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Cartilage repair evolution in post-traumatic osteochondral lesions of the talus: From open field autologous chondrocyte to bone-marrow-derived cells transplantation☆

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ABSTRACT

The aims of this study are to describe evolution in cartilage repair from open field autologous chondrocyte implantation to regeneration by arthroscopic bone-marrow-derived cells (BMDCs) "one step" technique; to present the results of a series of patients consecutively treated and to compare in detail the different techniques used in order to establish the advantages obtained with the evolution in cartilage regenerative methods.

81 patients (mean age 30 ± 8 years) were evaluated in this study. Patient assessment included clinical AOFAS score, X-rays and MRI preoperatively and at different established follow-ups. All the lesions were >1.5 cm^2 and received open autologous chondrocyte implantation (10 cases), arthroscopic autologous chondrocyte implantation (46 cases), and "one step" arthroscopic repair by BMDC transplantation (25 cases). For arthroscopic repair techniques a hyaluronic acid membrane was used to support cells and specifically designed instrumentation was developed. Patients of all the three groups underwent a second arthroscopy with a bioptic cartilage harvest at 1 year follow-up.

Mean AOFAS score before surgery was 57.1 ± 17.2 and 92.6 ± 10.5 ($P < 0.0005$) at mean 59.5 ± 26.5 months. A similar pattern of AOFAS improvement in results was found in the three different techniques. Histological evaluations highlighted collagen type II and proteoglycan expression.

The cartilage repair techniques described were able to provide a repair tissue which closely approximates the characteristics of the naive hyaline cartilage. Evolution in surgical technique, new biomaterials and more recently the use of BMDCs permitted a marked reduction in procedure morbidity and costs up to a "one step" technique able to overcome all the drawbacks of previous repair techniques.

Introduction

Osteochondral lesions are defects of the cartilaginous surface and underlying subchondral bone of the talar dome.[41,54] These lesions are often caused by a single or multiple traumatic event, mostly inversion or eversion ankle sprains in young, active patients.[7,13,55]

Due to poor hyaline cartilage repair capability, osteochondral lesions of the talus may lead to chronic symptoms with a reported frequency ranging from 17% to 50%.[3,11,14,34,48] In fact, deep ankle pain associated with weight bearing, limited range of motion, stiffness, catching, locking and swelling of the affected joint, are widely documented as a consequence of an osteochondral lesion. These symptoms place the ability to walk, work and perform sports at risk, and early osteoarthritis may develop.[3,11,14,34,48]

Cartilage repair in osteochondral lesions is now more than ever a hot topic for research.

Various surgical options have been proposed to restore an adequate cartilaginous layer in osteochondral lesions, but amongst them, only few have shown the ability to provide repair of the lesion site with hyaline cartilage.[8,9,40,47,56] Hyaline cartilage repair may be obtained by cartilage replacement (OATS, mosaicplasty),[2,31,32,48] or with techniques aimed to generate a newly formed cartilage: autologous chondrocyte implantation (ACI) or bone marrow derived cells (BMDCs) transplantation.[19,23,24,35,42,50,51,57]

☆ Level of Evidence: Level IV, therapeutic study. See Guidelines for Authors for a complete description of levels of evidence.

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Table 1
Characterisation of patients and lesions.

	Cases	Age	Follow up	Size (cm^2)	Previous surgery	Associated procedures	Location of the lesion
Open field ACI	10	25.8 ± 6.4	119.3 ± 6.1	3.1 ± 1.3	2 reparative techniques (micro-fractures) 2 arthroscopic debridment	1 sovramalleolar osteotomy 1 cancellous bone grafting	5 medial 5 lateral
Arthroscopic ACI	46	31.4 ± 7.6	57.5 ± 14.5	1.6 ± 0.6	10 reparative techniques (microfractures, chondroabrasion, drilling) 4 arthroscopic debridment 1 mosaicplasty 1 lateral ligament reconstruction	6 cheilectomy 5 cancellous bone grafting 1 first metatarsal osteotomy for cavus foot correction	35 medial 7 lateral 4 double
BMDCs implantation	25	28.2 ± 9.4	39.2 ± 1.9	2.18 ± 0.5	4 microfractures 3 debridement 2 ACI	1 calcaneal osteotomy for flat foot correction	

