ABSTRACT

Objective: To show the first Brazilian autologous chondrocyte implantation. Methods: Young patients with localized lesions in femoral condyle and talus were selected. The clinical evaluation was complemented with the imaginologic resources. Results: The neoformed cartilage tissue occupies the defect. The patients showed improve in the quality life, returning to the daily and sport activities. Conclusions: It was possible to concluded that the autologous chondrocyte implantation is a promising technique for the treatment of femoral condyle and talar condral lesions.

Keywords: Traumatology; Cartilage; Chondrocytes; Biotechnology; Cell culture techniques

INTRODUCTION

The difficulty in repairing chondral lesions is a challenge for orthopedic surgeons. Conventional(1) and surgical(2) alternatives usually result in relief of symptoms for patients, albeit without formation of hyaline tissue at the lesion site. Surgical techniques, such as microfracture, are based on the principle of filling the chondral lesion with mesenchymal cells from the bone marrow(3), resulting in formation of fibrous tissue with structural and biomechanical properties distinct from those of the original hyaline tissue(4).

Autologous chondrocyte implantation, also called autologous chondrocyte transplant by some authors, is a technique developed in the late 1980s, in Sweden, which uses the principles of Biotechnology for treating chondral lesions(5-7). The technique enables regeneration of the injured hyaline cartilage, restoring articular biomechanics. Cells used in the technique are autologous, obtained by arthroscopy with biopsy of a low load-bearing area of the joint(6). This cartilage sample is submitted to enzymatic digestion in order to isolate chondrocytes – i.e. the cartilage cells(8-9).

Chondrocyte proliferation is performed in vitro, since this condition is not obtained in vivo, due to the absence of innervation and vascularization of the tissue associated with the high level of chondrocyte specialization. Cell culture protocols that allow efficient cell proliferation have been established. A substantial quantity of cells (10⁶) is injected directly into the chondral lesion site(6-7).
For the inoculation of cultivated cells, a specific surgical technique was described creating a closed cavity in the chondral lesion, with a perilesional suture of a periosteal membrane removed from the patient’s tibia. The implanted chondrocytes have the capacity of reorganizing to produce a hyaline matrix with the same characteristics as the original hyaline cartilage. Neoformed tissue integration is noted, filling in the lesion to its depth \(^{(6)}\). Data demonstrated 71% filling during the first eight weeks after treatment with basically hyaline-type tissue, simulating embryological formation of cartilage \(^{(7)}\).

As of the first clinical results published in 1994 \(^{(6)}\), other scientific reports have demonstrated regeneration of the damaged cartilage and a restoration of the patient’s quality of life \(^{(10-13)}\). Later studies enabled improvement of the technique, called the “sandwich technique” with the use of two periosteum layers to prepare the lesion \(^{(14)}\), the use of growth factors in chondrocytes cultures \(^{(15)}\), use of bioreactors \(^{(16)}\) and, especially, the development of biomaterials utilized as cell carriers for the chondrocytes \(^{(17-18)}\).

**OBJECTIVE**

The objective of this study was to report the first clinical cases of autologous chondrocyte implantation performed in Brazil.

**METHODS**

**Patients**

This study enrolled six young patients with mean age of 30 years (ranging from 20 to 39 years old) presenting with a traumatic chondral lesion; three patients had lesions of the femoral condyle and three had osteochondral lesions of the talus (Chart 1). These patients had been previously submitted to conservative and surgical treatments with no satisfactory results.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Lesion</th>
<th>Side</th>
<th>Size</th>
<th>Previous treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>39</td>
<td>F</td>
<td>Femoral condyle</td>
<td>R</td>
<td>40 mm</td>
<td>Arthroscopy + arthroscopy</td>
</tr>
<tr>
<td>II</td>
<td>36</td>
<td>F</td>
<td>Femoral condyle</td>
<td>L</td>
<td>48 mm</td>
<td>Arthroscopy</td>
</tr>
<tr>
<td>III</td>
<td>29</td>
<td>M</td>
<td>Femoral condyle</td>
<td>R</td>
<td>50 mm</td>
<td>Conservative</td>
</tr>
<tr>
<td>IV</td>
<td>25</td>
<td>M</td>
<td>Talar</td>
<td>R</td>
<td>24 mm</td>
<td>Arthroscopy</td>
</tr>
<tr>
<td>V</td>
<td>20</td>
<td>F</td>
<td>Talar</td>
<td>R</td>
<td>23 mm</td>
<td>Arthroscopy + arthroscopy</td>
</tr>
<tr>
<td>VI</td>
<td>35</td>
<td>F</td>
<td>Talar</td>
<td>R</td>
<td>20 mm</td>
<td>Arthroscopy</td>
</tr>
</tbody>
</table>

In short, the criteria for selection included: absence of articular degenerative processes, young active patients (aged between 15 and 55 years), ineffective prior treatment (surgical or conventional), and persistence of pain and limitation of movement.

