Autologous chondrocyte implantation in a novel alginate-agarose hydrogel

OUTCOME AT TWO YEARS

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Autologous chondrocyte implantation is an established method of treatment for symptomatic articular defects of cartilage. CARTIPATCH is a monolayer-expanded cartilage cell product which is combined with a novel hydrogel to improve cell phenotypic stability and ease of surgical handling. Our aim in this prospective, multicentre study on 17 patients was to investigate the clinical, radiological, arthroscopic and histological outcome at a minimum follow-up of two years after the implantation of autologous chondrocytes embedded in a three-dimensional alginate-agarose hydrogel for the treatment of chondral and osteochondral defects.

Clinically, all the patients improved significantly. Patients with lesions larger than 3 cm² improved significantly more than those with smaller lesions. There was no correlation between the clinical outcome and the body mass index, age, duration of symptoms and location of the defects. The mean arthroscopic International Cartilage Repair Society score was 10 (5 to 12) of a maximum of 12. Predominantly hyaline cartilage was seen in eight of the 13 patients (62%) who had follow-up biopsies.

Our findings suggest that autologous chondrocyte implantation in combination with a novel hydrogel results in a significant clinical improvement at follow-up at two years, more so for larger and deeper lesions. The surgical procedure is uncomplicated, and predominantly hyaline cartilage-like repair tissue was observed in eight patients.

In recent years a number of surgical techniques have been developed for the treatment of chondral and osteochondral defects of the knee. Their aim has been a long-lasting reduction of pain and improvement of function by the regeneration of hyaline cartilage integrated with native cartilage and bone. These techniques may be categorised as bone-marrow stimulation, monolayer-expanded autologous chondrocyte transplantation and autologous or allogeneic osteochondral plug transfer. A number of randomised short-term comparative trials have provided no clear evidence that autologous chondrocyte transplantation gives better results. Secondly, cell suspension injection resulted in an inhomogenous distribution of cells within the defect and leakage of cells from the defect was possible, and finally there was a lengthy operating time.

To address these drawbacks, we developed a novel agarose-alginate hydrogel scaffold (CARTIPATCH; Tissue Bank of France, Lyon, France). It was tested in a prospective phase-II clinical trial. We hypothesised that the transplantation of this cell-scaffold combination for the treatment of chondral and osteochondral defects in the knee would result in stable fixation of the scaffold and clinical improvement. The final outcomes were assessed at two years post-operatively using the International Knee Documentation Committee clinical evaluation scoring system, MRI, arthroscopy and histological examination.

Patients and Methods
Between August 2002 and August 2006, an open, multicentre prospective trial was conducted to evaluate the safety and efficacy of CARTIPATCH for the treatment of isolated chondral or osteochondral defects of the femo-
Table I. Details of the inclusion and exclusion criteria for the study

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
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<tr>
<td>Age 16 to 45 years</td>
<td>Pregnancy or breastfeeding</td>
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<tr>
<td>Isolated lesion of the femoral condyle of grade III and grade IV according to the International Cartilage Repair Society classification</td>
<td>History of anaphylactic reaction to gentamicin and amphotericin B</td>
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<td>Size of lesion 1 to 5 cm²</td>
<td>Presence of associated intra-articular pathology such as osteoarthritis, axial malalignment (varus or valgus &gt; 6°), ligamentous (non-corrected) or meniscal lesion, patellar pathology, excessive anteroposterior laxity (Lachmann &gt; 3)</td>
</tr>
<tr>
<td>Presence of incapacitating symptoms (International Cartilage Repair Society score &lt; 60)</td>
<td>Presence of a kissing lesion on the patella or tibia</td>
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Between October 2002 and March 2004, 20 patients were enrolled in the study. Trauma was considered to have caused seven lesions, whereas 13 were secondary to osteochondritis dissecans. The inclusion criteria were waived on two occasions. One patient had an initial subjective International Knee Documentation Committee score of 64 while another had only a defect of 0.6 cm² caused seven lesions, whereas 13 were secondary to osteochondritis dissecans. The inclusion criteria were waived on two occasions. One patient had an initial subjective International Knee Documentation Committee score of 64 while another had only a defect of 0.6 cm² in size. At the time of inclusion this defect area was measured at 2.4 cm² by MRI.

