



## Autologous chondrocyte implantation : A comparison between an open periosteal-covered and an arthroscopic matrix-guided technique

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In this prospective study, the authors compared the Carticel® method of autologous chondrocyte implantation with the Hyalograft®C technique. The aim of the study was to compare the clinical outcomes of the two methods, to identify any complications and to analyse MRI images of the repair process. Seventeen patients who had received autologous chondrocyte implantation with the Carticel® technique and ten treated with Hyalograft®C were assessed. A statistically significant improvement was observed in both groups at six months ( $p < 0.001$  for Carticel® and  $p = 0.002$  for Hyalograft®C) and at twelve months after surgery ( $p < 0.001$  both for Carticel® and Hyalograft®C), according to HSS (Hospital for Special Surgery) and ICRS (International Cartilage Repair Society) scores. There were no statistically significant differences between the two groups. MRI images obtained one year after surgery revealed the formation of well-integrated tissue similar to the surrounding healthy cartilage in most cases, in both Carticel® and Hyalograft®C groups.

Six patients treated with Carticel® technique were also assessed by second-look arthroscopy and histology of biopsies. The newly-formed tissue presented structural features similar to normal cartilage in most cases (84%).

Molecular analysis was performed to assess mRNA levels of the various collagen molecules and proliferation and differentiation factors : the results showed that the implanted material undergoes progressive remodelling to regenerate hyaline cartilage.

Both Carticel® and Hyalograft®C implantation techniques seem to lead to comparable short- and medium-term results. Moreover, this study confirmed that

**MRI is a valid tool in the follow-up evaluation of autologous chondrocyte implantation.**

**Keywords :** autologous chondrocyte implantation (ACI) ; Carticel® ; Hyalograft®C ; cartilage MRI ; arthroscopic biopsy.

### INTRODUCTION

Symptomatic cartilage defects that fail to respond to non-invasive treatment represent a serious challenge for the orthopaedic surgeon. Although major progress has been made in stimulating intrinsic repair mechanisms, cartilage regeneration and in other substitutive techniques, no procedure has yet been devised that would enable lesions to be repaired with normal joint cartilage.

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Hyaline cartilage is a special type of tissue, able to resist pressure and to spread load over the underlying bone. Any substitute used has to respond to a variety of functional requirements and withstand considerable stress.

The treatment of cartilage lesions is aimed at mitigating pain, reducing inflammation, restoring function, limiting disability and the need for prosthetic replacements.

Although the natural evolution of cartilage defects is unknown (17), it is assumed that both chondral and osteochondral defects may spread progressively over time, eventually contributing to the development of more generalised osteoarthritic changes. The incidence of such lesions, both symptomatic and asymptomatic, is also unknown, because the harmful effect of an isolated trauma to the cartilage may not manifest for some time, and standard radiography and magnetic resonance are not fully able to pick up partial or full-thickness lesions. Even when examined by arthroscopy, the area of cartilage that has suffered trauma may appear healthy at first, only to degenerate later.

The aim of surgery is to mend these defects with cartilage-like tissue, thus interrupting the evolution of the lesion which might otherwise degenerate over time into osteoarthritis.

Although all repair techniques give good results, they only succeed in obtaining the formation of a new fibrocartilage tissue with bio-elastic properties that are considerably less satisfactory than those of the hyaline cartilage that covers normal, healthy joints. The clinical results of such methods usually worsen, as confirmed by Hangody (9) in a study that used the HSS (Hospital for Special Surgery) score to assess the medium- to long-term results in patients treated with subchondral drilling. The study confirmed that the HSS score five years after treatment was only slightly higher than immediately before surgery.

Short-term follow-up may show a more marked improvement in patients treated by repair techniques, when compared with a group treated with chondrocyte implantation. These results seem to be confirmed only in the case of small lesions, suggesting that the improvement may be due to the elimination of debris responsible for joint inflam-

mation, and to the early recovery of physiological conditions in the joint (14). This randomised and prospective study showed that the close relationship noted between good clinical results and smaller lesion size after microfracture was not demonstrated in the group treated with chondrocyte implantations. Considering the clinical and histological results confirmed by various studies, the methods used for implanting chondrocytes taken by biopsy and grown *in vitro* are now considered to be the most suitable techniques for the functional and anatomical restoration of large areas of cartilage.

