AUTOLOGOUS CHONDROCYTE TRANSPLANTATION–SERIES OF 3 CASES

Riccardo Gomes Gobbi[1], Marco Kawamura Demange[1], Ronald Bispo Barreto[2], José Ricardo Pécora[3],
Márcia Uchôa de Rezende[4], Tarcísio E.P. Barros Filho[5], Christiane Bertachini Lombello[6]

ABSTRACT

Hyaline cartilage covers joint surfaces and plays an important role in reducing friction and mechanical loading on synovial joints such as the knee. This tissue is not supplied with blood vessels, nerves or lymphatic circulation, which may be one of the reasons why joint cartilage has such poor capacity for healing. Chondral lesions that reach the subchondral bone (osteochondral lesions) do not heal and may progress to arthrosis with the passage of time. In young patients, treatment of chondral defects of the knee is still a challenge, especially in lesions larger than 4 cm. One option for treating these patients is autologous chondrocyte transplantation/implantation. Because this treatment does not violate the subchondral bone and repairs the defect with tissue similar to hyaline cartilage, it has the theoretical advantage of being more biological, and mechanically superior, compared with other techniques. In this paper, we describe our experience with autologous chondrocyte transplantation/implantation at the Institute of Orthopedics and Traumatology, Hospital das Clínicas, University of São Paulo, through a report on three cases.

Keywords – *Knee; Transplantation, autologous; Chondrocyte*

INTRODUCTION

Hyaline cartilage covers joint surfaces and plays an important role in reducing friction and mechanical loading on synovial joints such as the knee. This tissue is not supplied with blood vessels, nerves or lymphatic circulation, which limits its capacity for healing[1]. Injury to or degeneration of the joint cartilage diminishes its mobility and often causes pain on movement and, in cases of greater severity, causes deformities and constant pain[1,2].

Chondral lesions that reach the subchondral bone (osteochondral lesions) do not heal and may progress to arthrosis with the passage of time[3-6].

Excellent clinical results can be obtained among elderly patients with severe arthrosis when they treated with total knee arthroplasty. For young patients, no standardized treatment for chondral defects of the knee has yet been established in the literature, despite some attempts to organize such treatments in the form of management algorithms[7,8]. Among the therapeutic alternatives, simple joint lavage with or without debridement can be mentioned: through this, the substances and free bodies that degrade the cartilage and cause pain can be removed[9]. Perforations, microfractures and abrasion regenerate the joint surface with tissue similar to hyaline cartilage (fibrocartilage), from medullary mesenchymal cells[10]. Mosaicplasty (autologous osteochondral transplantation) and autologous chondrocyte transplantation, also known as autologous cartilage implantation (ACI)[9], are other treatment alternatives.

Functional units of joint cartilage are formed by different layers of chondral cells and by subchondral and spongy bone, below the cartilage. Techniques that interfere with the subchondral bone plate (perforations,

microfractures and mosaicplasty) may even reestablish the joint surface, but they do not restore the functional unit of the cartilage, especially the impact absorption function. Because the ACI technique does not violate the subchondral bone, and because it repairs the defect with tissue resembling hyaline cartilage, it theoretically has biological and mechanical advantages over other techniques[11], although such superiority has not been definitively proven. Thus, the use of this technique is still controversial, whether because of its high cost or because of the lack of definitive scientific evidence to justify its large-scale use.

In this paper, we describe our experience with ACI at the Institute of Orthopedics and Traumatology, Hospital das Clínicas, University of São Paulo School of Medicine (IOT-HCFMUSP), through a report on three cases.

SAMPLE AND METHODS

Firstly, we describe the technique that we use for chondrocyte transplantation.

The procedure is carried out in two stages. Initially, a biopsy is taken from the cartilage and is sent for chondrocyte culturing (cell proliferation) in the laboratory. Next, cell implantation is performed. This consists of arthrotomy, preparation of the chondral defect, harvesting of periosteum, hermetic fixation of periosteum over the lesion using stitches and fibrin glue, injection of chondrocyte concentrate and closure of the operative wound.

