INTRODUCTION

Hyaline cartilage covers joint surfaces and plays an important role in reducing friction and mechanical loading on synovial joints such as the knee. As it is not vascularized, this tissue has a very limited healing capacity. For the same reason, there is no inflammatory response to the tissue damage. Hence there is no invasion of macrophages that remove the devitalized tissues, and no cell migration with the ability to repair the damaged area.\(^1\)\(^,\)\(^2\) These lesions do not heal and may eventually progress to arthrosis. This evolutionary process is regulated, both by chemical mediators that mix with the synovial liquid such as Caspasases, Interleukin-1 and -6 and Nitric Oxide (enzymatic degradation)\(^3\)\(^,\)\(^4\)\(^,\)\(^5\), and by repetitive micro-trauma (mechanical overload)\(^6\)\(^,\)\(^7\) and the blocking of some of these substances can result in a clinical improvement of the joint with the lesion in the cartilage, as demonstrated by Rezende et al.\(^7\)

Excellent clinical results can be obtained among elderly patients with severe arthrosis when they are treated with total knee arthroplasty. In young patients, treatment of chondral defects of the knee is not yet standardized, despite some attempts to this end.\(^8\)\(^,\)\(^9\) The therapeutic alternatives include simple joint washing with or without debridement, aimed at removing chemical mediators and free bodies that degrade the cartilage and cause pain\(^10\); perforations, microfractures and abrasions, which regenerate the joint surface with scar tissue similar to hyaline cartilage (fibrocartilage), from medullary mesenchymal cells\(^11\); mosaicplasty (autologous osteochondral transplantation) and autologous chondrocyte transplantation, also known as autologous cartilage implantation (ACI).\(^10\)

Buckwalter\(^1\) describes the joint cartilage inside a functional unit with biological and mechanical properties. This model is composed of the different layers of chondral cells and by the subchondral bone plate. Techniques that interfere with the subchondral bone plate (perforations, microfractures and mosaicplasty) may even reestablish the joint surface, but do not restore the mechanical property of the cartilage, especially the impact absorption function. The ACI technique has shown biological and mechanical advantages in comparison to the others, since besides preserving the subchondral bone support, it repairs the chondral defect with tissue similar to that of the hyaline cartilage.

Since 1994, when Brittberg et al.\(^12\) described the ACI technique for the treatment of chondral knee lesions, many surgeons have begun to study the subject. Nowadays, there are many modifications of the technique, from the first generation to the second and third chondrocyte implant generations.\(^13\)\(^-\)\(^17\) This procedure has become one of the most important surgical alternatives for the treatment of chondral lesions of the knee, and its use has extended to the treatment of chondral lesions in other joints such as the ankle, shoulder, hip and wrist.\(^18\)\(^-\)\(^22\)

INDICATIONS

The ideal patient for autologous chondrocyte implantation presents a chondral lesion affecting the total thickness (Outerbridge\(^23\) or International Cartilage Repair Society [ICRS]\(^24\) degree III and IV) or osteochondral defect surrounded by normal and healthy cartilage in one knee without other lesions. The ideal lesion is an exception rather than the rule, since many knee lesions occur, simultaneously, in some degree of association with other pathologies. These combined lesions often present...
a rim of cartilage of doubtful quality around their circumference, thus compromising the chondrocyte implantation. ACI should be considered second-line treatment for chondral defects (<2cm²), and should only be used when other, simpler techniques such as microfractures fail. On the other hand, if the defects are larger than 2cm², ACI should be used as the treatment of choice. The defect should be located on the femoral or patellar joint surface and should be accessible by means of arthrotomy. The 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, besides evaluating the quality of the surrounding cartilage and the state of the chondral surface opposite the lesion. Patients should have their deformities (varus and valgus) and any ligament instability (anteroposterior, collateral and patellar) corrected before the ACI procedure, due to the risk of treatment failure.

CONTRAINDICATIONS

ACI is not indicated for patients with severe arthrosis or in the presence of bone-on-bone bipolar lesions. For this reason, the physical examination should be supplemented by obtaining a radiograph of the knee to exclude advanced degenerative joint disease. Other contraindications are rheumatoid arthritis or other active autoimmune disease of the connective tissue and patient with malignant neoplasia.