Whilst smaller size lesions may be satisfactorily repaired by fibrocartilage, chronic osteochondral lesions of the talus type II or IIA[5] (lesions with damage of the cartilaginous surface and underlying subchondral bone, $\geq$1.5 cm^2 in diameter and <5 mm or >5 mm in depth), are generally considered to require a cartilage repair with hyaline tissue, in order to prevent arthritis progression.[20,22,29]

Cartilage regeneration is a field in rapid evolution due to the investigation on cells, growth factors and biomaterials.[15,16,26–28,38,45] Recently, BMDCs have been indicated as a new option for the treatment of articular osteochondral defects.[46] Thanks to these advancements, regenerative techniques are quickly moving from traditional periostium based ACI to BMDCs transplantation in conjunction with platelet gel and engineered scaffolds able to support multipotent cells growth and differentiation.[46,53]

The aims of this study are to describe evolution in cartilage repair from open field autologous chondrocyte implantation to regeneration by arthroscopic BMDCs transplantation (“one step” technique), to present the results of a series of 81 patients consecutively treated by three different techniques, according to the state of the art at the moment of surgery, and to compare in detail the results, the morbidity and the costs of each technique used in order to establish the advantages obtained with the evolution in cartilage regenerative methods from open ACI to BMDC.

Materials and methods

81 patients (47 males and 34 females, mean age 30 ± 8 years), with focal osteochondral monolateral lesions of the talar dome, were treated between November 1997 and January 2007. Surgery was indicated in osteochondral lesions of the talar dome rated as Chronic Type II or IIA.[22]

The mean depth of the lesion was 4.0 ± 0.9 mm. The lesion was located medially in 55 and laterally in 26 cases. In all the patients the lesion had a definite post-traumatic origin. Two patients had a previous tibial fracture, and eight patients had an ankle fracture. The minimum follow up was 36 months, and the maximum was 130 months.

Patients younger than 15 years or older than 50 years, patients with osteoarthritis or kissing lesions of the ankle, and patients with rheumatoid arthritis were excluded from this study.

Malalignment of the lower limb and the presence of joint laxity were considered relative contraindications to be corrected if present.

Surgical techniques

Cartilage repair was performed according to the evolution of the technique at the moment of surgical treatment.

All the surgeries have been performed after the approval of the study protocol by an Independent Ethical Committee, and signed informed consent was obtained by all the patients.

Characterisation of patients and lesions is reported in Table 1.

Cartilage repair by ACI

First step surgery (56 cases)

A first step for cartilage harvesting was required in all the patients treated by ACI. A small sample of cartilage (15–25 mg cartilage tissue) was harvested arthroscopically from the ipsilateral knee for cell culturing in the first 14 cases. In order to minimise the harvesting site pathology, a different cell source was attempted: the osteochondral detached fragment was proven to be a viable source of cells for ACI.[21] In the last 42 cases, the cartilage was harvested directly from the affected ankle in a first step arthroscopy, which also allowed a direct evaluation and accurate measurement of the lesion.

Cartilage was sent to the laboratory for cell expansion and was available for implantation 4 weeks later.

Second step surgery

Open field ACI (10 cases)

Surgical approach to the lesion was transmalleolar, medial or lateral depending on the location of the osteochondral defect. Once the lesion was adequately exposed the fragment and the damaged cartilage were removed up to a sharply-defined rim of healthy cartilage. A measurement of the lesion and determination of the diameter and shape of the periosteal flap to be obtained was performed. A periosteal flap of adequate size, harvested either from the proximal or distal tibia, was fixed over the cartilaginous gap with reabsorbable 6-0 suture Vicryl® (Polyglactin 910) threads and the suture was sealed with Tissucol® fibrin glue. The chondrocytes in liquid media were, then transplanted through a hole deliberately left open and finally sutured and sealed with fibrin glue. The malleolar osteotomy was finally repaired (Fig. 1).

