Autologous chondrocyte implantation using a bilayer collagen membrane: A preliminary report

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ABSTRACT

Purpose. To present preliminary clinical experience with Matrix-induced autologous chondrocyte implantation, a new tissue-engineering technique for treatment of deep cartilage defects, in which autologous chondrocytes are seeded on a tri-dimensional scaffold provided by a bilayer type I–III collagen membrane.

Methods. From December 1999 to January 2001, 13 patients underwent implantation procedure for deep cartilage defects. Age of patients ranged from 18 to 49 years (mean age, 35 years). The mean defect size was 3.5 cm² (range, 2.0–4.5 cm²). Clinical and functional evaluation were performed using various score systems for the ankle and the knee, and magnetic resonance imaging was performed at 6 and 12 months postoperatively. Membrane structure and cellular population were investigated by light microscopy, scanning electron microscopy, and electrophoresis before implantation.

Results. The mean follow-up was 6.5 months (range, 2–15 months). No complications were observed in the postoperative period. The 6 patients with a minimum follow-up of 6 months showed an improvement in clinical and functional status after surgery. Magnetic resonance images showed the presence of hyaline-like cartilage at the site of implantation; there was evidence of chondroblasts and type II collagen inside the seeded membrane.

Conclusion. Matrix-induced autologous chondrocyte implantation offers several advantages with respect to the traditional cultured cell procedure. These include technical simplicity, short operating time, minimal invasiveness, and easier access to difficult sites. It appears to be a reliable method for the repair of deep cartilage defects.

Key words: chondral defect; chondrocytes; tissue engineering

INTRODUCTION

During the past decade, interest in the management of cartilage lesions has grown owing to the introduction of new therapeutic options. Autologous chondrocyte implantation is a well-established procedure for the treatment of large, full-thickness chondral defects of different joints. Its main field of application is the knee, with the first clinical experience reported in 1987.¹

It has been shown that autologous chondrocyte implantation can lead to the formation of hyaline-like cartilage in sites of repair; this represents a significant advantage with respect to conventional techniques relying on the repair potential of bone marrow, such as abrasions, drilling, and microfractures.²⁻⁵ Moreover,
autologous chondrocyte implantation can overcome the limits of more recent techniques—namely, mosaicplasty—for the treatment of large chondral lesions, where dead spaces between grafts are filled with fibrocartilage and surface irregularities can be present.6 In the autologous chondrocyte implantation technique, a suspension culture of autologous chondrocytes is injected into the defect, which is previously covered with a periosteal patch; satisfactory medium- and long-term clinical results have been achieved.2–4,7

Matrix-induced autologous chondrocyte implantation (MACI; Verigen, Leverkusen, Germany) can be considered an evolution of conventional autologous chondrocyte implantation. It is a tissue-engineering technique that requires the use of a type I–III bilayer membrane of collagen seeded with cultured autologous chondrocytes. MACI has been adopted at the Institute of Orthopaedics and Traumatology, University of Insubria, Italy since December 1999. This study reports the results of using MACI on 6 patients, with a minimum follow-up of 6 months.

MATERIALS AND METHODS

The first surgical step was the arthroscopic assessment of the joint and the harvesting of articular cartilage (5 mm x 10 mm) from a non-weightbearing area of the knee. In our series of patients, all biopsies were performed at the superior medial edge of the femoral trochlea. The biopsy material was immediately placed in a nutrient medium tube and forwarded to a cell laboratory (Verigen, Germany), along with 100 ml of autologous blood, distributed in 10 tubes. An enzymatic process that separates the chondrocytes from the matrix, and cell culture (using autologous serum and growth factors) over 3 to 4 weeks induced them to multiply to become about 15 to 20 million dedifferentiated cells. The cells were seeded on a type I–III collagen bilayer membrane and sent back to the hospital for the scheduled date of implantation.

The implant procedure was performed in a tourniquet-controlled bloodless field. In cases of knee lesions, the joint was approached through a small parapatellar skin incision and arthrotomy (Fig. 1a). For the ankle, osteotomy of the malleolus was usually performed to gain good exposure of the chondral defect. The softened borders of the defect were debrided without any bleeding of the subchondral bone plate. In cases of accidental bleeding, haemostasis was achieved by local application of epinephrine and thrombin.