In 1987, Lars Peterson from Göteborg University discovered that chondrocytes can be brought to proliferate *in vitro* and then be implanted to fill cartilage defects in the joint from which they originated. The procedure was developed by Genzyme laboratories in Cambridge (Mass, USA), where cells taken by arthroscopy are still being sent for culture. This method (Carticel®) involves isolation of chondrocytes, their *in vitro* proliferation and finally their implantation into the knee by open surgery.

For some years now, researchers have attempted to reconstruct cartilage in the laboratory using tissue engineering, a technique by which a living tissue can be reconstructed by associating the cells that it is made of with biomaterials that provide a scaffold on which they can proliferate three-dimensionally, as occurs in physiological conditions.

At FAB (Fidia Advanced Biopolymers) research laboratories in Abano Terme (PD, Italy), a new use for hyaluronic acid, a fundamental component of the synovial fluid and extracellular matrix, has been discovered, with the development of HYAFF®11, an esterified derivative of hyaluronic acid. Three-dimensional non-woven scaffolds based on HYAFF®11 support the *in vitro* growth of highly viable chondrocytes and promote the expression of their original chondrogenic phenotype. Chondrocytes, previously expanded on plastic and seeded into an HYAFF®11 scaffold produce a characteristic extracellular matrix rich in proteoglycans and express typical markers of hyaline cartilage, such as collagen II and aggrecan.

There are now many centres worldwide where chondrocyte implantation techniques are being

applied. The aim of our study is to compare the two implantation methods, analysing the clinical results obtained in both cases, any complications that may occur and MRI findings, as MRI has been recognised as a satisfactory non-invasive method of following up treated joints (10, 24).

**MATERIALS AND METHODS**

Between October 1998 and June 2004 at the Department of Orthopaedics and Traumatology of the “del Delta” Hospital at Lagosanto, Italy, 17 patients received autologous chondrocytes implantation according to the Carticel® technique, to treat knee cartilage lesions. Fifteen patients were male and 2 were female. Their average age was 32.3 years (range, 17 to 51). The cartilage lesions were classified as Outerbridge grades III or IV, and they measured between 2 and 9 cm². The average follow-up time was 48.5 months.

Between April 2002 and July 2004 a total of 15 chondrocyte implantation procedures were done according to the Hyalograft®C method in 13 male and 2 female patients with an average age of 35.2 years (range, 20 to 55). The chondrocytes were grown on a scaffold (HYAFF®11) and implanted without any kind of covering. The lesions were classified as Outerbridge grade III and IV and measured between 1.5 and 6 cm². The follow-up time was 13.5 months.

Table I gives a brief description of the location of chondral lesions in the two groups of patients.

**First step : cartilage biopsy**

Under peridural or general anaesthesia, the cartilage defects were assessed by arthroscopy. At the same time concomitant pathologies were treated and a cartilage biopsy taken. Fragments of cartilage (300-500 mg) were

harvested from a non-weight bearing area of the knee, such as the upper part of the medial condyle or from the side of the intercondylar notch. The cartilage samples were placed in sterile tubes containing transport medium at room temperature, and delivered to either Genzyme Tissue Repair laboratories in Cambridge, Massachusetts, USA or to FAB laboratories in Abano Terme, Italy within 48 hours. The implantations were performed no less than three weeks after biopsy. The cell cultures underwent quality control procedures consisting in sterility tests and photographic recording of their morphological characteristics. The cells were released only when they demonstrated 85% viability on Trypan blue staining.

**Second step : implantation of chondrocytes**

The implantation surgery was done under epidural or general anaesthesia. Patients received prophylactic antibiotics and thromboembolism prophylaxy.

The Carticel® technique required a medial or lateral parapatellar arthrotomy under tourniquet control. The cartilage lesion was thoroughly cleansed, and all damaged tissue removed to create a clear margin around the lesion and down to the subchondral bone, without damaging the latter to avoid bleeding.

The chondrocytes were injected under a periosteal flap taken from the tibia and stitched to the margin of the lesion bed with interrupted suture using Vicryl 6.0 and fibrin glue. The flap was placed with the deep cambium layer facing the subchondral bone.

Joint capsule, retinaculum and skin were then sutured in separate layers and the knee was dressed with an elastic bandage.