Arthroscopic ACI (46 cases)

An important evolution in the surgical technique was permitted by introduction, thanks to tissue engineering, of a biodegradable scaffold based entirely on the benzylic ester of hyaluronic acid for cell support and proliferation (HYAFF 11, Fidia Advanced Biopolymers, Italy). This non-woven three-dimensional structure consists of a network of 10–15 μm thick fibres with interstices of variable sizes which constitute an optimal physical support to allow cell-to-cell contact, cluster formation, and extracellular matrix deposition.[1,10]

In order to permit an entirely arthroscopic procedure in the ankle, where the tangential perspective makes it uneasy compared

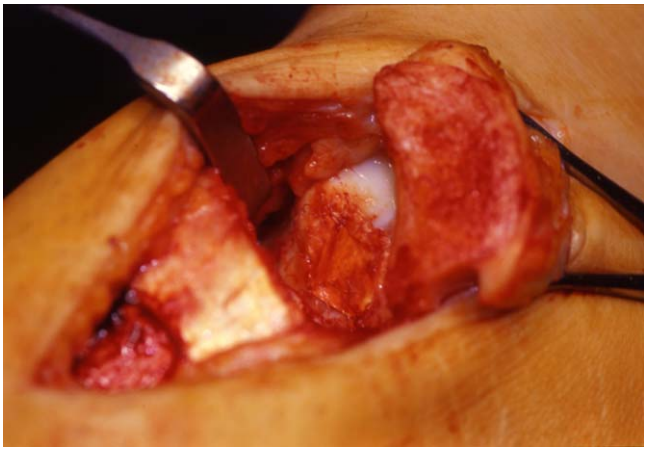

Fig. 1. Autologous chondrocytes implanted into the lesion site and covered by a periosteal flap.

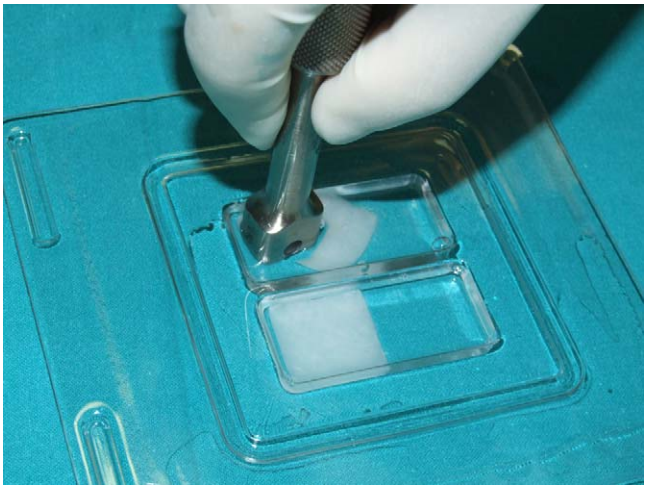

Fig. 3. A portion of hyaluronic acid auto-adesive membrane containing the cultured autologous chondrocytes is cut into the appropriate size.

to the knee, a custom made specific instrumentation was designed by the authors (CITIEFFE, Calderara di Reno, Italy). This consisted of an 8 mm diameter and 111 mm long stainless steel cannula with a window on one side and a positioner specifically designed to slide inside the cannula delivering the scaffold directly to the site of lesion.[23]

Arthroscopic antero-lateral and antero-medial approaches were used. The lesion site was trimmed to safeguard the integrity of the subchondral bone and a sharp rim of healthy cartilage was defined and measured using a millimetred probe (Fig. 2). The autologous chondrocytes, seeded on hyaluronic acid auto-adhesive membrane (HYAFF-11®) (Fig. 3), were arthroscopically positioned on the lesions with a specific custom-made cannula (Figs. 4 and 5).

BMDCs "one step" arthroscopic transplantation (25 cases)

The necessity of two operations and the high costs required by ACI procedure prompted the search of new methods for cartilage repair. Autologous bone marrow derived cells are multipotent cells able to differentiate into both cartilaginous and osseous lineages, and can safely be harvested from the patient's iliac crest and concentrate in the operating room.

Platelet gel production

In order to provide a supplement of growth factors,[30,37] autologous platelet gel was used in this technique. 120 mL of the patient's venous blood were harvested and processed the day before surgery with the Vivostat System® (Vivolution, Denmark) to provide 6 mL of platelet-rich fibrin gel (PRF).

Bone marrow aspiration

A total of 60 mL bone marrow aspirate was harvested from the posterior iliac crest, with the patient in prone decubitus (Fig. 6).

Bone marrow concentration

The harvested bone marrow was processed directly in the operating room, by removing most of the red cells and plasma with a cell separator (Smart PReP®, Harvest Technologies Corp., USA) in order to obtain 6 mL of concentrate containing nucleated cells (stem cells, monocytes, lymphocytes, and other bone marrow resident cells) (Fig. 7).