CLINICAL EVALUATION

The evaluation of the patient with chondral or osteochondral defect begins with the history of symptoms (joint effusion, joint block and pain) and of trauma (old sprains can be the cause of the problem). Family history (osteoarthritis, metabolic disorders etc.) and previous surgeries (meniscal resection, ligament reconstruction etc.) are important due to the subsequent damage to the cartilage. The physical examination should be focused on the following aspects: valgus or varus deformities, patellar alignment or instability, ligament instability (anterior, posterior and lateral), pain on palpation, effusion, crepitation, range of motion and joint blocks. The radiographic evaluation is also crucial. For this reason we take conventional radiographs of the knee (AP, lateral and axial view of the patella). Other special views are necessary to obtain information on the alignment of the lower limbs (panoramic radiograph of the patient standing and with knees extended) and of possible signs of degenerative osteoarthritis (Rosenberg’s AP).

Magnetic Resonance (MR) is a very useful tool in the diagnosis of chondral lesion. High resolution images obtained by modern devices (1.5 or 3 Tesla) help surgeons in the preoperative planning, providing detailed information about the chondral defect and the adjacent subchondral structure. ACI candidates should undergo an arthroscopic evaluation, an essential stage for preoperative planning. MR images do not yet have sufficient sensitivity or specificity to evaluate certain chondral lesions. In addition, only arthroscopy enables direct viewing and palpation of the joint cartilage, and thus allows us to diagnosis changes to its consistency and possible partial de-lamination. 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 surrounding cartilage.

SURGICAL TECHNIQUE

This is a procedure carried out in two stages. Initially, a biopsy is taken from the cartilage and is sent for chondrocyte culturing (cell proliferation) in the laboratory. Cell implantation is performed in the following stage, consisting of arthrotomy, preparation of the chondral defect, harvesting of periosteum, hermetic fixation of periosteum over the lesion with stitches and fibrin glue, injection of chondrocyte concentrate and closing of the operative wound. (Figure 1)
During this procedure, 200ml of venous blood should be taken from the patient. This blood is used to extract the serum to be used with the culture medium for cell proliferation. The cartilage harvesting procedure is safe, without complications and without late symptoms at the collection harvesting sites described in literature. Adequate harvesting is essential for the success of the cell culture, and good quality of cells is necessary to obtain the best results with the procedure.

In vitro cell expansion

The main objective of in vitro manipulation of chondrocytes is to increase the number of cells. This process starts with enzymatic digestion of the extracellular matrix of the cartilage that corresponds to approximately 90% of the tissue and is formed by a dense three-dimensional network containing associated collagen and proteoglycan molecules. The controlled use of proteolytic enzymes such as hyaluronidases, collagenases and trypsin allows the degradation of the elements that form the extracellular matrix, resulting in the isolation of a suspension of viable chondrocytes from a cartilaginous fragment.45 (Figure 2A) The viability of the chondrocytes can be obtained with the use of stains, such as Trypan blue, followed by counting in a hemocytometer chamber (Neubauer counting chamber). The primary cultures are initiated with a minimum concentration of 5 X 10^6 chondrocytes in 25cm^2 culturing flasks with the DMEM/HAMF12 culture medium supplemented with 10% autologous serum.10,11 The autologous serum is used as a source of hormones and growth factors for the cultured cells. In some protocols bovine fetal serum can be used to supplement the chondrocyte cultures, as it is a richer source of these cell inducer molecules and produces more uniform results for primary cultures. The cells are kept at 37°C (98.6°F) with 5% CO_2. 29 Under these conditions, monolayer system with medium supplementation, the chondrocytes adhere to the cell culture dishes, losing the characteristic rounded morphology and acquiring morphology typical of fibroblasts. (Figure 2B) This phenotypic alteration of the chondrocytes is characterized as cell dedifferentiation and results in cellular metabolic changes. The cells divert the preferential synthesis of collagen II, replacing it with the synthesis of collagen I, besides other alterations.30,31 Due to the morphological and functional alterations, the chondrocytes acquire proliferative capacity.22,33 (Figure 2C)

