The treatment of osteochondral lesions using a combination of autologous chondrocyte implantation and autograft

THREE-YEAR FOLLOW-UP

In this study a combination of autologous chondrocyte implantation (ACI) and the osteochondral autograft transfer system (OATS) was used and evaluated as a treatment option for the repair of large areas of degenerative articular cartilage. We present the results at three years post-operatively. Osteochondral cores were used to restore the contour of articular cartilage in 13 patients with large lesions of the lateral femoral condyle (n = 5), medial femoral condyle (n = 7) and patella (n = 1). Autologous cultured chondrocytes were injected underneath a periosteal patch covering the cores. After one year, the patients had a significant improvement in their symptoms and after three years this level of improvement was maintained in ten of the 13 patients. Arthroscopic examination revealed that the osteochondral cores became well integrated with the surrounding cartilage. We conclude that the hybrid ACI/OATS technique provides a promising surgical approach for the treatment of patients with large degenerative osteochondral defects.

The limited capacity for physiological regeneration of articular cartilage has long been recognised. Osteochondral lesions can be the cause of significant patient morbidity and can lead to the early onset of osteoarthritis. Surgical techniques such as debridement, which involves the mechanical removal of damaged or diseased tissue, have provided temporary relief from painful symptoms. Drilling, abrasion arthroplasty and microfracture have all been used in an attempt to recruit bone marrow cells to form repair tissue in the cartilage defect.

Fibrocartilage, characterised by a high concentration of collagen type I, rather than hyaline cartilage, which comprises collagen type II, is usually formed when these techniques are used. Fibrocartilage lacks the mechanical integrity of hyaline cartilage and often breaks down in the long term. These techniques appear to provide temporary relief of pain and are often employed as a first procedure before more invasive and complex surgery.

In order to restore hyaline cartilage to the defect area, two techniques have gained popularity over recent years, osteochondral autograft transfer (OAT) and autologous chondrocyte implantation (ACI). The osteochondral autograft transfer system (OATS), or mosaicplasty, involves the removal of small cores of healthy cartilage and subchondral bone from non-weight-bearing areas of the joint and transplanting them to the defect site. This delivers an immediate, stable and biomechanically sound cartilage repair. The osteal component of the plugs integrates well. The cartilage retains its hyaline nature and provides restoration of surface height and contour. This technique has shown good to excellent results for between three and six years in 92% of patients. The mean lesion size treated in that study was 1 cm² indicating that the use of the OATS procedure alone is more suitable for the treatment of smaller defects.

ACI has also been developed to provide an effective means of cartilage repair. Chondrocytes from an arthroscopic biopsy are cultured in vitro to deliver a large number of chondrogenic cells. These cells are injected into the area of the defect underneath a periosteal flap which covers the defect. This method has been shown to produce repair tissue with the appearance of hyaline-like cartilage and has undergone extensive development. A Swedish study, in which 101 patients were assessed retrospectively with two to nine years’ follow-up, indicated that good to excellent clinical results could be obtained in groups with lesions in different sites. The size of the defects ranged from 1.5 to 12.0 cm². Histological evaluation showed a correlation between the hyaline nature of the tissue and the good to excellent clinical results.
We investigated the use of a hybrid method, combining the two techniques, that provides a more effective treatment for patients with larger degenerative defects and patients with osteoarthritis. Our hypothesis is that the osteochondral cores provide restoration of the condylar contour and restore mechanical function, while the implantation of chondrocytes will fill the remaining areas with a functional covering and improve chondral integration. A small number of osteochondral cores are moved to the defect site so that cartilage height is restored. The cores are then overlaid with a periosteal graft under which chondrocytes are injected. In this investigation 13 patients underwent surgical repair of cartilage lesions by this method and were assessed for up to three years to determine the quality of the repair. Owing to the limited number of patients which met the criteria for inclusion in this study it was conducted as a case series trial.

**Patients and Methods**

**Patient details.** Thirteen patients (eight men, five women) who had been symptomatic for at least five years were recruited after informed consent was obtained. The mean age of the patients was 42 years (24 to 48). The mean size of the lesions was 4.84 ± 3.45 cm² (SD, 2.2 to 15.3). They had an initial mean Knee Society for pain and mobility score (KSS) of 64 ± 19. Patients who had undergone previous surgery including meniscectomy were not excluded from the trial. Where there was a co-existing remediable cause for progression of the degeneration this was treated at the same time; two patients had concomitant ACL reconstruction and one had previously undergone patellar realignment treatment for excessive lateral pressure syndrome.