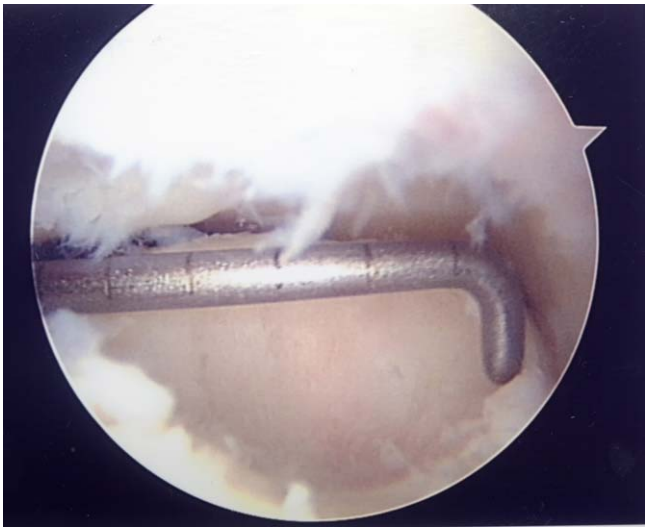

Fig. 2. The lesion site is measured using a millimetred probe.

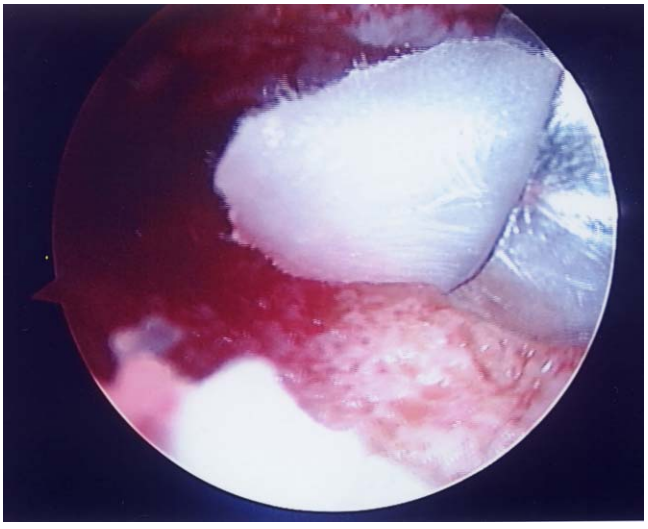

Fig. 4. The membrane patch is arthroscopically positioned on the lesions with a specific custom-made cannula.

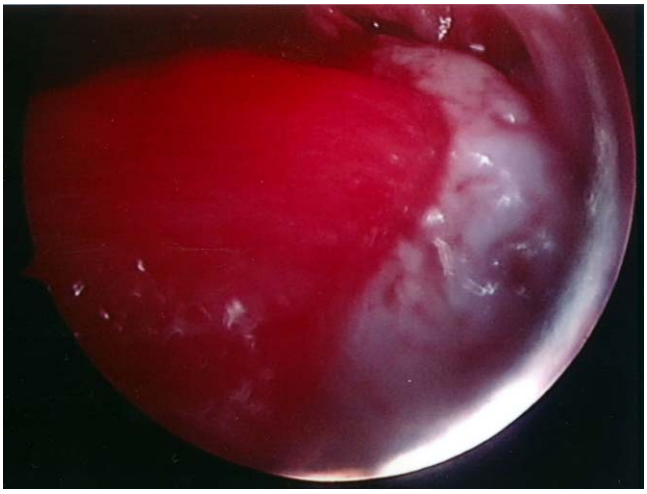

Fig. 5. Final positioning of the biomaterial.

Arthroscopic BMDC transplantation

After the bone marrow harvesting phase, a standard anterior ankle arthroscopy was performed, with the patient in the supine position. Lesion was detected and a curettage is performed. The scaffold used was either a collagen powder (Spongostan1 Powder; Johnson & Johnson Medical Ltd, Gargrave, Skipton, UK[54]) or the same hyaluronic acid membrane already used for arthroscopic ACI (HYAFF-11).[12,23,24] The scaffold used was loaded with 2 mL of bone marrow concentrate and with 1 mL of PRF, and cut or shaped in an appropriate size following the area of the lesion previously measured (Fig. 8). The composite was placed using the same instrumentation as previously described for the arthroscopic autologous chondrocyte implantation (CITIEFFE, Italy).[23] Multiple sagittal ankle movements were performed under arthroscopic control to verify the stability of the implant.