The term monolayer is used as the cells do not overlap in culture; if this occurs it gives rise to the process called contact inhibition, due to the presence of very close cells, which entails cell death. When the number of cells increases, resulting in the occupancy of the culturing flask, it is said that the monolayer is confluent (Figure 2D); at this time the cells are transferred to new flasks, in the process denominated cell replication and occupational digestion of the extracellular matrix of the cartilage that corresponds to approximately 90% of the tissue and is formed by a dense three-dimensional network containing associated collagen and proteoglycan molecules. The controlled use of proteolytic enzymes such as hyaluronidases, collagenases and trypsin allows the degradation of the elements that form the extracellular matrix, resulting in the isolation of a suspension of viable chondrocytes from a cartilaginous fragment.45 (Figure 2A) The viability of the chondrocytes can be obtained with the use of stains, such as Trypan blue, followed by counting in a hemocytometer chamber (Neubauer counting chamber). The primary cultures are initiated with a minimum concentration of 5 X 10^6 chondrocytes in 25cm^2 culturing flasks with the DMEM/HAMF12 culture medium supplemented with 10% autologous serum.10,11 The autologous serum is used as a source of hormones and growth factors for the cultured cells. In some protocols bovine fetal serum can be used to supplement the chondrocyte cultures, as it is a richer source of these cell inducer molecules and produces more uniform results for primary cultures. The cells are kept at 37°C (98.6°F) with 5% CO_2. 29 Under these conditions, monolayer system with medium supplementation, the chondrocytes adhere to the cell culture dishes, losing the characteristic rounded morphology and acquiring morphology typical of fibroblasts. (Figure 2B) This phenotypic alteration of the chondrocytes is characterized as cell dedifferentiation and results in cellular metabolic changes. The cells divert the preferential synthesis of collagen II, replacing it with the synthesis of collagen I, besides other alterations.30,31 Due to the morphological and functional alterations, the chondrocytes acquire proliferative capacity.22,33 (Figure 2C)

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Nowadays there are three generations of chondrocyte cultures. In the first, the cell culture is performed as a monolayer and the cell implant in the defect is covered with a piece of autologous periosteuem (ACI-P)12 or using a manufactured membrane of collagen I/III (ACI-C).7 In the second generation, after cell expansion in a monolayer, the cells are deposited on a carrier membrane/matrix, obtaining a membrane sown with MAC® (chondrocytes (Verigen AG, Leverkusen, Germany).17 In the third generation of ACI, the chondrocyte culture is deposited on a matrix of hyaluronic acid structured in three dimensions (Hyalograft-C®, Fidia Advanced Biopolymers, Abano Term, Italy)37-38, thus enabling homogeneous distribution of the chondrocytes inside the lesion. Both in the second and in the third generation of chondrocyte culturing systems for autologous implantation, the technical advantages are translated as decrease of complexity and of surgical time. This system can be performed by arthroscopy alone and using fibrin glue for fixation only, avoiding the removal and suturing of the periosteuem. Nevertheless the technique that employs the periosteuem is being widely used due to its safety and efficacy.

Surgical approach and lesion debridement

The patient is prepared and habitual anesthetic precautions are taken. A pneumatic tourniquet is positioned on the thigh and inflated after exsanguination of the limb with an Esmarch bandage. A standard parapatellar, medial or lateral incision is made and the knee is opened up by means of mini-arthrotomy. (Figure 3A) After achieving adequate exposure, the lesion should be debrided to remove all unviable tissue. The diseased 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 of the defect is to obtain a lesion surrounded by healthy
cartilage and with the base free from blood. If bleeding occurs during this preparation, it is necessary to perform hemostasis, either with a gauze plug with adrenaline, or with fibrin glue. This avoids the migration of fibroblasts or mesenchymal cells from the bone marrow to the interior of the lesion, which compromises the results of the ACI. (Figure 3B) 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 help remove the periosteum graft in the next stage.

Figure 3. (A) View of complete chondral lesion, in the femoral condyle of the left knee of a soccer player, after medial parapatellar arthrotomy; (B) Regularization and debridement of the fibrous tissue present at the base of the lesion.

Figure 4. Removal of the periostium graft from the medial side of the proximal tibia.

Figure 5. Injection of autologous chondrocyte concentrate under the periosteum plug of the lesion.

PROTECTION OF THE CHONDAL REPAIR

The isolated chondral lesion is rarely the only cause of symptoms and dysfunction of patients. These lesions are frequently associated with other knee pathologies. Misalignment of the limb and ligament instability can overload the chondral defect, affecting the result of the chondrocyte transplant. Accordingly, satisfactory clinical results cannot be expected from the cartilage repair methods until the coexisting pathologies are treated and conditions are created to protect the chondral repair.