Among the patients with femoral condyle lesions (chondromalacia grade IV), two were female and one was male. The mean dimension of the lesions was 46 mm (ranging from 40 to 50 mm). In the patients with an osteochondral lesion of the talus, two were female and one was male, and the mean dimension of the lesions was 22 mm (ranging from 20 to 24 mm). The lesions were located in the medial half of the talus, involving the central and posterior portions.

Treatment consisted of three distinct phases: arthroscopy for biopsy with cartilage collection, cell culture for chondrocyte proliferation and arthroscopy for cell implantation.

**Arthroscopy**

Selected patients were submitted to arthroscopy for sample collection of cartilage from a low load-bearing area of the upper medial femoral condyle (same as for the talus lesions). The cartilage samples, weighing approximately 400 mg, were collected with an arthroscopic grasper and deposited in the culture medium \(^{(6)}\). During this phase, the lesion was prepared with debridement and the need for bone grafts was assessed.

**Chondrocyte isolation and culture**

The articular cartilage samples were transported to the tissue engineering laboratory, where they were submitted to the enzymatic digestion process in order to isolate the cells \(^{(8-9)}\). The chondrocytes were then cultivated in HamF12 medium containing 1% penicillin/streptomycin and 10% autologous serum for periods of approximately 30 days. Microbiological evaluations and identification tests were performed on the tissues. At the end of culture time, cells were trypsinized, centrifuged and concentrated in 0.3 ml medium.

**Chondrocyte implantation – knee**

Cultivated chondrocytes were implanted in the femoral condyle using the surgical technique described above \(^{(6)}\). The patients, under general anesthesia, underwent arthroscopy with a tourniquet for access to the chondral lesion. The cartilage around the lesion was debrided and small bleedings were contained using noradrenaline. A mold of the chondral lesion was made on sterile paper for removal of a portion of the tibial periosteum membrane through a second incision. The periosteum was sutured.
over the chondral lesion with 6-0 absorbable thread (Vicryl, Johnson & Johnson®) dampened in sterile glycerin in order to create a closed cavity. The suture was sealed with fibrin glue, allowing one small orifice for injection of the suspension of cells. After inoculation of the cells and conclusion of the periosteal suture, the articular capsule was sutured, followed by the retinaculum and skin.

Chondrocyte implantation – talus
Cultivated chondrocyte implantation in the ankle was performed using the surgical technique described above. Patients under general anesthesia were submitted to arthrotomy of the ankle with osteotomy of the medial malleolus. The cartilage around the lesion was debrided and a mold was made in sterile paper. Using the “sandwich technique”, two periosteal membranes obtained from the tibia (through a second incision) were used to create a cavity in the chondral lesion. The first periosteal membrane was placed at the base of the lesion with fibrin glue, maintaining parietal orientation towards the subchondral bone. The second membrane was sutured around the lesion creating a closed cavity. This suture followed the steps described above. After inoculation of the cells, the medial malleolus was fixed in its original site and the superficial layers were closed as usual.

Rehabilitation
The initial phase of rehabilitation should be approached with caution and adapted specifically for each lesion. Articular movement with continuous passive motion (CPM) or a knee-ankle-foot device was initiated during the immediate postoperative period. Isometric exercises were initiated early and load-bearing was allowed in the first eight weeks after surgery. After the seventh month, low-impact activities were resumed. After 12 months, patients were able to resume even repetitive impact and high impact activities.

Assessment
Patients were evaluated clinically according to the Lysholm questionnaire specific for knee symptoms and the generic quality of life instrument (SF-36). To assess ankle symptoms, Student’s t test enabled comparing the means of the Analog Pain Scale, and the Wilcoxon test, the values of the American Orthopaedic Foot and Ankle Score (AOFAS). A p < 0.05 significance level was adopted. Magnetic resonance images were performed for patient follow-up, with post-implantation arthroscopies performed in five patients along with biopsies of the treated area.

RESULTS
The cell culture phase was performed in 30 days, on average. No contaminants were detected in any of the cell cultures. Operations transcribed normally, with no events. Operative time of the second phase, arthrotomy, was approximately two hours, primarily due to the suture of the periosteal layer over the chondral lesion (Figure 1). The symptoms of patients treated in the knee were considerably reduced after surgery, according to the Lysholm questionnaire (Chart 2), and all domains of SF-36 showed a significant improvement, with mean values of 90-100 (maximum of 100). Similar results were noted in patients treated in the ankles, based on data related to pain and on the AOFAS score (Chart 3). Patients obtained consolidation of the malleolus osteotomies between the sixth and eighth postoperative weeks.

