed at latest follow-up ($p < 0.05$).

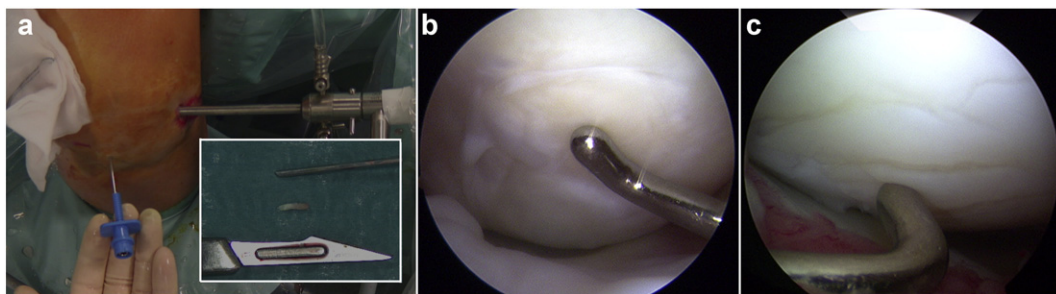


Fig. 1. Second-look arthroscopy and biopsy harvest. a) The Jamshidi needle is inserted from the appropriate portal and the bioptic cylinder is harvested. b) Second look biopsy showing a repair in level with the surrounding cartilage, completely integrated, with a smooth surface and a slightly fibrillated border. c) Repair in level, with a slightly demarcating border and minute fissurations.

Table 1
Patient demographics and patient-reported outcome data.

Patient	Age	Sex	FU	Side	Size (cm ²)	Previous surgery	Associated pathology	Associated procedure	Lysholm pre-op.	Lysholm post-op.	IKDC pre-op.	IKDC post-op.	VAS pre-op.	VAS post-op.	Tegner pre-injury	Tegner pre-surgery	Tegner post-surgery
1	52	f	32	MFC	2.1	2: IM, Sh	Chondral lesion II° P/F	-	67	90	51	86	7	3	4	2	4
2	48	f	32	MFC	2.8	1: IM	chondral lesion II° P/F	-	40	96	38	91	7	2	3	2	3
3	36	m	28	MFC	3.0	1: IM	-	-	67	99	57	95	7	3	7	4	7
4	53	m	28	MFC	2.3	3: IM, LCA Cyclop les.	Cyclop lesion, Lat. meniscus rupture, chondral lesion II° F/R	Meniscectomy	-	-	-	-	-	-	-	-	-
and shaving	70	78	61	72	9	6	4	2	4	4	32	84	9	0	4	2	4
5	41	f	35	MFC	1.8	2: IM, Sh	Synovitis	Synovectomy	40	94	32	84	9	0	4	2	4
6	48	f	28	LFC	2.3	1: IM	Chondral lesion II° F/R and LTP	-	51	100	39	98	8	0	6	2	6
7	35	m	14	MFC	3.0	1: IM	-	-	75	68	63	61	6	8	4	2	2
8	48	m	14	MFC	2.8	3: LCA + IM + MFX, ACL, IM	-	Mini arthroscopy	50	81	54	78	8	3	7	3	6
9	28	m	51	LFC	2.2	1: LM	-	-	59	82	49	78	7	2	9	3	9

FU = follow-up; MFC = medial femoral condyle; IM = internal meniscectomy; Sh = chondral shaving; LCA = anterior cruciate ligament reconstruction; P/F = patello-femoral; MFX = microfracture; ACL = autologous chondrocyte implantation; LTP = lateral tibial plateau; LFC = lateral femoral condyle; LM = lateral meniscectomy.

One patient (# 9) has met the definition of failure, having undergone a successive surgical procedure for persisting effusion. The patient underwent another cartilage repair procedure in the lateral femoral condyle at another institution; this patient (latest VAS = 2, latest Lysholm 82) was not in pain and had a significant improvement from baseline (VAS 7, Lysholm 58), however complained frequent knee effusions after practicing competitive soccer. Another patient (# 7) who did not meet the definition of failure had a worsening of pain and symptoms from baseline (VAS 6, Lysholm 75) to the latest follow-up (VAS 8, Lysholm 68). However his knee function was improved after a cycle of intra-articular platelet rich plasma injections.

Three post-operative MRI scans were retrieved with a mean of 7 SD(1.5) month follow-up (range 6–9 months). They have showed in all cases the reconstitution of the original cartilage level (Fig. 2). Similarly, bone marrow edema and/or subchondral irregularities were observed in all the cases (Fig. 2). Non-homogeneous cartilage signal was observed in two out of three cases; fissurations were noted in two out of three cases, surface irregularities in one out of three cases.