Of these 20 patients, three did not undergo the final transplantation procedure for various reasons. One was diagnosed with chondrocalcinosis at the time of transplantation. In another the in vitro cell expansion failed, while a third showed development of fibrocartilaginous tissue induced by a debridement performed during the biopsy procedure. Because of the quality of the repair tissue, we decided not to perform the transplantation procedure. This left 12 men and five women available for follow-up. Their mean age at the time of transplantation was 30 years (17 to 42). Before transplantation, 13 patients had already undergone a surgical procedure including two failed mosaicplasties. Details of the patients are shown in Table II.

The clinical outcomes were evaluated using the International Knee Documentation Committee score, which includes an objective and a patient-related, subjective section. The objective evaluation was performed by an independent, experienced orthopaedic surgeon (PV). The patients were evaluated at the time of inclusion in the study and subsequently at 3, 6, 12 and 24 months.

MRI. A 1.5T MR unit (Symphony; Siemens AG, Erlangen, Germany) was used. A positioning device for the ankle and knee allowed postural consistency. In order to compare the initial defect with the final repair, a validated MRI protocol (proton density-weighted 3D fat-suppressed gradient echo T1, dual echo steady state and T1 sequencing) for visualisation of the articular cartilage was used at the time of inclusion and at the final follow-up of 24 months. The extent and quality of the reparative tissue were assessed using the following variables: the size of the lesion, the homogeneity of the signal of the reparative tissue compared with that of the native cartilage and the presence and extent of bone-marrow oedema. The evaluation was performed by an independent, experienced radiologist in a single session.

Arthroscopy. This was performed at the time of inclusion to assess the defect and obtain a biopsy for cell isolation. At the final follow-up, arthroscopy was undertaken again to assess the repair and to obtain a biopsy from its central region.

The size of the defect was measured in the horizontal and vertical directions using a graded arthroscopic probe.

The reparative tissue was macroscopically assessed at final follow-up using the International Cartilage Repair Society assessment form. This includes separate sections for the extent of the repair, integration to the border zone and macroscopic appearance, each scoring from 0 to 4 points, with a maximum overall score of 12 indicating normal, hyaline-like tissue. Additionally, the extent to which the defect was filled with tissue was expressed as a percentage of the total area of the defect.

Using a bone-marrow biopsy needle, an osteochondral core biopsy of 2 mm in diameter was obtained from the central zone of the site of transplantation.

Histological examination. The core biopsy was immediately transferred in formalin and embedded in paraffin.

An independent, experienced histologist examined four distinct regions within the specimens: a global area, a superficial zone, an intermediate zone and a deep zone and macroscopic appearance, each scoring from 0 to 4 points, with a maximum overall score of 12 indicating normal, hyaline-like tissue. Additionally, the extent to which the defect was filled with tissue was expressed as a percentage of the total area of the defect.

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Histological examination. The core biopsy was immediately transferred in formalin and embedded in paraffin.

An independent, experienced histologist examined four distinct regions within the specimens: a global area, a superficial zone, an intermediate zone and a deep zone and calcified layer/bone transition. Sections were stained with haematoxylin and eosin, Safranin-O and Methylene Blue to evaluate the cellular morphology, visualise the proteoglycan content of the extracellular matrix and to
calculate the percentage of hyaline-like tissue. The presence of type-II collagen and aggrecan in the extracellular matrix was evaluated by immunohistochemistry. The histological sections were scored using the system of O’Driscoll et al.17 and the International Cartilage Repair Society method.16

**Trial endpoint.** The primary endpoint was an improvement of at least ten points on the subjective International Knee Documentation Committee score. Secondary endpoints included an improvement in the objective International Knee Documentation Committee score, the percentage of filling of the defect, the histology and side-effects caused by the use of the scaffold.