Harvesting of cartilage for cell expansion

During the arthroscopic evaluation, the surgeon should perform delicate debridement of the lesion and remove possible free bodies and cartilage fragments from the joint. Only then should cartilage harvesting for cell expansion be performed, from areas of the femur that do not receive loads (superomedial and superolateral edges of the femoral condyles and the lateral wall of the intercondylar notch).

To harvest the cartilage, curettes or intervertebral disc biopsy tweezers are used, and three or four small cartilage fragments are obtained, with their thickness totally or partially free of subchondral bone. To achieve enzymatic digestion and adequate cell culturing, around 200 to 300 mg of joint cartilage are required, corresponding to around 1 cm^2.

During this same procedure, 200 ml of venous blood should be taken from the patient. From this blood, the serum that will be used with the culture medium for cell proliferation is extracted.

In vitro cell expansion

The main objective of *in vitro* manipulation of chondrocytes is to increase the number of cells. This process begins with enzymatic digestion of the cartilage matrix, which corresponds to around 90% of the tissue. To proliferate the chondrocytes, monolayer culturing should be used. In this system, the cells are cultivated in 25 cm^2 culturing flasks with the DMEM/HAMF12 culture medium supplemented with 10% autologous serum[11]. The autologous serum is used as a source of hormones and growth factors for the cultured cells. Under these condition, because of the morphological and functional changes, the chondrocytes acquire proliferative capacity. They are kept in this monolayer culturing system for a mean period of four weeks, in order to obtain around 10 X 10^6 cells, i.e. a concentration that is considered to be a therapeutic dose[11].

Today, there are three generations of chondrocyte cultures. In the first generation, the cell culture is performed as a monolayer and the cell implant in the defect is covered with a piece of autologous periosteum (ACI-P) or with a manufactured membrane of collagen I/III (ACI-C). In the second generation, after cell expansion in a monolayer, the cells are deposited on a carrier membrane/matrix, thus obtaining a membrane sown with chondrocytes. In the third generation of ACI, the chondrocyte culture is deposited on a matrix of hyaluronic acid that is structured in three dimensions, thereby enabling homogenous distribution of the chondrocytes inside the lesion. Our technique consists of the first generation.

Second surgical stage

A standard parapatellar, medial or lateral incision is made and the knee is opened up by means of mini-arthrotomy. After achieving adequate exposure, the lesion should be debrided, to remove all dead tissue. The diseases cartilage surrounding the lesion is removed, the chondral fissures and erosions inside the defect are regularized and the fibrous tissue present at the base of the lesion is debrided. The aim of this initial preparation on the defect is to obtain a lesion surrounded by healthy cartilage and with its base free from blood. Once the defect has been prepared, a mold of the lesion should be made, using a sheet of aluminum or sterile paper. This mold is used to assist in removing the periosteum graft in the next stage.