3.2. Arthroscopic evaluation

At the time of the second-look arthroscopy (Fig. 1) all the patients but one were asymptomatic. According to the ICRS CRA evaluation all the four C-CMBMC patients were classified as nearly normal (grade 2), the median of the overall assessment being 9.5 (range 9–11).

3.3. Histological evaluation

According to the ICRS II score, the four C-CMBMC biopsies scored a mean overall of 64 SD(13), with a mean tissue morphology of 63 SD(19). Hyaline-like matrix was found in only one case (Fig. 3d), fibrocartilage was found in two cases (Fig. 3a, c) and a mixture of hyaline/fibrocartilage was found in one case (Fig. 3b), with hyaline-like cartilage next to the osteochondral junction (Fig. 3j) and fibrocartilage next to the articular surface (Fig. 3f). Even when hyaline-like cartilage was found, the columnar cell arrangement typical of normal articular cartilage was never observed (Fig. 3d). The collagen membrane was completely reabsorbed in all the bioptic samples cases.

4. Discussion

With a mean of 29 months follow-up, the C-CMBMC technique was shown to be safe and effective in improving symptoms of patients affected by isolated condylar cartilage lesions, and has the potential to induce hyaline-like cartilage repair.

In recent years one-step cartilage repair has become increasingly adopted to treat chondral knee defects [8,9,17–20]. The diverse techniques in use mainly differ for the type of scaffold adopted (collagenic [6,17,19,20] or PGA/hyaluronan based [8,9,18]), for the eventual platelet rich plasma (PRP) augmentation [8,17] and for the surgical approach (arthroscopic [8,9,17] or mini-open [8,9,17–20]).

The original AMIC (autologous matrix-induced chondrogenesis) associated MFX, a porcine collagen type I/III membrane and the injection of fibrin mixed with autologous serum underneath the scaffold [21].

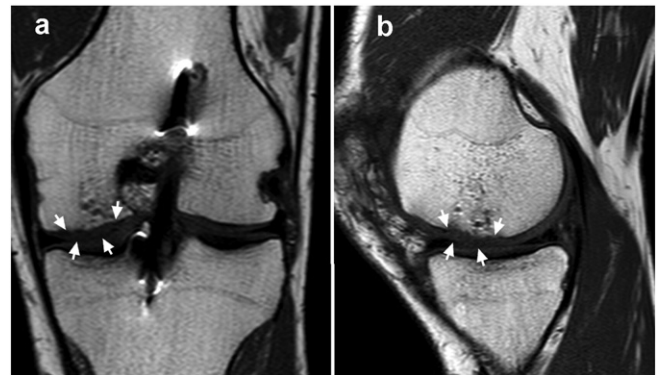


Fig. 2. Postoperative MRI scans representative of the average quality of cartilage repair. a, b) The T1 coronal and sagittal sections (6 months post-operatively) of the left knee show complete defect filling of the medial compartment defect (arrows), isointense cartilage signal, minor subchondral junction irregularities and the presence of small bone cysts. Moderate marrow edema was visible with T2 sequences (not shown). The patient (48 years old at the time of the C-CMBMC procedure) had previously undergone both microfracture and a ACL procedure on the same site.