**CARTIPATCH scaffold processing.** After inclusion of the patients, autologous serum was prepared for the cell cultures. Arthroscopy for tissue harvesting was carried out approximately three weeks later.18 Between 200 mg and 300 mg of cartilage tissue were harvested from the region of the intercondylar notch or the medial aspect of the trochlea and immediately stored in a specially-designed container at 4°C at the Good Manufacturing Practice facility (Tissue Bank of France Laboratory) for cell isolation and culture. Upon arrival at the laboratory, the specimens were tested for sterility. The cartilage fragments were rinsed, diced (0.5 mm by 0.5 mm) and enzymatically digested. The isolated chondrocytes were washed three times and cultured under monolayer conditions with culture medium (DMEM/HAM’s F12 1:1; Biochrom, Berlin, Germany) supplemented with 10% autologous serum, 50 mg/l of ascorbic acid (L-aroscorbine; Laboratoire Roche, Neuilly sur Seine Cedex, France), and with fungicide (2.5 mg/l amphotericin B; Fungizone; Bristol-Myers Squibb, Rueil-Malmaison Cedex, France) and antibiotics (gentamicin 50 mg/l; Gentalline; Schering-Plough, Levallois-Perret, France). The antibiotic/fungicide supplement was stopped after the first passage.

The cells underwent a maximum of three passages, after which they were isolated and suspended in an ultrapurified agarose-alginate suspension (GelForCel; Tissue Bank of France) at a final minimum concentration of 10 × 10^6 cell/ml of hydrogel. Ultrapurified agarose and alginate are biocompatible vegetal components of algae of clinical grade. The number and sizes of plugs were dictated by the morphology of the defect as determined at the initial arthroscopy and by the pre-operative MRI. Quality control before transplantation included cell viability, microbiology and immunohistological evaluation for type-II collagen and aggrecan.

**Surgical implantation.** The implantation was performed as a mini-open procedure with the use of a tourniquet through a longitudinal skin incision over the affected femoral condyle. The defect was re-evaluated and debrided. Sub-

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<th>Table II. Clinical details of the 17 patients in the study</th>
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<tbody>
<tr>
<td>Gender</td>
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<tr>
<td>Mean age in years (range)</td>
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<tr>
<td>Mean subjective IKDC* score (range)</td>
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<tr>
<td>Clinical IKDC grade (n = 16)</td>
</tr>
<tr>
<td>A</td>
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<tr>
<td>B</td>
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<tr>
<td>C</td>
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<tr>
<td>D</td>
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<tr>
<td>Pre-operative diagnosis</td>
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<tr>
<td>Osteochondritis dissecans</td>
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<tr>
<td>Post-traumatic</td>
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<tr>
<td>Mean delay between first symptoms and inclusion (range)</td>
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<tr>
<td>Surgical knee history</td>
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<tr>
<td>First intention/second intention</td>
</tr>
<tr>
<td>Autograft</td>
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<tr>
<td>Microfracture</td>
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<tr>
<td>Screwed fragment</td>
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<tr>
<td>Other</td>
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<tr>
<td>Mean surface area of the lesion in cm² (range)</td>
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<tr>
<td>MRI</td>
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<tr>
<td>Arthroscopy</td>
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<tr>
<td>After debridement</td>
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* IKDC, International Knee Documentation Committee

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sequently, the defect area was templated to obtain optimal cover. Ideally, a single hydrogel plug should be used, but the use of multiple plugs was also acceptable. The plugs were available in three different diameters: 10 mm, 14 mm and 18 mm. They were implanted in close contact with each other and with the edges of the defect.

The debrided defect was drilled to a depth of 4 mm using specially-designed drill bits with depths and sizes corresponding to the plugs. One or more trial components were fitted into the pre-drilled holes and their primary stability was tested during flexion-extension of the knee. The cell-loaded hydrogels were delivered in a container with transportation medium. Each plug was taken out of the container using a needle and carefully transferred directly to the pre-drilled defect. The hydrogel(s) could then be gently squeezed into the defect with forceps. After complete covering of the defect, the stability of the plugs was tested through repeated flexion-extension movements of the knee. Expulsion or dislocation of the plugs was never observed.