When using Hyalograft®C method, the lesion was accessed by mini-arthrotomy or arthroscopy. The implantation site was prepared in the same way as in the Carticel® technique. The implant was simply cut to size,

Table I. — Location of chondral lesions

Carticel (n = 17)		Hyaff 11 (n = 15)	
Site of lesion	Number of patients	Site of lesion	Number of patients
Medial condyle	10	Medial condyle	5
Lateral condyle	1	Lateral condyle	4
Patella	2	Trochlea	1
Trochlea	1	Patella + trochlea	1
Patella + trochlea	1	Medial condyle + trochlea	2
Medial + lateral condyle	1	Medial + lateral condyle	1
Medial condyle + patella	1	Medial condyle + patella + trochlea	1

applied to the lesion and the site was left uncovered. In some cases a few stitches of Vicryl 6.0 or fibrin glue was used around the edges to fix it. Limited bleeding could be an advantage for a Hyalograft®C since it favours adhesion of the biomaterial to the implantation site, unlike Carticel® technique where bleeding should be avoided. Excessive bleeding may lift the scaffold out of the defect.

To apply the matrix arthroscopically a lip cannula and a plunger were used. We did not use a drill to clean damaged cartilage as described by Marcacci *et al* (15). We used temporary stitches in the synovial membrane and capsule covering the lesion, entering through arthroscopic portals, to improve access and to imbed the cell matrix in the chondral lesion free from any intra-articular fluid. Stitches in the patellar tendon also allow mobilisation of the patella and greatly facilitate the arthroscopic treatment of trochlear and patellar lesions.

### Follow-up

All the patients treated according to the Carticel® technique and 10 patients who had received Hyalografts®C over a year, were re-examined after 6 and 12 months. A clinical assessment was made using Hospital for Special Surgery (HSS) and ICRS scoring systems for subjective evaluation. MRI assessment was done at 12 months in all and a biopsy was taken by arthroscopy for histological analysis in 6 Carticel® cases.

#### MRI assessment (6)

The MRI analysis was with a 1.5 Tesla (Philips) with the use of a specific coil for the knee, in order to improve resolution. Images were taken in the three standard planes.

The protocol we used provided for Spin-Echo T1 and T2 and Gradient-Echo T1 and T2 sequences. The interface between periosteum and cartilage, the interface between substituted and native cartilage, the intrinsic signal of the cartilage and the thickness of the implant site were evaluated and scored according to Roberts *et al* (20) (fig 1, 2 and 3).

#### Arthroscopic evaluation

Second-look arthroscopy was possible only for 6 patients (respectively after 23 months, 18 months, 17 months (2 patients), 15 months and 12 months).

We used the Brittberg scoring system (table II), which analyses the outcome of the repair, the integration of the



**Fig. 1.** — ACI on the medial femoral condyle. Good cartilage signal, thickness comparable to the surrounding cartilage, good integration, minimal subchondral reactive oedema.

graft with the surrounding native cartilage and its macroscopic appearance. For a satisfactory outcome, the minimum score for a biologically acceptable cartilage is 8 points.

### Histological and immunohistochemical evaluation

Six patients from the Carticel® group consented to undergo to a second-look arthroscopy with full-depth biopsies for investigative purposes from the area of the implantation. The biopsy samples were then analysed at the laboratory of Immunology and Genetics at the *Codivilla Putti* Research Institute in Bologna. The specimens were embedded in paraffin, sliced with a microtome and stained with haematoxylin/eosin, safranin-O, Alcian blue ; immunohistochemical tests were performed for specific markers, such as collagen type I, collagen type II and proteoglycans.



**Fig. 2.** — Subchondral oedema under the site of ACI : good integration, thickness and signal of the newly formed cartilage.



**Fig. 3.** — Inhomogeneous thickness in the ACI site with fibrotic areas.

### Molecular assessment

Cartilage biopsies from 4 patients were tested by the Real-Time PCR technique for the expression of collagen I, II, X messenger RNA, aggrecan, cathepsin B and the Early Growth Response Protein (Egr-1) and Sry-type high-mobility-group box transcription factor-9 (Sox-9) as gene transcription regulating factors.