Misalignment

If the physical examination or the initial x-rays indicate some form of misalignment of the limb, it is necessary to take a panoramic radiograph of the lower limb, including hip and ankle. If the mechanical axis passes through the compartment in which the lesion is located, it is recommended to perform osteotomy to divert the forces of the impaired compartment. In the case of large condylar lesions, without misalignment of the limb, the chondral repair should be protected with load diverting...
orthosis made to order. Repairs that are not protected can present a good appearance for two years, but overload in the lesion area evolves with tapering and destruction of the repair. Instability

Ligament instability produces excessive shear force in the knee and this mechanical imbalance damages the repair tissue. If ACL reconstruction is performed concomitantly, the ACI should only be finished after ACL fixation. Amin et al. published their experience in the treatment using ACI in combination with ACL reconstruction. They report that the combination of the two procedures is viable and that the clinical results of ACL reconstruction are improved when this procedure is associated with ACI.

Meniscal Lesion

The lack of meniscal protection of the cartilage overloads the chondrocyte transplantation region. Whenever possible, the meniscus should be preserved or repaired. If the patient has undergone total or partial meniscectomy leaving the meniscus without protective function, the possibility of meniscus transplantation should be considered. The meniscal allograft will help reduce the concentration of forces in the compartment involved and will protect the recently formed repair tissue.

When the meniscus transplantation is performed at the same time as the ACI, attention should be paid to a technicality. First of all the meniscal allograft is positioned and fixed, then the ACI procedure is completed, thus avoiding undesirable manipulations and accidents in the chondrocyte transplantation.

Bone Defect

Superficial bone lesions up to 8mm in depth evolve well when treated just with ACI. Peterson et al. monitored 58 patients diagnosed with osteochondritis dissecans and treated with ACI over an average period of 5.6 years. 91% of the patients had good or excellent clinical results, some with bone defect above 10mm in depth. However, the current recommendation is to graft bone defects above 8mm. The bone graft can be performed at the time of the arthroscopic evaluation or of the cartilage biopsy. Another option is to perform the procedure all at once by means of the “sandwich” technique. In this procedure, the bone defect is filled with spongy bone graft up to the height of the subchondral bone and covered with a piece of periosteum at this level. A second piece of periosteum is fixed at the top of the cartilage, then the chondrocyte suspension is injected between the two periosteum layers.

REHABILITATION

The basic principles for success of the postoperative ACI rehabilitation program should be focused on protection of the graft, joint mobilization exercises, muscle strengthening and load progression. Rehabilitation should be based on the state and needs of the patient, on the lesion size and location and on whether there has been any surgical procedure in association with the ACI. During the initial postoperative period it is mandatory to protect the repair tissue against excessive intra-articular forces, mainly avoiding rotating movements and friction on the repair. The CPM (continuous passive motion) machine usually starts on the first postoperative day and continues while the patient remains in hospital (2 to 3 days). The load is gradually increased and the patient starts to take their first steps. The isometric strengthening of the quadriceps and of the flexors is introduced early in the rehabilitation program and should progressively advance to exercises against resistance.

Some closed chain exercises are introduced in the program in the 3rd postoperative week. Open chain exercises are started in the 2nd postoperative month. It is recommended to start running eight months after surgery and high impact activities 12 months after surgery.

The rehabilitation of femoropatellar lesions requires special considerations. Pressure in the femoropatellar joint reaches its peak between 40° and 70° of knee flexion and these degrees of range of motion (ROM) should be avoided until the repair tissue is mature enough to bear the friction force. CPM should be used and open chain exercises are allowed from the 3rd postoperative month of the patellar or trochlear lesion repair. If the lesion is large, the use of a brace can be considered for load alleviation.

INTERNATIONAL EXPERIENCE

The first clinical study published using ACI for the treatment of chondral lesions was in 1994, in which 23 patients were included. Since then, thousands of patients have been treated by this technique and various authors have published their results around the world.

In patients with small and acute bone lesion, ACI is generally applied after other techniques have been tried and failed after 6 months. ACI and osteochondral transplantation should be used in large acute lesions (>3cm²), precisely because for these large lesions, it is hard to obtain adequate coverage using other techniques. (Table 1)

Table 1. Recommendations among the various alternatives for biological reconstruction of the joint surface.