At the end of the procedure a suction drain was placed in the suprapatellar pouch.

**Statistical analysis.** The hypothesis of the trial was a clinical improvement according to the subjective International Knee Documentation Committee score in at least 50% of the patients and a desired improvement in 75%. Alpha- and beta-errors were calculated to be 10% each. If 13 of 19 patients improved by at least ten points, the CARTIPATCH treatment could be considered to be effective.

The statistical analysis was performed by an independent evaluator (3Es, Paris, France). Pearson’s correlation and the chi-squared or Fisher exact tests were performed to compare the qualitative variables (International Knee Documentation Committee subjective). Quantitative variables (International Knee Documentation Committee knee form) were tested using Student’s t-test. All the tests were performed in a two-tailed manner. Statistical significance was set at \( p \leq 0.05 \).

**Results**

**Surgical implantation.** The mean size of treated defect was 3 cm\(^2\) (1.0 to 5.1). In three patients the defect was only partially treated amounting to 73%, 88% and 90% of the whole, respectively. The number of implants which were fitted into the pre-drilled holes and their primary stability was tested during flexion-extension of the knee. The stability of the plugs was tested through repeated flexion-extension movements of the knee. Expulsion or dislocation of the plugs was never observed.

**Clinical findings.** The mean subjective International Knee Documentation Committee score increased significantly from 37 (18 to 64) pre-operatively to 77.8 (42 to 96) at the final follow-up (matched \( t \)-test, \( p < 0.001 \)). This value did not differ from that of the normal age-matched population (Wilcoxon matched-pairs signed ranks), this improvement was attained progressively during the first 12 months (mean International Knee Documentation Committee score at 12 months; 76 (39 to 97)) to remain stable at 24 months (matched \( t \)-test, \( p = 0.34 \); Fig. 1). At the final follow-up, 16 patients (94%) had improved by at least ten points and 14 (82%) by at least 40 points. Table III shows an overview of the different patients’ characteristics relative to the final subjective clinical outcome. One significant predictor of the final clinical outcome was identified. Lesions larger than 3 cm\(^2\), as calculated by MRI had a significantly better mean final clinical outcome score than smaller lesions (90.8 (84 to 96) vs 75.1 (44 to 94), Kruskal-Wallis test, \( p = 0.027 \)). Patients with osteochondritis dissecans lesions (mean International Knee Documentation Committee score 83.3 (42 to 91)) tended to have a better clinical outcome than those with traumatic lesions (mean International Knee Documentation Committee scores 69.9 (45 to 96) (Kruskal-Wallis test, \( p = 0.06 \)) and those with grade-IV lesions (mean International Knee Documentation Committee score 78.8 (42 to 91)) fared better than those with grade-III lesions (mean International Knee Documentation Committee score 72.3 (42 to 96); Kruskal-Wallis test, \( p = 0.613 \)).

Objective evaluation at final follow-up placed all patients in category A on the International Knee Documentation Committee assessment.

**MRI findings.** At follow-up MR scans were available for 15 of the 17 patients. One patient refused to undergo MRI while another was excluded because she was pregnant. Defects were filled by repair tissue to a mean extent of 81% (60% to 100%). Complete filling was observed in eight patients. Six patients had partial filling and one had no repair tissue within the defect. The mean size of the defect decreased from 2.7 cm\(^2\) (0.5 to 5.9) pre-operatively to 0.4 cm\(^2\) (0 to 1) at the final follow-up (matched \( t \)-test, \( p < 0.001 \)).
The transition zone of the repair tissue with the adjacent normal cartilage was smooth and regular in 13 patients. In 11, the repair tissue could no longer be distinguished from the adjacent normal cartilage, indicating perfect integration. In ten, the MRI signal of the repair tissue was comparable with that of normal articular cartilage. The subchondral bone had a normal appearance in nine patients. Bone-marrow oedema was present in six patients and subchondral cysts were observed in three of these.