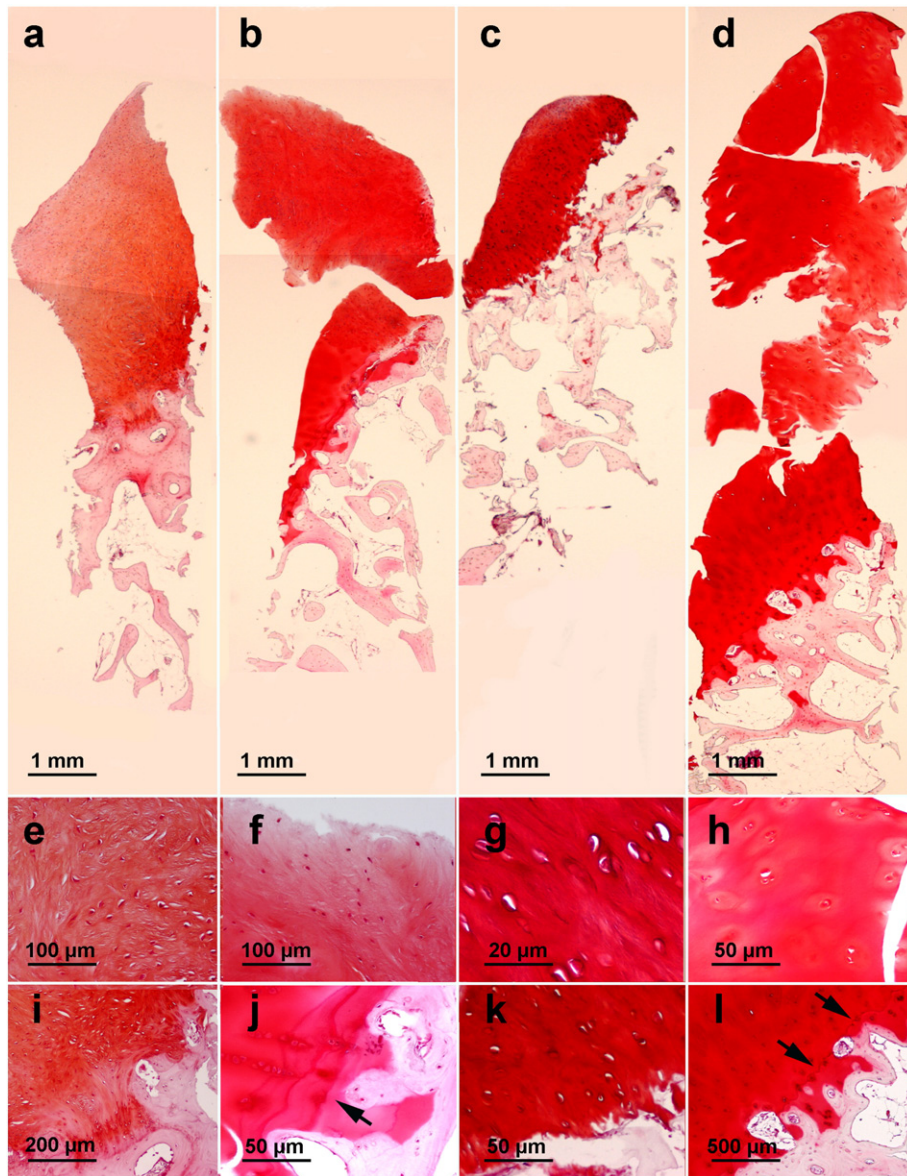


Fig. 3. Biopsies stained with Safranin-O. Each column represents a single biopsy; lines a–d represent the entire bioptic cylinder; lines e–h represent the chondral matrix; and lines i–l represent the osteochondral junction. Biopsies a and c represent a fibrocartilaginous repair. Cells are in fact clearly chondrocytes, but collagen fibers can be seen running within the matrix in different directions (e, g). It has to be noted that the thickness of biopsy c is reduced. Biopsy d represents hyaline-like cartilage repair. It shows chondrocytes in large, round lacunae and a glassy matrix with a metachromatic staining for Safranin-O (h) and tide mark reconstitution (l, arrows). Biopsy b represents a mixed hyaline-like/fibrocartilaginous repair where fibrocartilage may be observed toward the articular surface (f) and a glassy matrix with the tide mark reconstitution may be observed close to the osteochondral junction (j, arrows). Picture d shows large clefts in the chondral substance which may be artifacts from biopsy harvesting or sample cutting.

Large (mean area 4 cm²) chondral defects have been treated with such technique obtaining a significant clinical improvement at an average of 37 month follow-up. However, the quality of the regenerated tissue and the level of tissue filling were not ideal. Around one out of three MRI scans revealed incomplete defect filling and subchondral bone abnormalities [6].

Kusano et al., in a retrospective study on autologous matrix-induced chondrogenesis (AMIC) did not observed a significant clinical improvement in patients treated for condylar cartilage defects. Moreover, half of the patients treated for patellar defects required mobilization under anesthesia due to knee stiffness. The authors found inconsistent tissue regeneration. MRI scans revealed some complete filling, some empty defect and some hypertrophic repair [20].

Efe et al. reported on the prospective clinical and MRI follow-up of a three-dimensional collagen gel. The surgical technique did not involve microfracture and relied on chondrocyte migration from the surrounding

healthy cartilage. The authors treated 1 cm² lesions and reported significant clinical and MRI improvements [19].

Siclari et al. treated 52 patients with the association of subchondral perforations and PRP-augmented PGA/hyaluronan scaffold. The authors reported a statistically significant clinical improvement at 12 month follow-up [8]. Dhollander et al. reported on a pilot study on the association of microfracture and a PGA/hyaluronan coverage scaffold enriched by autologous serum. The authors observed noticeable clinical improvement, however MRI scans revealed different percentages of incomplete filling, subchondral bone irregularities, subchondral cysts and intralesional osteophytes [18]. The same group analyzed patients treated with the original AMIC technique in association with PRP. Again, the favorable clinical outcomes were not matched by MRI improvements. At 2 years follow-up the authors reported persistence of subchondral bone abnormalities, incomplete filling or hypertrophy of the repair and intralesional osteophyte formation [17].

In a pilot study similar to one being described, our group has tested the same CMBMC technique adopting a PGA/hyaluronan scaffold rather than a collagen membrane, showing good clinical and functional outcome [22].