**Autologous chondrocyte culture.** The joints were evaluated arthroscopically and biopsies taken from the intercondylar notch. The mean size of the biopsies that were harvested was 135 ± 23 mg. Tissue was transported at room temperature to the laboratory in F12 nutrient mixture (Ham) with GlutaMax (Invitrogen Ltd, Paisley, Pennsylvania), 10 mM N-(2-Hydroxyethyl) piporazine-N¹-(2-ethanesulfonic acid) (HEPES), 50 µg ml l-ascorbic acid, and 50 IU ml penicillin and 50 µg streptomycin/ml. The chondrocytes were cultured according to the methods of Brittberg et al.7 After washing three times in Hank’s buffered saline solution, the tissue was minced with a scalpel and incubated overnight at 37°C in 0.3% collagenase type II (Worthington Products; Lorne Laboratories Ltd, Reading, UK), with gentle agitation. The liberated cells were cultured in monolayer in F12 nutrient mixture (Ham) with GlutaMax with 10% fetal calf serum, 10 mM HEPES, 50 µg/ml l-ascorbic acid, 50 IU/ml penicillin and 50 µg streptomycin/ml. The cells were cultured in monolayer for approximately 30 days during which three trypsin passages were made at a 1:3 split ratio. The chondrocytes were isolated, washed and resuspended in the cell culture medium but without fetal calf serum. The total cell yield was between 3 x 10⁶ and 3 x 10⁷ cells. The suspension was maintained at room temperature until implantation, which took place after approximately four hours.
Implantation. The joints were explored through an arthrotomy 28 to 34 days after the initial biopsy and the condylar lesion was exposed (Fig. 1). The defect was debrided to calcified cartilage and measured. Osteochondral cores were harvested using the Arthrex osteochondral autograft transfer system (Arthrex, East Sheffield, UK) and a percussion-based harvester and corder to reduce heat necrosis of bone. Between one and five circular cores were evenly spaced in the defect to cover approximately 20% of the defect area. A single periosteal patch was harvested from the lateral femoral condyle and was secured over the lesion and the osteochondral grafts using either sutures or biodegradable PLA tacks (Biosorb, Bionx Implants Inc, Tampere, Finland) where the adjacent cartilage was not healthy enough to take sutures. The chamber was made watertight with Beriplast P. Generations were injected into the space under the periosteum. The joint was closed routinely. The patients commenced continuous passive motion the next day for 24 hours, gradually increasing the amount of weight-bearing to reach full weight-bearing by the next day for 24 hours, gradually increasing the amount of weight-bearing to reach full weight-bearing six weeks. The total inpatient stay for this second procedure was four days.

Evaluation. Patient mobility and pain were evaluated using the KSS. Although this system is normally used for the evaluation of knee replacements, it was found suitable for this study as it incorporated clinical findings as well as aspects of daily activity. Scores were obtained six months, one, two and three years post-operatively.

A one-year arthroscopic examination was also carried out to evaluate the repair visually, which was graded using the International Cartilage Repair Society (ICRS) grading system. In criterion 1, which describes the degree of defect repair for both protocols A (ACI) and B (OATS), both protocols were scored. Only nine of 13 patients were scored in this way because of the unavailability of the relevant clinician.

Where possible, a small, fibrillated area at the edge of the repair was trimmed and used for histology to avoid damage to the repaired surface. Full thickness biopsies were removed from two patients. Sections were stained in Williams acid haematoxylin for five minutes, washed in running water, stained in 0.02% aqueous fast green FCF (C_{37}H_{64}N_{2}Na_{2}O_{10}S_{3}) for four minutes, followed by immersion in 1% acetic acid. Sections were then stained with 0.1% Safranin-O for four minutes, washed in 95% alcohol, dehydrated in xylene and mounted.

Results

Assessment of patient's response to treatment. The results of the ACI/OATS surgery on the KSS for pain and mobility in the 13 patients are summarised in Table I. The KSS was found to increase from a pre-operative mean of 63.9 ± 18.9 to 84.6 ± 12.3 at six months, 90.2 ± 8.3 at one year, and 90.6 ± 6.8 at two years post-operatively. A slight reduction to a KSS of 88 ± 14.1 was found at three years (Table I). A paired samples t-test was performed on complete data sets comparing pre-operative scores with follow-up at six, 12, 24 and 36 months and KSS were found to differ significantly (p < 0.05) from pre-operative scores at six, 12, 24 and 36 months (p = 0.031, 0.009, 0.005 and 0.007, respectively).