Periosteum graft

The periosteum graft is obtained through an incision over the medial proximal tibia, around 4 cm distally from the goosefoot (Figure 1). The periosteum is dissected, removing all the fat and fascia on top of it. The mold of the lesion that were obtained earlier is positioned and the periosteum is marked out with the addition of 1-2 mm on the edge. This precaution is taken because there is a tendency for the periosteum to shrink after harvesting. Next, the graft is cut on the mark that was made and, using a periosteum detachment device, the periosteal membrane is removed from the bone. The thinner the membrane is, the lower the risk of hypertrophy and fibrillation of the periosteum is. This also makes it possible to inject a greater volume of chondrocyte concentrate. Graft perforation during the harvesting should be avoided. A mark should also be made on the graft to identify the internal layer of the periosteum.
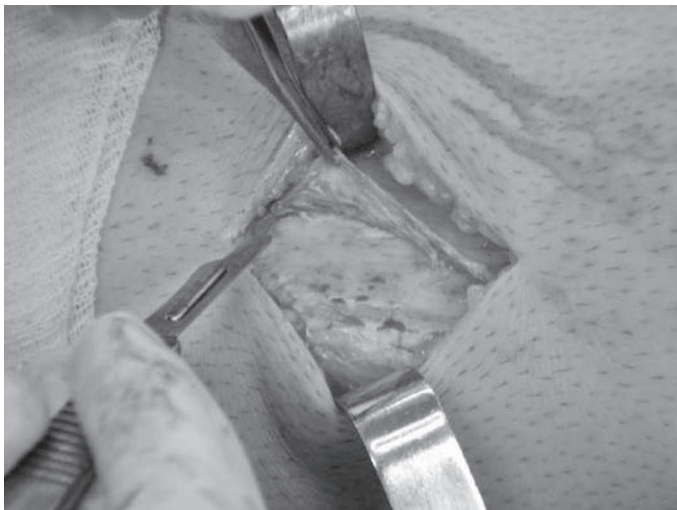
Figure 1 – Removal of the periosteum graft

Suturing of the periosteum graft and chondrocyte implantation

The internal layer of the periosteum contains chondrogenic cells that, in combination with the implanted chondrocytes, help in producing the repair tissue. This layer, which was identified earlier, should be placed facing the bony part of the lesion and be anchored using separate stitches of 5-0 or 6-0 thread. These stitches should be made using small cutting needles, starting by going from outside to inside in the periosteum and ending by going from inside to outside in the cartilage. The knot is tied on the side of the periosteum, thereby avoiding cutting the cartilage with the thread. These stitches should be spaced 3-4 mm from each other, and

the intervals should be sealed with fibrin glue. Next, a check for any leakage sites is made by gently injecting physiological serum under the periosteum. Once the hermetic closure of the lesion has been verified, the surgeon should inject the autologous chondrocyte concentrate into the defect (Figure 2).

Figure 2 – Injection of the chondrocyte culture into the prepared defect

The procedure is summarized in Figure 3.

During the years 2006 and 2007, three ACI procedures were performed by the Knee Group of IOT-HCFMUSP. All of these cases had chondral lesions that affected the entire thickness of the cartilage (Outerbridge[12] or International Cartilage Repair Society [ICRS][13] grade IV) and had not presented a satisfactory response to clinical treatment (of minimum duration three months) or to other forms of surgical treatment.

CASE 1

26-year-old male patient

This patient had presented a complaint of pain in his left knee for one year, without any history of trauma or twisting of this knee. He had the antecedent of having sequelae of poliomyelitis in his right leg since infancy.