### Statistical analysis

The clinical results and scores obtained by assessing the magnetic resonance images of the two implanting techniques were assessed using the Wilcoxon Signed Ranks test and the Mann-Whitney-test. These two methods were used to compare the clinical results at 6-month and 1-year follow-up times, and the scores obtained from assessment of MRI images. We assessed any statistical correlation that emerged between the scores, relative to the ICRS result at 12 months and the NMR scores described by Roberts *et al* at 12 months (20).

## RESULTS

### Clinical assessment

Table III and IV show the HSS and ICRS scores at follow-up.

Only the Hyalograft®C cases presented a statistically significant clinical improvement 6 months after surgery ( $p = 0.1$  for Carticel® and  $0.005$  for Hyalograft®C) while there was significant improvement in both groups 12 months after surgery ( $p = 0.001$  for Carticel® and  $p = 0.005$  for Hyalograft®C).

The clinical results of the two study groups did not present any statistically significant differences at baseline ( $p = 0.675$ ) and at follow-up after 6 ( $p = 0.334$ ) and 12 months ( $p = 0.537$ ), according to the Mann-Whitney test.

Table II. — The Brittberg score and the criteria to be analysed

Criteria	Points	
Degree of Defect Repair (1) I Protocol A	* level with surrounding cartilage	4
	* 75% repair of defect depth	3
	* 50% repair of defect depth	2
	* 25% repair of defect depth	1
	* 0% repair of defect depth	0
I Protocol B (2)	* 100% survival of initially grafted surface	4
	* 75% survival of initially grafted surface	3
	* 50% survival of initially grafted surface	2
	* 25% survival of initially grafted surface	1
	* 0% (plugs are lost or broken)	0
II Integration to Border zone	* Complete integration with surrounding cartilage	4
	* Demarcating border < 1 mm	3
	* 3/4 of graft integrated : 1/4 with a notable border > 1 mm width	2
	* 1/2 of graft integrated with surrounding cartilage : 1/2 with a notable border > 1 mm	1
	* From no contact to 1/4 of graft integrated with surrounding cartilage	0
III Macroscopic Appearance	* Intact smooth surface	4
	* Fibrillated surface	3
	* Small, scattered fissures or cracks	2
	* Several : smal or few but large fissures	1
	* Total degeneration of grafted area	0
Overall Repair Assessment	Grade I normal	12 P
	Grade II nearly normal	11-8 P
	Grade III abnormal	7-4 P
	Grade IV severely abnormal	3-1 P

Table III. — HSS Score

Carticel (n = 17)		Hyaff 11 (n = 10)	
Results	Number of patients	Results	Number of patients
Excellent	9	Excellent	6
Good	6	Good	3
Fair	2	Fair	1
Poor	0	Poor	0

The results obtained with the HSS score revealed comparable data for the two methods in the two groups.

No patients had infections or thromboembolic complications. The cases in the Carticel® group were specifically evaluated for donor site problems at the site of periosteal flap preparation on the tibia. None had local tenderness.

After surgery, three patients experienced joint effusion (2 treated by the Carticel® technique and 1

with Hyalograft®C), but all resolved within 4 to 5 days.

### MRI assessment

MRI images one year after surgery showed the formation of tissue with characteristics similar to the surrounding healthy cartilage and signs that the implants had become well integrated in most cases in both Carticel® and Hyalograft®C groups. In

Table IV. — ICRS score and MRI evaluation

PATIENT	Technique	Preop	6 months	12 months	MRI at 12 months
1	carticel	42	43	62	2
2	carticel	42	47	64	3
3	carticel	45	52	72	3
4	carticel	51	54	77	4
5	carticel	52	57	74	3
6	carticel	50	47	83	2
7	carticel	56	58	79	3
8	carticel	53	58	75	3
9	carticel	57	61	81	4
10	carticel	57	61	79	4
11	carticel	51	57	79	3
12	carticel	49	57	72	3
13	carticel	61	59	75	3
14	carticel	64	62	77	4
15	carticel	61	63	80	4
16	carticel	66	59	77	3
17	carticel	64	58	73	3
	average	54.18	56.06	75.24	3.1
1	fidia	50	53	79	4
2	fidia	52	57	80	4
3	fidia	58	59	78	3
4	fidia	51	64	80	3
5	fidia	50	60	75	3
6	fidia	55	58	75	3
7	fidia	55	59	78	2
8	fidia	53	59	76	4
9	fidia	59	61	72	2
10	fidia	47	55	77	3
11	fidia				
12	fidia				
13	fidia				
14	fidia				
15	fidia				
	average	53	58.5	77	3.1

some cases, and in particular in one patella implantation, inhomogeneous areas were visible, probably due to fibrous tissue (fig 4). In general, the images of patients treated with Carticel® showed a greater tendency towards hypertrophic growth of the repair tissue, particularly marked in 4 patients.