Fig. 6. Bone marrow is harvested from the posterior iliac crest, with the patient in prone decubitus.

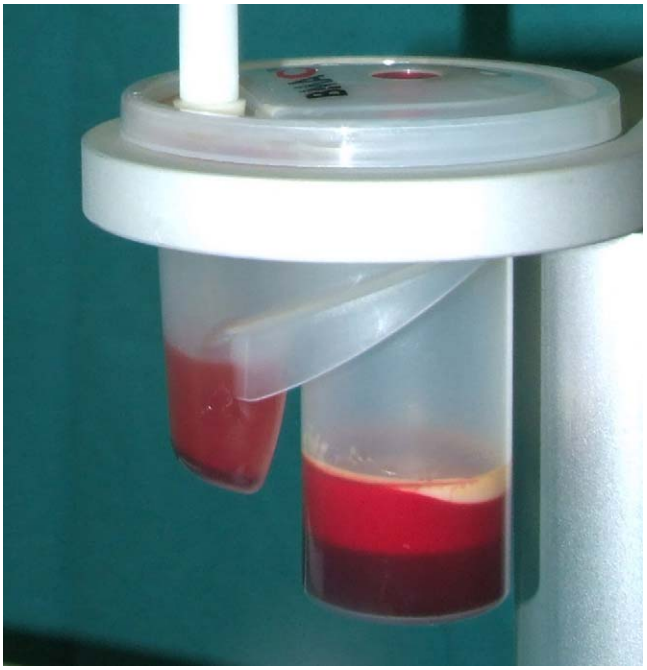

Fig. 7. Bone marrow concentrate in the lower portion of the smaller anterior chamber, containing the nucleated cells.

Post-operative treatment

Plaster cast for 15 days was required in all the open field cases. Continuous passive motion was advised at plaster cast removal in the open field cases, or immediately the day after surgery in all the other cases, and gradually increased as tolerated.

Partial weight-bearing and swimming were advised at 6 weeks; increasing to complete weight-bearing at 8 weeks was allowed. Patients who received open ACI were maintained in a boot during weight bearing up to 8 weeks.

At 4 months after surgery, all the patients were allowed to resume low impact sport activities. At 10 months after surgery, running and progressive training for high impact activities such as tennis and soccer can be resumed.

Follow-up evaluation

All patients were examined clinically preoperatively, at 12, 36 months and at maximum follow-up for pain, function, range of motion and alignment (AOFAS score).[39] X rays and MRI scan were also taken preoperatively and at the scheduled follow-up.

Assessment of cartilage was performed by the Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scoring system.[44] The MRI acquisition protocol – proton-density fast spin echo, with or without fat suppression; T2 fast spin echo, with or without fat suppression; and three-dimensional spoiled gradient-recalled using a dedicated phased array coil and 1.5-T MRI scanner – was recommended by the International Cartilage Repair Society.[6]

After one year, in the 10 open field ACI cases, the hardware was removed and, at the same time, an arthroscopy with biopsy was performed in order to assess the transplant condition.