On physical examination, he did not present any discharge or increased volume in the affected knee. His muscle strength was normal and he presented slight genu varum. He had a normal range of motion and did not present any signs of instability or meniscal lesions. The preoperative clinical assessment showed a subjective IKDC of 31.03 (percentile < 5) and a Lysholm score of 40.
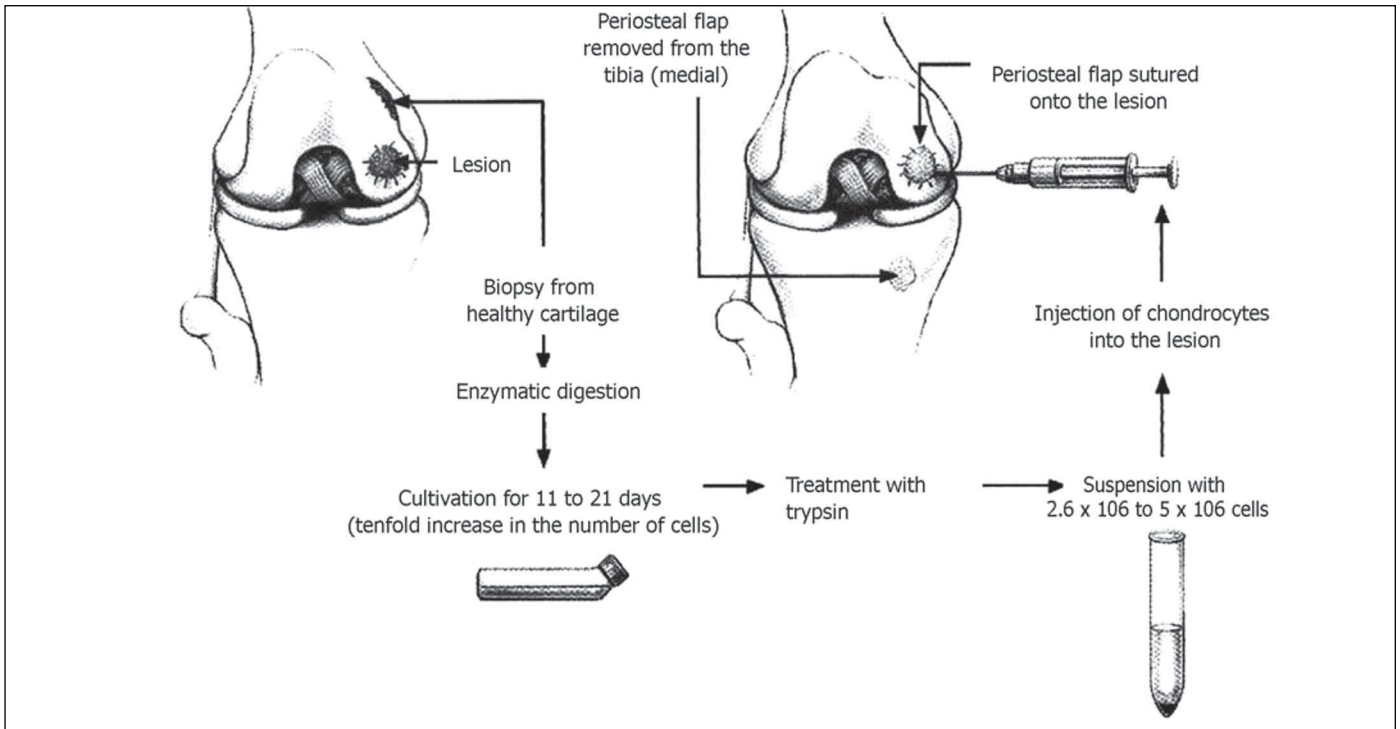
Figure 3 – Schematic drawing of the autologous chondrocyte transplantation/implantation procedure (Brittberg, 1994)[11]

On imaging examinations, he presented a lesion compatible with osteochondritis dissecans in the medial femoral condyle, in the loading area. This lesion was confirmed by magnetic resonance examination, which also showed that fixation of the fragment would be impossible. A panoramic radiograph on the legs showed a mechanical axis of eight degrees of varus.

Initially, non-operative treatment methods were instituted, using analgesic and chondral protective medications, physiotherapy and removal of the mechanical overload, but without success. Since the patient did not present any improvement, it was decided to proceed with surgical treatment.

The patient underwent arthroscopic surgery to remove a cartilage sample outside of the loading area, from the lateral part of the femoral trochlea. During this procedure, the lesion could be viewed: it presented a total area of 5 cm^2 of detached cartilage, without any significant subchondral bone defect (< 5 mm). The cartilage sample was sent for culturing in order to multiply the chondral cells.

Thirty-five days later, the patient underwent the second stage of chondrocyte transplantation, together with osteotomy for varus correction.

Six months later, the patient underwent surgery again, to remove the plate, with arthroscopy again, to inspect the transplanted area.

CASE 2
40-year-old male patient

This patient had a history of pain in his left knee that began seven years earlier, after twisting his knee during sports practice.