For both techniques the average score was 3.1, according to the scoring system for resonance imaging described by Roberts *et al* (20) (table IV).

Comparison of the MRI analysis scores did not reveal any significant differences between the groups (p = 0.824). A relationship could be seen between the clinical results using the ICRS score, and the MRI analysis scores (correlation = 0.67).

### Arthroscopic results

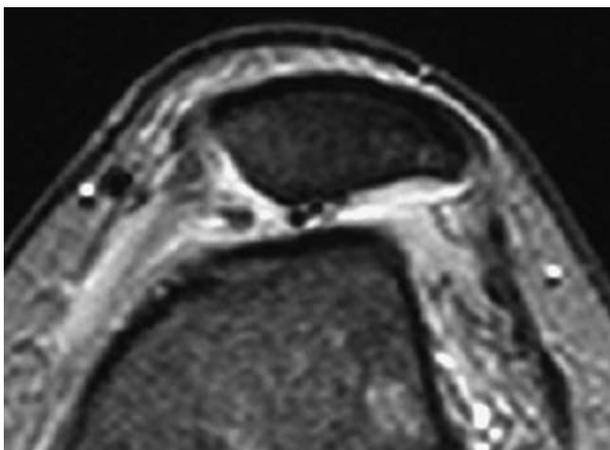
The Brittberg scores in the 6 arthroscoped knees are listed in table V. Figure 5 shows the arthroscopic image of patient # 1, seventeen months after surgery.

### Histological results

In accordance with the literature (8), immunohistological evaluation confirmed that the newly-formed tissue had morpho-structural features similar to normal cartilage (fig 6) and presented immunohistochemical reactions for type I and II

Table V. — Brittberg scores of the 6 patients who underwent a second-look arthroscopy

Patient	Follow-up (mths)	Score
1	17	12
2	15	11
3	18	10
4	23	10
5	17	9
6	12	8



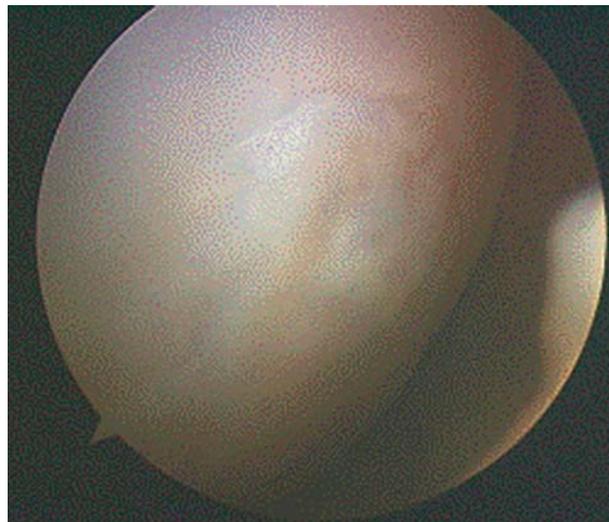
*Fig. 4.* — Fibrotic tissue areas in the ACI site

collagen and proteoglycans in 5 cases. In one case the newly-formed tissue had disorganised structural features, unlike those of normal cartilage.

We used molecular analysis to look at mRNA expression for the various collagen molecules and proliferation and differentiation factors. The results were correlated with the levels of expressed molecules and showed the presence of cartilage with similar features to those observed in healthy cartilage, but in a remodelling phase. Low levels of Egr-1 and Sox 9 and the marked presence of cathepsin B and mRNA for type I collagen show that low differentiation was still present in the chondrocytes, compared to control values.

## DISCUSSION

Joint cartilage is considered to be a noble tissue because it is highly differentiated. Cartilage has no proliferative and self-regenerating properties,



*Fig. 5.* — ACI site (with Carticel). Second look after 17 months.