By taking an impression, the outline of the defect was transferred to the seeded membrane, which was cut out with scissors (Fig. 1b). Using fibrin glue (Fig. 1c), the membrane was implanted in the defect and its stability was tested by making some movements of the joint (Fig. 1d). After wound closure, the joint was covered with a compressive elastic bandage. Continuous passive motion was started on the first postoperative day for the knee and on the third postoperative day for the ankle, achieving daily increases in range of motion. Isometric strengthening of the quadriceps began in the first week, while partial weightbearing was allowed after 2 months and full weightbearing shortly thereafter. Return to sports was related to the type of joint impact required by the

Figure 1 The Matrix-induced autologous chondrocyte implantation procedure. (a) Exposure of the chondral defect on the medial femoral condyle through a small arthrotomy of the knee, (b) cutting of the seeded membrane using an impression of the defect, (c) fixation of the membrane with fibrin glue, and (d) final appearance of the graft.
specific activity: sports requiring low loading stresses on the knee (e.g. swimming and cycling) were allowed 3 months after surgery, while sport activities that required jumping, twisting, and running with quick stops and starts (e.g. soccer and basketball) were delayed for at least 10 months.

**Membrane analysis before implantation**

At low magnification, light microscopy showed the double layer of the membrane — namely, the smooth, thin and denser surface, covering the loose collagen network of the porous portion. Scanning electron microscopy revealed the presence of chondroblasts that produced collagen fibres in the tridimensional scaffold (Fig. 2). The nature of the newly synthesised fibres was investigated by sodium dodecylsulphate-polyacrylamide gel electrophoresis (with a 7.5% gel). Type II collagen was demonstrated in both the buffer medium and the membrane after enzymatic digestion.

**Clinical series**

Between December 1999 and January 2001, MACI was used to treat 13 patients with chondral defects. This series included 9 males and 4 females, whose mean age was 35 years (range, 18–49 years). The sites of the defects were the following: medial femoral condyle (n=8), lateral femoral condyle (n=2), femoral trochlea (n=1), and talar dome (n=2). The average size of the defects was 3.5 cm² (range, 2.0–4.5 cm²). Five patients had undergone prior surgical procedures for the treatment of the cartilage defects, but they still presented with severe symptoms. Patient characteristics are summarised in the Table.

![Figure 2](https://via.placeholder.com/150)

**Figure 2** Photomicrograph showing chondroblasts (marked *) synthesising collagen fibres (marked >) in the seeded membrane (SEM, x980).

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years)/Sex</th>
<th>Lesion type</th>
<th>Location/side</th>
<th>Defect size (cm²)</th>
<th>Previous operation</th>
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<tr>
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<td>MFC/L</td>
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<tr>
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<tr>
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<td>TL†</td>
<td>MFC/R</td>
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<td>Mosaicplasty</td>
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<tr>
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<td>49/M</td>
<td>TL†</td>
<td>FT* /R</td>
<td>4.0</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>44/F</td>
<td>TL†</td>
<td>TD** /R</td>
<td>3.5</td>
<td>-</td>
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<tr>
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<td>39/M</td>
<td>TL†</td>
<td>TD, Tibia/L</td>
<td>2.5, 2.0</td>
<td>Debridement, lavage</td>
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<td>MFC/L</td>
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<tr>
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<tr>
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<td>3.0</td>
<td>Microfractures</td>
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<tr>
<td>13</td>
<td>38/M</td>
<td>TL†, ACL†</td>
<td>MFC/L</td>
<td>3.0</td>
<td>-</td>
</tr>
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* OCD osteochondritis dissecans
† TL traumatic lesion
‡ ACL anterior cruciate ligament
§ LFC lateral femoral condyle
¶ MFC medial femoral condyle
* FT femoral trochlea
** TD talar dome

Table

**Patient characteristics**
In case 4, MACI to treat a medial femoral condyle was combined with a tibial osteotomy to correct a genu varum. In case 6, a concomitant lateral retinacular release was performed for patellar realignment. Clinical and functional evaluation were performed using the International Cartilage Repair Society (ICRS) evaluation form, the modified Cincinnati rating system, the Lysholm function score, and the Tegner activity scale for the knee, while the American Orthopaedic Foot and Ankle Society (AOFAS) score system was used for assessing the ankle. Statistical comparison between preoperative and postoperative values was not performed, owing to the limited data. Magnetic resonance images were taken preoperatively (except for cases 1 and 2), as well as at 6 and 12 months postoperatively. Fat-suppressed T1-weighted and T2-weighted sequences were performed.