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<thead>
<tr>
<th>Lesion Size</th>
<th>Microfractures</th>
<th>OCT</th>
<th>ACI</th>
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<tr>
<td>Children and Adolescents</td>
<td>+++</td>
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<td>1-2cm² lesion</td>
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<td>3-14cm² lesion</td>
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In a study that evaluated 244 patients, with clinical follow-up for 2 to 10 years, noteworthy subjective and objective clinical improvements were observed when ACI treatment was used. A large portion of these patients had femoral condyle lesions or osteochondritis dissecans. There was a high percentage 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).40

To study the long-term durability of ACI, 61 patients were monitored for 5-11 years (mean of 7.4 years) after 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/excellent results. The total failure rate was 16% (10/61 patients), among which all the ACI failures occurred in the first two years. Thus, the high percentage of patients with good/excellent results in the first two years remained well for a long period of postoperative follow-up.42

Many authors have compared the ACI technique with other cartilage repair procedures, yet only a few of them were able to design studies with a relevant degree of clinical evidence. Based on the systematic review of literature published in 2006 by Wasiak et al.5 in the Cochrane Database Systematic Review, we selected some of these prospective, randomized clinical studies.

ACI versus Microfractures

Knutsen et al.46 studied 80 patients with symptomatic cartilage lesion of the femoral condyle requiring repair, measuring 2-10 cm². The patients were randomized between two groups treated with ACI and microfractures and followed up for 12 and 24 months. Based on the SF-36 questionnaire applied two years after surgery, the microfractures group presented a significant improvement in comparison to the ACI group (p=0.004). Although both groups presented improvements of the Lysholm score and of the Tegner score (p<0.05). The arthroscopic evaluation, carried out after one year of treatment, showed that 82% of the patients treated with ACI and 34% of the OCT group presented excellent or good quality of the joint repair (p<0.001).

MACI versus Microfractures

Basad et al.49 studied 46 patients with post-traumatic chondral lesions (mean defect of 2-10 cm²) and compared treatment through MACI or by microfractures. After 2 years of follow-up, the MACI group presented better results based on the Lysholm score (p=0.049) and the Meyer’s index (p=0.024). However, the differences based on the Tegner index (p=0.064) and ICRS score (p=0.32) were not significant. In the same study, Basad performed a MR two years after surgery and demonstrated complete equalization of the sign between the regenerated tissue and the normal cartilage surrounding the repair in the MACI group. In the microfractures group, the sign obtained in the repair was different when compared with the normal cartilage.

ACI versus OCT (Mosaicplasty)

Horas et al.47 conducted a quasi-randomized, prospective clinical study (<80% of follow-up) with 40 patients to compare the results of the ACI and OCT techniques for the treatment of complete chondral defects (mean of 3.75 cm²). Both treatments reduced the patients’ symptoms, but a statistically significant difference was reported in the Lysholm score at 6, 12 and 24 months, which favored the OCT group. However, the other indices used for clinical evaluation by the study (Meyers score and Tegner score) did not present significant difference in comparing the two techniques. Bentley et al.48 published a randomized and controlled clinical study with 100 patients (58 patients treated with ACI and 42 with OCT). The majority were posttraumatic lesions with chondral defect size averaging 4.76 cm². After a mean follow-up period of 19 months (12-26 months), it was shown that 88% of the patients treated with ACI and 69% treated with OCT presented good or excellent clinical results, according to a clinical and functional evaluation (p<0.05). The arthroscopic evaluation, carried out after one year of treatment, showed that 82% of the patients treated with ACI and 34% of the OCT group presented excellent or good quality of the joint repair (p<0.001).

CONCLUSIONS

Autologous chondrocyte transplantation has become an alternative for treating complete chondral lesions since when the first case series were published, and several authors have reported excellent and good results in the use of this surgical technique. Tissue engineering has developed new methodologies and has presented improvements and refinements of the original ACI technique. However, all this technology entails a high cost and, moreover, there is not yet sufficient evidence in literature to affirm that ACI is superior to the other complete chondral defect treatment strategies. New studies are required to obtain further information that can help define the conduct for chondral lesions, pursuing the goal of better quality of life for patients.