He started to be followed up at our service three years after the event. At that time, he was diagnosed as presenting a medial meniscal lesion and, for this reason, he underwent partial meniscectomy of the posterior cornu of the medial meniscus. Initially, he responded well to the treatment, but one year later, he evolved with new complaints of pain in the knee. It was decided to perform arthroscopy again, and revision of the meniscectomy was performed. A diagnosis of complete chondral lesion (grade IV), measuring 1 cm^2 on the medial femoral condyle in the loading area, was made. During the same operation, the chondral defect was perforated (microfracture technique) as a form of treatment.

The evolution was unsatisfactory and the patient continued to present a condition of medial pain in the knee. Thus, two and a half years later, he was again referred to the surgical center to undergo valgizing osteotomy of the tibia, in order to correct the varus, along with the first stage of chondrocyte transplantation, for harvesting of healthy cartilage. The preoperative assessment at this time revealed a subjective IKDC 28.74 (percentile < 5) and a Lysholm score of de 75.

The second stage of chondrocyte transplantation was performed 45 days later, with implantation of cultured cells into the chondral defect.

CASE 3
33-year-old patient

The patient had a history of pain in the left knee that started 20 years earlier, after mild trauma during adolescence. The pain had been mild, without functional limitations until six months before treatment started at our service, when a progressive worsening of the pain began.

Magnetic resonance performed at the start of the follow-up revealed a large osteochondral lesion in the loading area of the medial femoral condyle, with a bone fragment that had detached and dislocated. Since the anatomical lesion was significant and there had been no improvement over the six months of treatment outside of our service, it was decided to treat the case surgically, by means of chondrocyte transplantation. The preoperative clinical evaluation revealed a subjective IKDC of 24.14 (percentile < 5) and a Lysholm score of 39.

The first stage of ACI was undertaken with removal of healthy cartilage for culturing.

The second stage of the chondrocyte transplantation was carried out 34 days later, by means of knee arthrotomy. Since the lesion was not just cartilaginous and there was bone loss from the base of the lesion, it was decided to perform grafting from the iliac to fill this bone defect. The spongy bone was covered by a layer of periosteum that was sutured to the surrounding cartilage. The cultured cells were implanted on this periosteum and were, in turn, covered by another layer of periosteum ("sandwich" technique). During this same operation, the detached osteochondral fragment (free body) was removed, for which fixation was impossible.

RESULTS

Case 1 was 26 years of age at the time of the chondrocyte transplantation surgery and had a chondral lesion of non-traumatic origin (osteocondritis dissecans) that had been present for around one year. The lesion had a large area, of 5 cm^2, on the medial femoral condyle in a loading area. The patient presented associated conditions of genu varum and sequelae of poliomyelitis in the contralateral limb. The preoperative clinical assessment revealed a subjective IKDC of 31.03 (percentile < 5) and a Lysholm score of 40. In the postoperative clinical evaluation, with 12 months of follow-up, the patient presented an IKDC score of 57.47 (percentile < 5) and a Lysholm score of 70. Although the patient reported improvements in the symptoms, the painful condition was maintained.

Case 2 was 40 years of age at the time of the chondrocyte transplantation surgery and had a chondral lesion of traumatic origin that had been present for a long time (around eight years). The lesion area was 1 cm^2, on the medial femoral condyle in a loading area. In association with this, the patient had a medial meniscal lesion and genu varum. The preoperative clinical assessment showed a subjective IKDC of 28.74 (percentile < 5) and a Lysholm score of 75. In the postoperative clinical assessment, after 18 months of follow-up, the patient presented an IKDC score of 49.43 (percentile = 5) and a Lysholm score of 94. Although the patient reported improvements in the symptoms, a significant painful condition was maintained.

Case 3 was 33 years of age at the time of the chondrocyte transplantation surgery and had a chondral lesion of non-traumatic origin that had been present for a long time (around 20 years). The lesion area was 5 cm^2, on the medial femoral condyle in a loading area. There were no associated lesions. The preoperative clinical assessment revealed a subjective IKDC of 24.14 (percentile < 5) and a Lysholm score of 39. In the postoperative clinical evaluation, with 16 months of follow-up, the patient presented an IKDC score of 60.92 (percentile 10) and a Lysholm score of 84. Although the patient reported improvements in the symptoms, the painful condition was maintained.