RESULTS

All patients were evaluated at a mean follow-up of 6.5 months (range, 2–15 months). No complications were observed in the postoperative period and all patients followed the rehabilitation protocol. Clinical and functional results were reported for the 6 patients who were treated for knee lesions, with a minimum follow-up of 6 months. Preoperatively, all 6 patients but one had severely abnormal knees. At the latest follow-up, according to the ICRS evaluation form, there were 4 normal knees and 2 nearly normal knees. The mean modified Cincinnati score (maximum, 10) increased from 4.7 (range, 2–6) preoperatively, to 8.5 (range, 6–10) at the time of this study, as regards the clinical evaluation. Patients’ mean evaluation score (maximum, 10) increased from 2.6 (range, 2–4) to 8

Figure 3  Histograms showing preoperative and postoperative scores of various clinical and functional evaluation scales of 6 patients with a minimum follow-up of 6 months.
The Lysholm function score (maximum, 100) at the time of biopsy showed a mean value of 46.5 (range, 18–67), while at the latest follow-up, it was 94 (range, 87–97). Finally, the Tegner activity scale (maximum, 10) averaged 2.6 (range, 1–4) before MACI, and increased to 6.5 (range, 5–7) [Fig. 3]. Magnetic resonance images taken 6 and 12 months after MACI showed the presence of hyaline-like cartilage in the site of the defect, with restoration of the articular surface, in all the knees (Fig. 4).

**DISCUSSION**

Chondral or osteochondral defects are joint lesions that can cause pain and functional disability in young individuals, with a potential progression to early osteoarthritic changes. Because of the low capacity of intrinsic repair, several methods for inducing healing of the cartilage injuries have been proposed.

It is well known that surgical methods relying on bone marrow stimulation (e.g. drilling and microfractures) lead to the formation of fibrocartilage that fills the defect but has poor mechanical properties, not being capable of resisting cyclic loading and shearing forces for a long time. Mosaicplasty is a reliable method for restoring the height and shape of the articular surface in cases of small focal osteochondral defects. However, if the lesion is large (>2 cm²) and if the patient has high functional demands, this technique has some limitations. The need for harvesting several grafts may also have negative consequences on the donor site. The presence of dead spaces between the grafts in the recipient site and differences in thickness of the transposed osteochondral tissue are further concerns regarding clinical results. Cell therapy with autologous cultured chondrocytes seems to solve these problems. In the medium and long term, conventional autologous chondrocyte implantation gives good clinical and functional results in knee lesions.

An adequate selection and preoperative assessment of the patients are fundamental for successful treatment outcome. Compliance to rehabilitation protocols, as well as correction of aetiological factors of the chondral lesions (e.g. tibiofemoral and patellofemoral malalignment, and instability), should be verified before autologous chondrocyte implantation is performed. Technical limits related to conventional autologous chondrocyte implantation, however, include non-homogeneous distribution of chondrocytes in the recipient site owing to the use of a cellular suspension, and the risk of leaking out if sealing is inadequate. Moreover, complications related to the use of the periosteal patch

![Figure 4](a) Computed tomography scan of the left knee of patient one with an osteochondritic lesion of the lateral femoral condyle. The size of the defect measured 4.5 cm². (b) Magnetic resonance image (T1-weighted) taken 6 months after implantation procedure: the defect was filled with hyaline-like cartilage, with good recovery of the articular surface despite a small area of posterior hypertrophy of the graft. An increased signal in the subchondral bone indicates localised oedema. (c) Magnetic resonance image one year after surgery: tissue remodelling is evident and subchondral bone oedema has disappeared.
REFERENCES