**Arthroscopic findings.** Of the 17 patients, 13 agreed to undergo a second-look arthroscopy at the final follow-up. The mean International Cartilage Repair Society arthroscopy score and the subjective International Knee Documentation Committee score at the final follow-up (Spearman’s rank correlation test, $r = 0.499$, $p = 0.083$). The repair tissue was level with the adjacent normal cartilage in ten patients while in three a defect filling up to 75% of the adjacent normal cartilage was found.

The peripheral zone of the repair tissue was completely integrated with the normal cartilage or showed a small (< 1 mm) gap in nine patients. In two, 75% of the peripheral margin was integrated with the surrounding cartilage and in another two 50% was integrated.

The surface of the repair tissue was graded as smooth and intact in five patients (Fig. 2) and fibrillated in two. In four patients, small cracks and fissures were observed. The repair tissue had a normal cartilage-like consistency in four patients while in eight it was softer. In one patient, no repair tissue was seen.
Historical examination. This was performed in 13 patients. The mean O’Driscoll histology score was 16 (10 to 20) of a maximum of 21. The mean International Cartilage Repair Society histology score was 14 (9 to 18) of a maximum of 18. No correlation could be found between the histology and the clinical outcome scores (Spearman’s rank correlation test, p = 0.363) (Fig. 3).

In eight biopsies, the repair tissue consisted predominantly of hyaline-like cartilage. The cells were round or chondrocyte-like and were frequently located in lacunae. A columnar cellular organisation was observed. Cellularity was normal in 12 biopsies. The extracellular matrix stained positive for type-II collagen in the deep and intermediate zones of 11 specimens, while in two type-I collagen was found. Intracellularly, type-II collagen and aggrecan were detected in nine biopsies, including those which stained positive for type-I collagen in the extracellular matrix. In 11 biopsies, the presence of a transitional zone and calcified layer was detected. The surface layer was described as smooth and intact or having minor cracks and fissures in 12 biopsies.

Discussion

Autologous chondrocyte transplantation has been shown to be a viable option for the treatment of symptomatic chondral and osteochondral lesions of the knee.3,5,20,21 The inherent drawback of cell-suspension-based autologous chondrocyte transplantation methods has stimulated many attempts to improve the surgical handling and stabilisation of the chondrocyte phenotype in order to enhance a homogeneous distribution of cells within the defect and to avoid leakage of cells from the defect. Most efforts have focused on the development of an implantable, biodegradable, cell-laden three-dimensional (3D) scaffold.7,8

In our study the clinical outcome based on the subjective International Knee Documentation Committee scoring system improved significantly from a mean of 37 pre-operatively to 77.8 at final follow-up. This latter value did not differ from that of the normal, age-matched population.22 Therefore, the primary objective of our study was achieved. The clinical outcome of our 3D autologous chondrocyte transplantation technique compares favourably with that of studies using cell-suspension-based techniques and with other 3D methods with a similar follow-up.7,8

The final follow-up of our study was set at two years since it is suggested that this is the minimum time period required for obtaining mature hyaline-like repair tissue.23 However, after the first year, the significant clinical improvement had already been achieved and remained stable at the two-year endpoint. Histological examination at the final follow-up showed hyaline-like or mixed hyaline-fibrocartilage repair tissue with complete integration to the subchondral bone in 11 of 13 patients. In most patients, the subchondral bone showed signs of ongoing remodelling. Complete columnar cellular organisation of the repair tissue was observed in only two patients. Both observations indicate that the repair tissue had not yet reached full maturity. On the other hand, the observed level of maturity was probably higher than that obtained with cell-suspension autologous chondrocyte transplantation techniques at a similar follow-up.4,5 In 11 biopsies, type-II collagen and aggrecan were present in the extracellular matrix of the middle and deep zones. In two biopsies, type-I rather than type-II collagen stained positive in these areas. In these patients, type-II collagen staining was observed intracellularly. The presence of type-I collagen did not necessarily imply a negative outcome, since positive intracellular staining for type-II collagen in these cases indicated ongoing remodelling. In all biopsies, no remnants of the agarose-alginate scaffold could be observed, indicating its complete biodegradation.