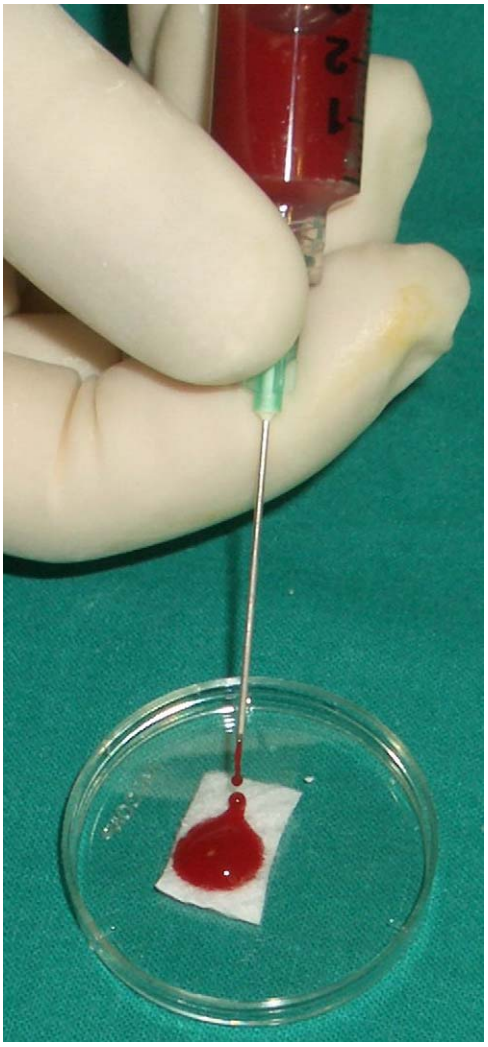

Fig. 8. The hyaluronic acid membrane is loaded with 2 mL of bone marrow concentrate and with 1 mL of PRF.

Patients who received arthroscopic repair (4 patients for the ACI group, and 5 patients for the BMDC group) underwent a second arthroscopy with a bioptic cartilage harvest at 1 year follow-up. A 2 mm cylinder of cartilage was removed from the transplant area for biopsy and histological and immunohistochemical analyses were performed.[19,22–24]

Histological and immunohistological anlysis of follow-up biopsies

Biopsies for histological analysis were fixed in 10% buffered formalin washed and decalcified. The samples were then dehydrated in alcohol graded series and embedded in paraffin. Sections of 5 μm were stained with 0.02% Fast Green and 0.1% Safranin-O. For immunohistochemical analyses mouse monoclonal anti-human type I collagen and mouse anti-human-collagen type II monoclonal antibody were used. All the samples were analysed using a Zeiss Axioscope Microscope.

Statistical analysis

Continuous data were described as means and standard deviations. The Kolmogorov–Smirnov test was used to test the normality of data distribution. The Levene test was used to test the homoscedasticity. Differences between preoperative and various follow-up data were evaluated with the paired t test for homoscedastic and normally distributed data; otherwise, the nonparametric Wilcoxon–Mann–Whitney test was used. Differences amongst groups were evaluated with the one-way ANOVA test for homoscedastic and normally distributed data; otherwise, the nonparametric Kruskal Wallis test was used. Scheffé test was used as pairwise post hoc analysis. The groups also were checked for differences considering the percentage of maximum possible improvement at each follow up, calculated as follows: improvement percentage at a set follow up = (AOFAS score at the follow up − AOFAS preoperative/100 − AOFAS preoperative) × 100. Statistical analysis was performed using SPSS® software (Version 15.0; SPSS Inc., Chicago, IL).

The preoperative clinical score evaluated with the AOFAS scoring system was related to the AOFAS score obtained at each follow-up for the total amount of patients. Then the patients were divided into three groups depending on the type of surgery: open field ACI, arthroscopic ACI and BMDCs transplantation, and correlations between follow up and AOFAS scores were considered for each group and between the groups themselves.

Costs of surgery were calculated for each procedure.

Results

Clinical

Mean AOFAS score before surgery of all the patients considered together was 57.1 ± 17.2 and 92.6 ± 10.5 ($P < 0.0005$) at mean 59.5 ± 26.5 months.

All the three groups had a statistically significant improvement from pre-operatively to 12 and 36 months of follow-up ($P < 0.0005$). A statistically significant improvement was also found for the arthroscopic ACI group and the BMDC's group from 12 to 36 months follow-up ($P < 0.0005$).

It is noticeable that the Open ACI group had a lower preoperative score with respect to other groups ($P < 0.0005$) and that the improvement of this group from 12 to 36 months of follow up resulted ns probably because of the small number of subjects in this group (Table 2).

Non-statistically significant differences were evident in the comparison of the percentages of improvement of each group at the different follow-up, so that the three groups improved with a similar pattern (Table 3).

A similar pattern of AOFAS improvement was found in the three different techniques (Fig. 9).

Table 2
Clinical scores at the different follow-up times.

	Open ACI	Arthroscopic ACI	BMDCs	Amongst the three groups
Pre-op AOFAS	38 ± 18	57 ± 14	65 ± 16	$P < 0.0005$
12 months	89 ± 14	87 ± 13	89 ± 9	NS
36 months	94 ± 8	89 ± 13	93 ± 8	NS
Pre-op vs. 12 months	$P < 0.0005$	$P < 0.0005$	$P < 0.0005$	
Pre-op vs. 36 months	$P < 0.0005$	$P < 0.0005$	$P < 0.0005$	
12 months vs. 36 months	NS	$P < 0.0005$	$P < 0.0005$	

Table 3
Percentages of clinical improvement at the different follow-up times.