DISCUSSION

Autologous chondrocyte transplantation/implantation (ACI) is considered to be a treatment option for lesions affecting the total thickness of the joint cartilage (Outerbridge[12] or International Cartilage Repair Society [ICRS][13] grade IV).

ACI should be considered to be second-line treatment for chondral defects < 2 cm^2 and should only be used when other, simpler techniques such as microfractures fail. On the other hand, if the defects are larger than 2 cm^2, ACI can be used as the initial treatment option[7]. The location of the defect should be on the femoral or patellar joint surface and should be accessible by means of open arthrotomy. A definitive indication for using ACI should only be considered during arthroscopic assessment. This procedure is the best determinant

of the location, depth and size of the defect, as well as proving assessments of the quality of the surrounding cartilage and the state of the chondral surface opposite the lesion[14,15]. For the best results to be obtained from the technique, it is fundamentally important not to have mechanical overload on the cartilage. Thus, any varus and valgus deformities that patients may present, and any ligament instability (anteroposterior, collateral and patellar), should be corrected before the ACI procedure, or else there may be a risk of treatment failure[16].

Conditions of severe osteoarthritis and the presence of bipolar lesions (kissing lesions) or bone-on-bone lesions (lesions through the joint, i.e. femur and tibia) are considered to be contraindications for ACI[14]. For this reason, in addition to the physical examination, a radiograph of the knee using the view described by Rosenberg et al[17] (anteroposterior view of the knee with loading and flexed at 45°) should be obtained in order to rule out advanced degenerative joint disease. Other contraindications are rheumatoid arthritis or other active autoimmune diseases of connective tissue, and malignant neoplasia[14].

It is essential for candidates for ACI to go through an arthroscopic evaluation: this is a fundamental stage for preoperative planning. Magnetic resonance images still do not have sufficient sensitivity and specificity to evaluate certain chondral lesions. In addition, only arthroscopy enables direct viewing and palpation of the joint cartilage, and thus is able to diagnose changes to its consistency and possible partial delamination. Only arthroscopic examination of the knee makes it possible to exactly determine the size and depth of the chondral defect, and the quality of the cartilage that surrounds it[8,14].

Among the cases presented here, there were two patients with a diagnosis of osteochondritis of large area (5 cm^2), while the remaining case had a traumatic chondral lesion of small area (1 cm^2). The first and third cases went straight for ACI treatment because of the size of the lesion, while the second case firstly underwent an unsuccessful attempt at treatment by means of microfractures, which is the preferred choice for lesions of that size. The first two cases presented genu varum with deformity < 10º, and both of them underwent correction of this deviation, in order to maximize the clinical results. In case 1, osteotomy with lateral wedge closure was chosen, since this patient presented shortening of the opposite side as a sequela of poliomyelitis. In case 2, valgizing osteotomy with Puddu medial wedge ope-

ning was chosen. None of the cases presented relevant postoperative complications.

Peterson et al[18] followed up 58 patients with a diagnosis of osteochondritis dissecans who were treated with ACI, for a mean of 5.6 years. Among these patients, some of whom with bone defects greater than 10 mm in depth, 91% had good or excellent clinical results. However, the current recommendation is to use grafts in bone defects larger than 8 mm[19]. In a study that evaluated 244 patients, with clinical follow-up for 2-10 years, notable subjective and objective clinical improvements were observed when ACI treatment was used. A large proportion of these patients had femoral condyle lesions or osteocondritis dissecans. There was a high rate of good and excellent results (84-90%) among the patients with isolated femoral condyle lesions. On the other hand, the rate was low (mean of 74%) among those with other types of lesion (patellar, trochlear and multiple lesions)[16]. To study the long-term durability of ACI, 61 patients were followed up for 5-11 years (mean of 7.4 years), after the surgery. After two years, 50 of the 61 patients had good or excellent results, and after 5-11 years of evolution, 51 of the 61 patients were graded as good and excellent results. The total failure rate was 16% (10/61 patients), among which all the ACI failure occurred in the first two years. Thus, the high percentage of patients with good and excellent results over the first two years remained well for a long period of postoperative follow-up[16].