*Fig. 6.* — Haematoxylin and eosin coloration : histological confirmation of the cartilaginous nature of the implantation.

because it has no blood or lymph supply. Even small lesions alter the microenvironment of the joint and cause overloading of the surrounding areas. Various techniques are used to treat cartilage lesions : regenerative techniques that stimulate the bone marrow to produce repair tissue, or substitutive techniques involving implantation of autologous/engineered cells or tissues to replace the damaged cartilage. The short- and medium-term clinical results obtained with regenerative or reparative techniques are very similar, as reported in the literature (12, 13, 18, 22, 23).

Chondro-abrasion and microfracture techniques are usually used in patients over 55 years of age, for which other forms of treatment are not recommended. It does not normally require any particularly rigorous rehabilitation, and the patient can soon return to a normal lifestyle. These techniques lead to a fibrocartilaginous repair tissue and the effect is short-term (12, 23).

Osteochondral plugs can be implanted in a single arthroscopic procedure. Disadvantages are the risks involving the donor site (4, 13, 22) and the fact that fibrocartilage repair tissue forms between the separate plugs. It therefore is only indicated for smaller lesions where a single osteochondral plug is sufficient.

A new approach to the problem comes from tissue engineering techniques. The aim is the restoration of the structural integrity and function of the damaged tissue, through regeneration of cartilage that is histologically identical to the original, capable of bearing weight and thus able to reduce or prevent the progression to osteoarthritis. Hyaline cartilage is indeed an ideal candidate tissue for these techniques, since it has no vascularisation or innervation and no intrinsic capacity for repair.

Autologous chondrocyte implants are indicated for the treatment of defects of the articular cartilage in patients with instability and/or malalignment, aged less than 55 years, in association with reconstructive and/or corrective surgery. At second-look arthroscopy we found a stable new tissue with a good consistency after chondrocyte implantation. Long-term results must confirm this. Biopsies for histological examination were taken only after Carticel® treatment. Other authors have reported on second-look biopsies taken after Hyalograft®C implantation, and have shown the formation of hyaline-like cartilage tissue, with the presence of type II collagen and the eventual absence of traces of the biomaterial, which has a degradation time *in vivo* of a few months (5). The results are in line with other data in the literature after autologous chondrocyte implantation (3, 4, 8, 10, 14, 19, 22, 24). However other authors observed fibrocartilage tissue growth both after ACI techniques and microfracture (12).

Recovery of joint function in the absence of pain and joint effusion was the rule except in 3 patients with an unsatisfactory result (two Carticel, one Hyalograft). One Carticel® case had multiple cartilage lesions and did not comply well with the physiotherapy prescribed. A second Carticel® case had a pre-existent patellar malalignment which was not corrected. The unsatisfactory Hyalograft®C case had a patellar and trochlear lesion and began rehabilitation very late.

It is fundamental for the success of the therapy, not only to treat any basic pathology, but also to follow the correct rehabilitation protocol in order to allow proper maturation of the new cartilage tissue (2, 8, 13, 22). The first step of the rehabilitation programme is centred on protecting the implant from axial load or tangential forces, as well as controlling inflammation and restoring the range of joint movement. Correct mobilisation of the joint in the absence of harmful loads, guides cell orientation in the implant. During the intermediate stage of rehabilitation the joint is gradually re-acclimatised to load, an important factor for the correct production of matrix and integration of the regenerated cartilage to the subchondral bone. Only in the final stages of rehabilitation is there a gradual return to normal activities, once muscular trophism and proprioceptive control have been restored and there are no longer any inflammatory phenomena. Patients are carefully selected before being assigned to this type of surgery. They have to be strongly motivated and determined to persevere, considering that they will have to continue the rehabilitation protocol for at least 5 months and stay in close contact with the surgeon throughout.

In the cases treated with Carticel®, the results of histological, arthroscopic and MRI analysis confirm the formation of cartilage that is morphologically comparable with and perfectly integrated with the surrounding healthy tissue, and presents complete continuity with the subchondral bone. It seems to take about 12 months for the cartilage defect to become completely filled (11, 16). We noted a tendency towards a slightly hypertrophic growth of the implanted cartilage, probably due to hypertrophy of the periosteal flap. None of the patients in our sample, however, required a second

arthroscopic treatment for cartilage debridement, a measure that could be used to resolve painful symptomatology.