	Open ACI	Arthroscopic ACI	BMDCs	Amongst the three groups
Percentage of improvement at 12 months	83 ± 25%	68 ± 31%	68 ± 26%	NS
Percentage of improvement at 36 months	90 ± 15%	74 ± 31%	82 ± 22%	NS

No statistically significant differences were found in the AOFAS score for the first and the second group from 36 months to follow up.

No major complications were observed. In the group treated by arthroscopic ACI two patients had a recurrence of pain at 28 and 31 months follow-up, and underwent a second surgery by BMDCs transplantation.

In the BMDCs group one patient had a superficial infection of an arthroscopic portal; the infection resolved with oral antibiotic therapy.

The costs associated to BMDC's transplantation were found to be lower with respect to ACI either open or arthroscopic and these values are summarised in Tables 4–6.

Radiographical

Radiographic results at follow up demonstrated no increase in arthritis in all the cases.

MRI performed at final follow-up according to the MOCART[44] scoring system showed nearly complete integration of the regenerated tissue with the surrounding cartilage in 76% of the cases. Subchondral oedema at the repair site was still evident in 12 cases, 2 treated with open field ACI, 4 by arthroscopic ACI and 6 treated with BMDCs transplantation. The regenerated tissue resulted nearly homogeneous in 82% of the cases.

In 5 patients, (2 open ACI and 3 BMDC's transplantation) MRI showed hypertrophy of the regenerated cartilaginous tissue. No intra-articular fluid was found.

Histological

Second-look arthroscopies revealed a continuous and intact cartilage layer in all the cases evaluated. Moderate hypertrophy of the periosteal flap was evident in 2 of the open ACI cases, a slightly demarcated border in 1 arthroscopic case and a moderate hypertrophy of the regenerated area in 2 of BMDC cases evaluated.

The histological evaluation of the biopsy specimens of all the three groups highlighted the presence of all the components of hyaline cartilage, and the tissues showed various degrees of tissue remodelling.[19,23,24]

Discussion

The ideal technique for a chondral defect repair would generate a repair tissue with biomechanical proprieties similar to normal hyaline articular cartilage. ACI was first used in the treatment of osteochondral lesions of the knee, becoming increasingly popular, and later was successfully applied to the ankle.[4,9,17,19,21,42]

The excellent durability of results obtained by ACI over time is well established and contrasts sharply with the long-term results reported for marrow stimulating techniques (such as abrasion, drilling or microfractures) which provide a fibrocartilaginous repair tissue.[22,33,52]

Open field ACI in the ankle was technically demanding since an open surgery with malleolar osteotomy, and a periosteal flap sutured to the surrounding cartilage were required, with considerable morbidity.[19] Nevertheless, impressive results even in large size lesion were obtained. Furthermore, these patients have the longest follow-up of the series and is noticeable the stability of their result over time.[25]

A biodegradable three-dimensional scaffold for cell support and proliferation, developed thanks to recent tissue engineering improvements, permitted then to shift to a completely arthroscopic procedure.[1,10]

Arthroscopic implantation, firstly implemented in the knee,[17,43] was modified to be used in the ankle, thanks to the development of an instrumentation able to overcome the disadvantages given by the tangential perspective and the narrow space available.[23]

Still, the need of two surgical operations and the high costs, due to cell expansion, were major drawbacks of ACI, which lead in search of new methods of repair.[23]

Mesenchymal stem cells, represent 2–3% of the total mononuclear cells in bone marrow and recently, have been indicated as a