Many authors have compared the ACI technique with other cartilage repair procedures, but only a few of them were able to design studies with a notable degree of clinical evidence. In general, the evidence does not prove that ACI is superior to the microfracture and mosaicplasty techniques, for example[2,20-22].

With regard to the clinical results from the cases operated in our service, we only observed a slight improvement in the patients' conditions. The indication of ACL in case 1 can be questioned, given that the presence of sequelae from poliomyelitis in the opposite leg was a significant overload factor for the operated knee, although this would be true for any other surgical option for chondral lesion treatment. Nevertheless, this overload may have compromised the clinical results from the treatment. Case 2 also presented an insignificant improvement in pain symptoms, although this patient also had an overload factor in the chondral repair: partial meniscectomy of the medial meniscus. Thus, we consider

that the results obtained are not discouraging, since it is likely that the improvement would have been even more significant in ideal cases, although ideal situations are rarely achieved in treating chondral lesions. We consider that the reported pain was due not only to the chondral lesion but also to other factors present in these patients, which limited the improvement in pain.

Another point that needs to be made is in relation to the costs and the low availability of the technique. There is no doubt that ACI should preferably be considered to be a second choice in treating chondral lesions, given that it costs much more than microfracture (which is considered to be the preferred technique for the initial surgical approach in most cases of complete chondral lesions) and requires two surgical procedures (including the fact that one of them is an open procedure). Moreover, it has extremely low availability in Brazil.

Maci® (Verigen AG, Leverkusen, Germany)[23], which is considered to be a second-generation technique, and Hyalograft-C® (Fidia Advanced Biopolymers, Abano Term, Italy)[24,25], which is considered to be a third-generation technique, are examples of advances in chondrocyte implantation. Maci® uses a matrix of collagen type I/III to sow chondrocytes in a double layer. Hyalograft-C® uses a 3-D matrix of hyaluronic acid, which functions as a support for the growth of chondrocytes *in vitro*. These matrixes containing chondrocytes are implanted on the chondral lesion and attached using fibrin glue. In this way, periosteum grafts are not needed, and hence no suturing onto healthy cartilage is needed, either. These techniques have been developed in an attempt to resolve one of the commonest problems shown by the ACI technique[19]: hypertrophy of the periosteum, which is a reason for complaints of localized pain among some patients.

CONCLUSION

We conclude that autologous chondrocyte transplantation/implantation is an option for treating extensive chondral lesions or after the failure of simpler techniques for smaller chondral lesions, even though the improvement was only partial in our patients.

We emphasize that we do not consider the ACI technique to be the preferred option for the initial management of complete chondral lesions, because of its high cost, greater complexity, need for two hospitalizations, low availability and lack of international consensus regarding its results, in relation to other techniques that are available.

REFERENCES

1. Buckwalter JA. Articular cartilage: injuries and potential for healing. J Orthop Sports Phys Ther. 1998;28(4):192-202.

2. Wasiak J, Clar C, Villanueva E. Autologous cartilage implantation for full thickness articular cartilage defects of the knee. Cochrane Database Syst Rev. 2006; 3:CD003323.

3. Buckwalter JA, Mankin HJ. Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. Instr Course Lect. 1998;47:487-504.

4. D'Lima DD, Hashimoto S, Chen PC, Lotz MK, Colwell CW Jr. Cartilage injury induces chondrocyte apoptosis. J Bone Joint Surg Am. 2001; 83(Suppl 2-Pt 1):19-21.