From a histological point of view, it was possible to demonstrate the presence of hyaline-like cartilage in 5 cases, while only 1 case showed poorly organised cartilage with islets of fibrous tissue.

It is known that chondrocytes exposed to mitotic cycles undergo dedifferentiation *in vitro* (1), synthesising molecules normally produced by fibroblasts such as collagen types I, III and V. However, when implanted *in vivo*, they tend to recover their original phenotype characteristics, probably by means of signals of various kinds. In fact, the results we obtained from immuno-histochemical and molecular analysis concur in defining the newly-generated tissue as hyaline-like, even if it is still in the remodelling stage. The strong presence of cathepsin B and mRNA for collagen type I, molecules known to increase during chondrocyte dedifferentiation, indicates that the cells are not yet completely mature. The levels of Sox 9, an important regulator of collagen type II expression, are lower than the control values, showing that it is down-regulated.

The Hyalograft®C technique involves mitosis of cells *in vitro* and subsequent seeding on a HYAFF®11 scaffold before implantation into the joint. *In vitro* studies have shown that chondrocytes grown in a three-dimensional matrix return to their phenotype in a time-dependent manner, thus restoring their capacity for secreting proteins and molecules characteristic of a hyaline cartilage (7). Indeed the metabolism of chondrocytes is influenced by various stimuli, such as their direct interaction with the molecules that constitute the cartilage matrix (25).

Our data seem to demonstrate that the two different methods of growing chondrocytes (Carticel® and Hyalograft®C) lead to similar short- to medium-term clinical results. It would however be informative to perform a longer follow-up to see to what extent the greater differentiation at the time of implantation affects how the implant takes and endures.

We can only compare the results obtained with the two techniques from a clinical point of view and from MRI analysis, because no second-look

arthroscopy was performed in the Hyalograft®C group of patients. The clinical and MRI results are similar to those obtained by Carticel® technique, even though no signs of cartilage hypertrophy were observed.

Statistical analysis revealed that the patients in both groups experienced a major clinical improvement after 6 months. There was no statistically significant difference between the two methods after 12 months of follow-up. After 6 months, patients treated with the Carticel® technique did not show a statistically significant improvement ( $p = 0.1$ ); five patients had a lesser IRCS score than preoperatively.

These data need to be further confirmed because of the small number of patients treated. Statistical analysis of the data showed no major statistical difference between the two methods, though in one case there was a trend towards statistical significance at the 6-month follow up. This is confirmed by looking at the mean improvement scores for the two groups at 6 months: Hyalograft®C group shows a mean improvement of 24, markedly higher than Carticel® patients (21.1).

We can use MRI to assess integration of the neocartilage to the subchondral bone and to the native adjacent cartilage and to evaluate the thickness of the implant zone, which are fundamental parameters for assessing the quality of the new tissue. This method can be considered as a non-invasive alternative to second-look arthroscopy in the follow-up evaluation of ACI procedures (6, 24).

However, to date no universally accepted MRI protocol has been drawn up for the study of cartilage defects and their repair, even though a special ICRC commission is working on it. The aim is to correlate MRI results to the histological/biochemical composition of the cartilage tissue and to clinical outcomes.

The role of bleeding at the site of implantation is still open to debate. It is agreed that it is better to minimise bleeding in the case of the Carticel® technique to avoid the risk of detachment of the periosteal flap, whereas for a Hyalograft®C some bleeding could be useful as it favours the adhesion of HYAFF®11. On the other hand, even if there have been no specific studies on autologous chondrocyte implants, preliminary experiments have

demonstrated that blood can damage chondrocytes, at least *in vitro* (11, 21).

Special attention should be paid to highlight a special property of Hyalograft®C to adhere to the lesion site without fixation. Indeed, we observed only in 2 cases transplant dislodgment after testing implant stability by flexing and extending the joint several times. However in both cases the problem was solved with fibrin glue.

In conclusion, it is clear that the ACI technique has become a valid therapeutic approach to repair cartilage lesions.

From the surgeon's point of view, the use of a biomaterial simplifies the technique, and markedly reduces the time required for the surgery.

However longer follow-up is needed to confirm clinical results obtained with both methods. It would also be interesting to explore the possibility to use ACI techniques to treat chondral lesions caused by chronic conditions such as osteoarthritis, and/or to associate implanting techniques to gene therapies to prevent the development of such chronic chondral pathologies.

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