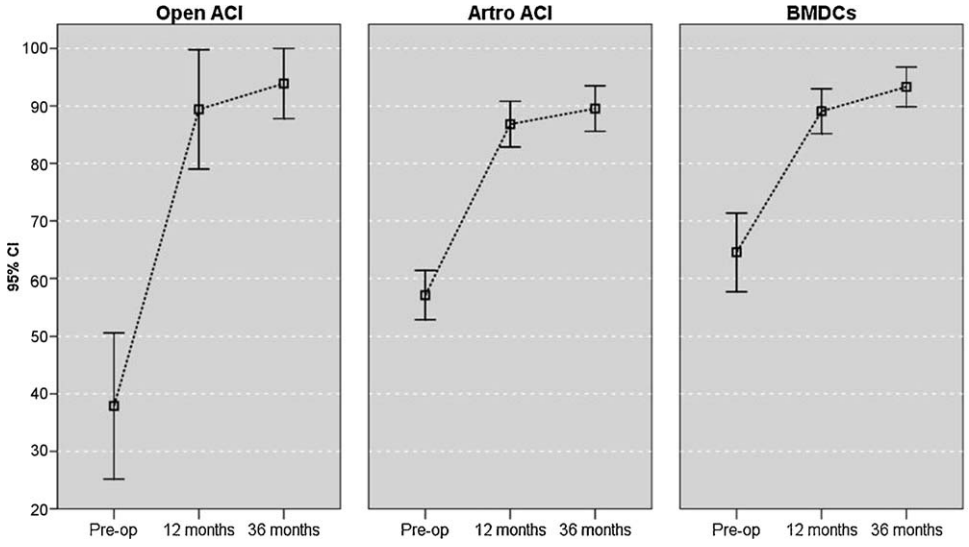

Fig. 9. A similar pattern of AOFAS improvement was found in the three different techniques.

Table 4
Open field ACI: cost details.

First step	
3 days recovery	475€ × 3 = 1425
30 min of operating room	327€
30 min of 2 orthopaedic surgeons	80€
Cell expansion in liquid media (Carticel)	9000€
Second step: ACI implant	
4 days recovery	475€ × 4 = 1900
90 min of operating room	981€
90 min of 3 orthopaedic surgeons	360€
1 plate or 1 cancellous screw	35€
2 mL Tissucol fibringlue	197€
Total	14,305€

Table 5
Arthroscopic ACI: cost details.

First step	
3 days recovery	475€ × 3 = 1425
30 min of operating room	327€
30 min of 2 orthopaedic surgeons	80€
Cell expansion and seed on Hyaff scaffold	6032€
Second step: ACI implant	
3 days recovery	475€ × 3 = 1425
30 min of operating room	327€
30 min of 2 orthopaedic surgeons	80€
Total	9696€

Table 6
BMDC's transplantation: cost details.

PRF production	
60 min of 1 haematologist for PRF production	80€
30 min of a bone and tissue bank operator and 30 min of a nurse	29€
1 Kit for PRF production	500€
BMDC's implant	
3 days recovery	475€ × 3 = 1425
60 min of operating room	654€
60 min of 2 orthopaedic surgeons	160€
30 min of 1 haematologist	40€
1 Kit for cells concentration	500€
1 Hyaff scaffold	960€
Total	4348€

new option for the treatment of articular osteochondral defects, because of their ability to differentiate into various lineages, including osteoblasts and chondroblasts.[5,9,18,43,44] The idea to transplant the entire bone marrow cellular pool, permits the cells to be processed directly in the operating room, without the need for a laboratory phase, and allowing BMDCs transplantation to be performed in "one step" instead of the two required for ACI.[36,49]

Cartilage regeneration history in osteochondral lesions of the talar dome is now 10 years long.

Over this time a thrilling series of evolution steps rendered this technique less invasive, less expensive and simpler overcoming all the major drawbacks over time.

The results obtained with the described procedures were excellent or good in more than 80% of cases and did not show any negative tendency over time. The clinical results remained stable after 36 months in the ACI groups, both open and arthroscopic. Since the follow-up for the BMDCs is still mid term, further studies are required in order to understand if also in this technique the score remain stable over time.

The MRI and histological results showed in most of the cases, a good restoration of the cartilaginous layer and a regenerated tissue in reorganisation which approximates the characteristics of the original hyaline cartilage. Furthermore, the improvements described, significantly extended the applicability of the technique from the bigger size of the first 10 open ACI to the smaller size of the last BMDC's transplant.

Conclusions

The ultimate goal of clinicians and scientists involved in cartilage is restoring a mechanically functional repair tissue reducing pain and progression to arthritis.

Even if further research are required to regenerate a hyaline cartilage indistinguishable from native articular cartilage, the techniques proposed permitted high rate satisfactory results durable over time. Advancements achieved with the advent of tissue engineering and stem cell technologies reduced time costs and morbidity and made the goal closer.

Conflict of interest

All authors disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) this work. No fundings were received by any of the authors of the paper concerning the work described in the paper itself.

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