The improvements in the KSS were also analysed with respect to the location of the defect and no significant differences could be found (analysis of variance) between lateral femoral condylar, medial femoral condylar and patellar groups. The patient who was treated for a patellar lesion had a very low one-year score; this was, however, against a background of a relatively low pre-operative score. The size of lesion varied between 2.2 and 15.3 cm² (mean 4.8 cm² ± 3.5). The effect of the lesion’s surface area was also examined in all groups the area of the lesion appeared to be unrelated to the KSS. The KSS was also unaffected by age,
which would perhaps be expected because of the narrow age range (27 years). Both the number of cores and the number of cells impaired (4 x 10^6 to 3 x 10^7 cells) did not correlate with the KSS. Patient 1 required a total knee arthroplasty after three years owing to severe deterioration. This patient had received multiple other unsuccessful procedures, including carbon rods and a periosteal graft before our surgery. The results from this patient are included for completeness.

**Arthroscopy.** After one and three years the joint surfaces were evaluated arthroscopically. This enabled direct inspection of the repair site, allowed images to be captured and, in some patients, a biopsy was taken and debridement performed if required. The repair was scored in nine of 13 patients by using the ICRS cartilage repair assessment (Table II). In all cases the cores had become well integrated although their outlines could still be distinguished against the whiter coloured surrounding repair tissue. In some cases it was noted that the cartilage covering the cores was slightly softer than the healthy adjacent cartilage. The spaces between the cores were completely covered in all but one of the patients. This patient (number 2 in our series) had only patchy cover at one year, although the symptom score had improved from 55 to 88. In general, although the repair tissue seemed to be closely adherent to native and donor cartilage, it remained fibrillated at one year, was whiter than the cores and did not reach the same height as the cores after one year.

Arthroscopy after three years revealed that the repair tissue appeared to have changed little since one year post-operatively.

**Cell culture.** Studies using fluorescent immunocytochemistry showed that during the *in vitro* culture period the cells synthesised progressively less collagen type II and more collagen type I. Some collagen type II was detected after three passages indicating that the implanted cells retained some chondrogenic potential.

**Histological evaluation.** For simplicity and reproducibility Safranin-O staining was used which stains proteoglycans red and indicates the presence of hyaline cartilage. Fibrocartilage is stained blue. Tissue for histology was obtained at three different time points; at the time of repair, when the joint surface was debrided of diseased tissue, or at a one-year or three-year arthroscopy. In order to avoid damaging the joint surface the biopsy comprised loose tissue arising from the neocartilage that was not a full thickness biopsy. This was obtained in eight of 13 patients. Only two patients, patient 8 after one year and patient 13 after three years had a full thickness biopsy.

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*ICRS, International Cartilage Repair Society*
The debrided tissue was mainly fibrocartilage which had formed around the osteochondral cores, although areas of cartilage staining red with Safranin-O indicated the presence of some proteoglycan-rich and more hyaline-like tissue (Fig. 2a). The full thickness biopsies taken from patients 8 (Fig. 2b) and 13 (not shown) indicated that the repair tissue consisted mainly of fibrocartilage. This was stained reddish-brown by Safranin-O (Fig. 2b) which indicated the presence of some proteoglycan although the tissue did not have the appearance of hyaline cartilage and appeared to be fibrocartilage.

Discussion

Damage to hyaline articular cartilage can occur as a result of a variety of pathologies. It causes pain and other morbidities and can lead to the early onset of osteoarthritis. A number of options exist for the treatment of such lesions and currently the most popular and successful are ACI and OATS. Many of the patients treated for such lesions have undergone other unsuccessful surgical procedures. In this study it was proposed that the use of OATS could be augmented by the use of ACI. The combination of the two techniques was expected to be applicable to patients with large (> 2 cm) lesions and those with degenerative cartilage lesions. In deciding the design of the study we considered carefully the need for one or more control groups. The ideal study would have consisted of four groups: a control group, ACI only, OATS only and a combination of ACI and OATS.

However, insufficient patients are admitted to our centre each year. The four-arm clinical trial would have been a better study but would have been difficult to justify ethically as there would be a group of patients who were not treated. A three-arm trial with no control group was considered but was rejected owing to limited patient numbers. A two-arm trial seemed unjustified as it was not immediately obvious whether the control for ACI/OATS would be ACI only or OATS only. We elected to maximise our experience with the combination treatment while giving time for a three-year follow-up.