To date, no study has shown a significant correlation between clinical and objective outcome parameters, such as MRI or histology. Accordingly, we have been unable to identify a significant correlation between the International Knee Documentation Committee outcome score (objective and subjective) and MRI findings as measured using the method of Marlovits et al.23 A significant correlation was...
found, however, between MRI and arthroscopy in that the extent of the defect filling and the integration of the repair tissue with the surrounding healthy cartilage correlated significantly using both techniques. Therefore, we plan to include MRI as a non-invasive method of objective structural evaluation of the repair tissue in future research.

The clinical outcome of our patients is comparable with that of earlier studies on cell-suspension autologous chondrocyte transplantation.\(^1\)\(^{,}\)\(^{,}\)\(^{,}\)\(^{,}\)\(^{,}\)\(^{,}\)\(^{,}\)\(^{,}\) However, a number of interesting differences were observed. Certain patient characteristics, such as a surface defect > 3 cm\(^2\), a grade IV lesion and the type of lesion (osteochondritis dissecans), have been identified as significant predictors of clinical outcome, determined by the International Knee Documentation Committee scoring system. These can help the surgeon to define the optimal treatment option for the patient. In contrast to the microfracture technique,\(^2\)\(^{,}\)\(^{,}\) the body mass index (BMI) was not identified as a predictor of an inferior outcome in our study using hydrogel plugs. It appears that autologous chondrocyte transplantation combined with a 3D gel gives a satisfactory clinical outcome irrespective of the BMI. However, this observation may be biased by the

Fig. 3a

Fig. 3b

Fig. 3c

Fig. 3d

Fig. 3e

Photomicrographs of the examples of six patients (one column for each) showing the histological findings a) with haematoxylin and eosin, b) with Safranin-O, c) with haematoxylin and eosin superficial zone, d) with haematoxylin and eosin in intermediate level, and e) with haematoxylin and eosin, osteochondral junction.
fact that a high (> 30 kg/m²) BMI value was one of the exclusion criteria of our study. A similar observation was made concerning the age of the patients. In a recent study by Knutsen et al, a higher age was related to a worse clinical outcome in the microfracture arm of their study. Again, it appeared that the satisfactory clinical outcome of autologous chondrocyte transplantation in combination with a 3D gel was not influenced by the age of the patient.

Although no statistical significance was reached, there was a clear trend for patients with osteochondritis dissecans to have a better clinical outcome than those with lesions of traumatic origin. This finding is in keeping with the study by Peterson et al, indicating a long-term satisfactory outcome in this group of patients.

Interestingly, our clinical results were significantly better in lesions larger than 3 cm². We also observed a trend for better clinical results in patients with grade-IV lesions. These findings are in contrast to those of other treatment options such as the microfracture technique. The results of the latter method have been shown to be worse in larger lesions. Furthermore, other investigations using cell-suspension autologous chondrocyte transplantation techniques or mosaicplasty have suggested that the clinical outcome is inversely related to the size of the lesion. This observation could possibly be explained by the inherent advantages of the use of a 3D matrix, including a homogenous cell distribution within the defect, a perfect fit of the cell-laden scaffold to the prepared lesion and a predetermined cell concentration.

The major limitation of our clinical phase-II study is the limited number of patients involved. Furthermore, although all the patients were clinically reviewed at the final follow-up, only 15 patients (88%) and 13 patients (76%) were available for radiological and histological evaluation, respectively. These drawbacks are all inherent in the design of a prospective study like this and are accepted principles in the conduct of clinical trials.

We conclude that a cell-agarose-alginate gel combination with an alginate-agarose based hydrogel (CARTIPATCH) is a reduced implantation time and ease of surgical handling. This new technique has been shown to be safe, efficient and reproducible in a phase-II prospective clinical trial. Our findings may be attributed to a number of advantages associated with the use of this combination, such as the absence of cell leakage, a reduced implantation time and ease of surgical handling.

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References