In the present study the C-CMBMC patients obtained a statistically significant improvement in all the analyzed assessment tools from baseline to the latest follow-up. MRI scans commonly revealed the persistence of bone marrow edema and subchondral plate irregularities, but also showed a complete defect fill in all the cases (Fig. 2). Overall one patient, required a successive surgical intervention to treat persistence of knee effusions the day after practicing competitive soccer. This patient was not in pain having a VAS of 2 at latest follow-up. However this or a higher percentage of reoperations must be expected when performing cartilage repair procedures [17,18,20,23,24].

More recently peripheral blood progenitor cells or cultured MSCs have been associated with MFX with or without a coverage scaffold to treat knee cartilage defects [25–27]. These procedures have demonstrated to be safe and effective, however they require a first step for the Filgrastim administration and plasma apheresis or for the surgical marrow blood harvest, cell sorting and subsequent culture. Therefore these are not single-stage procedures, require two steps, autologous cell manipulation and are expensive. Moreover the indication for these procedures needs to be confirmed with a previous diagnostic arthroscopy.

Only a few studies have investigated the histological outcomes of one-step procedures in the treatment of articular cartilage lesions [7,8,13,28]. In particular, Giannini et al., associated BMC and PRP gel with a hyaluronic acid membrane or collagen powder to treat talar osteochondral lesions. In this study a functional improvement was observed for all the patients, and three biopsies harvested showed different degrees of tissue remodeling toward hyaline-like cartilage [13]. Siclari et al. performed 10 second look arthroscopies harvesting five biopsies. Macroscopic observation showed a whiter appearance of the repairs, a certain degree of hypertrophy and surface irregularity. Histological evaluation uniformly showed hyaline-like cartilage repair with good subchondral integration [8]. In the present study a nearly normal appearance of the repaired tissue was documented according to the ICRS CRA (Fig. 1b, c). The histological analysis revealed one hyaline-like repair, one mixture of fibrocartilage and hyaline-like cartilage and two fibrocartilaginous repairs (Fig. 3). The percentage of hyaline-like repair in this study is in line with that previously reported for ACI and ACI-related procedures [29,30]. The mean overall ICRS II score of both treatment groups of 64 SD(13) is in line with the one recently reported for ACI-related procedures and higher than that reported for microfracture [3,29]. These histological results indicate that cells derived from autologous BMC and seeded on a scaffold may differentiate into mature chondrocytes and produce a fibrocartilaginous and/or hyaline extracellular matrix when applied in human adult articular cartilage lesions. In particular the presence of hyaline-like cartilage next to the osteochondral junction in the mixed hyaline/fibrocartilage repair (Fig. 3b, f, j) could indicate progressive bottom-to-top cartilage remodeling and maturation [31,32]. These in vivo observations confirmed some in vitro results that demonstrated that human MSCs from bone marrow aspirate can proliferate on collagen scaffolds and differentiate into chondrocytes without growth factor supplementation [33]. Even though both MFX procedures and one-step procedures adopting scaffolds have been associated with bone osteophytes formation on the bed of the lesion [17,18], this was not observed in our patient group neither arthroscopically nor histologically. Since the addition of BMC to the mentioned procedures could theoretically increase this possibility, a longer follow-up is mandatory to explore the potential onset of such bone hypertrophy.

The mean age of the overall population was 43 SD(9) years (range 28–53). Therefore it may be hypothesized that some degree of degenerative changes occurred at least in some of the patients. However, cartilage repair techniques have been adopted to treat patients with early

OA, demonstrating the capability to improve the symptoms and delay the need for prosthetic replacement [34]. Moreover, if compared to the original ACI, one-step procedures are relatively inexpensive and have been used in older patients (up to 65 years-old) providing pain relief and good histological results [8].

Limitations of this study are small sample size, lack of control group, and short-term follow up. Moreover the patients were not stratified for presence of early OA with preoperative plain X-ray. The strength of the present study is that isolated condylar lesions of similar size were treated in the absence of limb malalignment and major associated confounding procedures such as ACL reconstruction or unloading osteotomies. This study also provides biopsies which represent an objective assessment of the C-CMBMC cartilage repair capabilities.

In summary our clinical and histological data suggest that the arthroscopically performed C-CMBMC procedure was safe and provided short-term significant pain relief and functional improvement. A nearly normal arthroscopic appearance of the repair and the potential to regenerate hyaline-like cartilage were documented. A complete fill of the defect was shown by MRI imaging and second-look arthroscopies. A complete re-evaluation of the patients with MRI, functional scores and failure rate has been planned at 5 years follow-up.

Conflict of interests

The authors declare no conflict of interests.

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