The results presented here demonstrated that ten of 13 (77%) patients treated with a combined ACI/OATS treatment showed an improvement in KSS three years. This compares with good to excellent clinical results, in patients treated with ACI alone, of between 65% and 92%. It is not possible, however, to determine whether one technique is superior to the other, particularly for the treatment of large degenerative lesions. To enable a proper comparison of the two techniques a fully randomised trial would need to be undertaken. As the number of patients with such large defects is limited it is difficult to draw any definite conclusions as to the comparative benefits of either treatment. However, the good clinical outcome in patient 2 would suggest that the combined ACI/OATS procedure provided an effective treatment for such a large defect.

Patient 4 showed an improvement after one and two years but by three years post-operatively showed a slight decrease in KSS. The repaired area in patient 1 showed pain-free improvement for a significant period but degenerated rapidly after approximately three years three months. This patient presented with the most severe lesion of the series and had undergone many other procedures, including implantation of carbon sponge and the application of a periosteal graft, both of which failed. During the three years following the ACI/OATS procedure the patient reported a good improvement that was reflected in his KSS. The subsequent degeneration of his repaired cartilage may bring into question the long-term stability of this type of repair. However, similar findings were not observed in the other cases and the severity of the lesion in this case may have brought about the late failure of the repair.

Peterson et al, in a study of autologous chondrocyte transplantation, showed a correlation between the hyaline nature of the cartilage and a good to excellent clinical outcome. The cartilage formed between the osteochondral cores in our study, as observed arthroscopically after one year, was more fibrous in nature, although the amount of information is limited due to our reluctance to retrieve biopsy material from the repaired lesion. A more recent study which is currently underway addresses this shortcoming by taking needle biopsies one year post-operatively. Histology of tissue trimmed from the defect during arthroscopy in eight of 13 patients indicates some of the properties of hyaline cartilage with a non-fibrous matrix rich in proteoglycans surrounding embedded cells (Fig. 2a). Fibrillation of the new cartilage between the cores was observed arthroscopically after one year which indicates that the
repaired tissue is not fully integrated hyaline cartilage. As the subchondral plate is breached during the OATS procedure, migration of stem cells from the marrow cavity may occur which has been shown previously to result in a more fibrous matrix. The osteochondral cores appear to become well integrated into their new position and are barely distinguishable from the surrounding cartilage after one year. The use of osteochondral cores may provide other benefits, particularly when cartilage has degenerated. The healthy subchondral bone that is also transplanted may prevent regression of the repair tissue. This is supported by the finding that the osteochondral cores remained healthy and retained their function as hyaline cartilage in the majority of patients. Patient 1 was an exception, because of the onset of severe osteoarthritis all repair tissue was subsequently lost (Fig. 3). Interestingly, no evidence of the implanted cores could be seen when the entire defect area was examined histologically. This would suggest that in this case the osteal component of the grafts was completely integrated and remodelled into the surrounding subchondral bone and that the cartilage component became fibrocartilage.

The actual contribution of each of the elements used in this procedure is unknown. The presence of the periosteal membrane may also be important as it provides chondrogenic stimuli to the underlying cells. It is thought that paracrine influences from the cambium of the periosteum may stimulate cell growth. The fate of the implanted cultured cells is also open to speculation. A recent study of green fluorescent protein-labelled chondrocytes in rabbits showed that after one week 100% of implanted cells could be detected. This decreased to 70% after two and three weeks and 15% after four which could indicate that implanted cells might not survive as well as previously thought.

The degree of improvement in the KSS with respect to the site of the lesion is also of interest. There was no significant difference between lateral femoral condyle (LFC), medial femoral condyle (MFC) and patellar groups, although this may be attributable to small group size. Subjectively, LFC lesions appear to be treated more effectively than lesions on the MFC, with the single patellar lesions scoring the lowest of all for our patients. This agrees with other studies using ACI which have shown that lesions on the LFC are more successfully treated than on the MFC and patella.

The post-operative regimen for patients treated using the combined procedure was continuous passive motion for 24 hours progressing to a gradual amount of weight-bearing with full weight-bearing after six weeks. In a study of ACI alone, the post-operative regimen was continuous passive motion for 48 hours progressing to a gradual weight-bearing for eight weeks with full weight-bearing after ten to 12 weeks. It is not known whether this slight difference in post-operative rehabilitation would contribute to any significant difference in clinical outcome.

While this study had no effective control the comparison of pre- and post-treatment scores indicated that an improvement had occurred. However, this combination treatment may not be superior to ACI alone for the treatment of smaller traumatic defects although we suggest that it provides excellent clinical results in the treatment of degenerative defects that would usually be considered too large for treatment.

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References