5. Borrelli J Jr, Tinsley K, Ricci WM, Burns M, Karl IE, Hotchkiss R. Induction of chondrocyte apoptosis following impact load. J Orthop Trauma. 2003;17(9):63541.

6. Buckwalter JA, Mankin HJ. Articular cartilage: tissue design and chondrocyte-matrix interactions. Instr Course Lect. 1998;47:477-86.

7. Scopp JM, Mandelbaum BR. A treatment algorithm for the management of articular cartilage defects. Orthop Clin North Am. 2005;36(4):419-26.

8. Fritz J, Janssen P, Gaissmaier C, Schewe B, Weise K. Articular cartilage defects in the knee--basics, therapies and results. Injury. 2008; 39(Suppl 1):S50-7.

9. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, et al. OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage. 2008;16(2):137-62.

10. Mithoefer K, Williams RJ, Warren RF, Potter HG, Spock CR, Jones EC, et al. Chondral resurfacing of articular cartilage defects in the knee with the microfracture technique. Surgical technique. J Bone Joint Surg Am. 2006;88(Suppl 1 Pt 2):294-304.

11. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med. 1994;331(14):889-95.

12. Outerbridge R. The etiology of chondromalacia patellae. J Bone Joint Surg Br. 1961;43:752-7.

13. International Cartilage Repair Society. Disponível em: www.cartilage.org, 2005.

14. Brittberg M. Autologous chondrocyte implantation--technique and long-term follow-up. Injury. 2008;39(Suppl 1):S40-9.

15. Petersen L. International experience with autologous chondrocyte transplantation In: Scott N, Insall J, editors. Insall & Scott - Surgery of the knee. 3rd ed. New York: Elsevier; 2006. p.341-56.

16. Brittberg M, Peterson L, Sjogren-Jansson E, Tallheden T, Lindahl A. Articular cartilage engineering with autologous chondrocyte transplantation. A review of recent developments. J Bone Joint Surg Am. 2003; 85(Suppl 3):109-15.

17. Rosenberg TD, Paulos LE, Parker RD, Coward DB, Scott SM. The forty-five-degree posteroanterior flexion weight-bearing radiograph of the knee. J Bone Joint Surg Am. 1988;70(10):1479-83.

18. Peterson L, Minas T, Brittberg M, Lindahl A. Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation: results at two to ten years. J Bone Joint Surg Am. 2003; 85(Suppl 2):17-24.

19. Marcacci M, Zaffagnini S, Kon E, Visani A, Iacono F, Loreti I. Arthroscopic autologous chondrocyte transplantation: technical note. Knee Surg Sports Traumatol Arthrosc. 2002;10(3):154-9.

20. Knutsen G, Engebretsen L, Ludvigsen TC, Drogset JO, Grøntvedt T, Solheim E, et al. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J Bone Joint Surg Am. 2004;86(3):455-64.

21. Bentley G, Biant LC, Carrington RW, Akmal M, Goldberg A, Williams AM, et al. A prospective, randomized comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. J Bone Joint Surg Br. 2003;85(2):223-30.

22. Horas U, Pelinkovic D, Herr G, Aigner T, Schnettler R. Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint. A prospective, comparative trial. J Bone Joint Surg Am. 2003;85(2):185-92.

23. Cherubino P, Grassi FA, Bulgheroni P, Ronga M. Autologous chondrocyte implantation using a bilayer collagen membrane: a preliminary report. J Orthop Surg (Hong Kong). 2003;11(1):10-5.

24. Grigolo B, Lisignoli G, Piacentini A, Fiorini M, Gobbi P, Mazzotti G, et al. Evidence for redifferentiation of human chondrocytes grown on a hyaluronan-based biomaterial (HYAff 11): molecular, immunohistochemical and ultrastructural analysis. Biomaterials. 2002;23(4):1187-95.

25. Marcacci M. Articular cartilage engineering with Hyalograft C: 3-year clinical results. Clin Orthop Relat Res. 2005;(435):96-105.