The magnetic resonance imaging showed the formation of tissue similar to the original tissue, which was confirmed by posterior arthroscopies (second look, Figure 2). It was possible to observe integration of the periosteum on the articular surface and formation of tissue below the periosteum filling in the chondral cavity. Tissue indentation did not show the same pattern as the areas neighboring

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Figure 1. Patient IV, talus lesion. Technique for autologous chondrocyte implantation. A. Measuring the chondral lesion; B. Chondral lesion debridment and preparation of the subchondral bone; C. Fixating the first periosteal membrane over the subchondral bone (only in surgeries of the talus); D. Suture of the upper periosteal membrane around the chondral lesion to create a closed cavity and enable the inoculation of cultivated chondrocytes

Chart 2. Numerical and nominal values of the Lysholm Scale – a specific questionnaire for knee symptoms

<table>
<thead>
<tr>
<th>Patient</th>
<th>Preoperative</th>
<th>Postoperative</th>
<th>Second look</th>
<th>Final assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>62 (bad)</td>
<td>95 (excellent)</td>
<td>9 months</td>
<td>30 months</td>
</tr>
<tr>
<td>II</td>
<td>37 (bad)</td>
<td>100 (excellent)</td>
<td>4 months</td>
<td>30 months</td>
</tr>
<tr>
<td>III</td>
<td>65 (regular)</td>
<td>89 (good)</td>
<td>---</td>
<td>24 months</td>
</tr>
</tbody>
</table>
the implant, especially before 12 months, but rather a pattern typical of grade I chondromalacia. The small time interval up to arthroscopy justified this finding, since tissue regeneration occurs from the subchondral bone to the articular surface. The patients who underwent arthroscopy at around 12 months after implantation showed indentation similar to that of the healthy neighboring cartilage.

The biopsies demonstrated integration of the periosteum and neoformation of hyaline cartilaginous tissue in the cell implantation area (Figure 3). In one of the patients, adhesion of the knee joint was reported, which was resolved by arthroscopy. Patients resumed their daily routine and sports activities within 12 months. The clinical and imaging results remained unchanged during the follow-up time with the patients.

**DISCUSSION**

The difficulty of chondral tissue regeneration continues today. Nevertheless, advances in Biotechnology brought alternative approaches, such as the use of growth factors\(^ {22} \) or biomaterials\(^ {23} \). The most significant progress in the area of chondral regeneration is related to cell therapy\(^ {6,8,19} \). As of 1987, chondral lesion repair has been proposed with the use of autologous cells\(^ {5} \). Experimental studies have demonstrated not only tissue repair, but regeneration of the hyaline cartilage originally present in the joint\(^ {7} \). These data were posteriorly confirmed in human studies\(^ {6} \).

Chondrocytes cultivated \textit{in vitro} suffer a cell dedifferentiation process induced by physical and biochemical factors\(^ {24-27} \). In this condition, chondrocytes adhere to the substrate of the cultures and display elongated morphology typical of fibroblasts. Phenotypical changes are also accompanied by changes in the gen expression, and chondrocytes start to preferentially synthesize type-I collagen, with decreased type-II collagen and proteoglycan aggregate syntheses\(^ {24-25} \). Phenotypic and genotypic changes favor cell proliferation. The proliferated cells are capable of returning to their differentiated condition by biochemical stimuli and by interaction with poorly adhesive substrates\(^ {8,25-26} \). Once implanted in a chondral lesion, cells have the capacity to adhere to the subchondral bone, reassuming the characteristic phenotype and genotype, and regenerating the damaged area. The presence of a high density of dedifferentiated cells in the chondral lesions simulates the embryological formation of cartilage\(^ {6} \). Published studies showed the formation of hyaline cartilage in the treated region, justifying the patient’s returning to normal activities and the relief of symptoms – results that are considered permanent\(^ {10-12,19-21,28-30} \).

A significant improvement in the quality of life of patients treated in this study, with a return to normal daily activities and an absence of symptoms after treatment, was noted. The rehabilitation period is extremely important for treatment success and should be carefully adhered to by the patient. Regeneration of the tissues was noted in imaging tests, and the results were confirmed by arthroscopies and biopsies. Posterior arthroscopies with biopsies enabled observing periosteal integration and tissue formation with full restoration of the injured area.
No adverse effects of the treatment were observed. One of the patients presented an adhesion of the joint treated by arthroscopy. After surgery, the patient did not experience any other symptoms. The clinical progress of patients treated followed the pattern described in literature, as did the images obtained during patient follow-up, both by magnetic resonance imaging and arthroscopy. These results indicate the technical adequation of the implantation performed at our institution.

CONCLUSIONS

Despite the number of patients, it is possible to conclude that autologous chondrocyte implantation is a promising technique to treat traumatic chondral lesions, enabling recovery of the injured area. New studies involving the development of biomaterials and improvement of surgical technique are future perspectives for this procedure.

ACKNOWLEDGEMENTS

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REFERENCES