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Original article

Fresh osteochondral knee allografts in Brazil with a minimum two-year follow-up☆

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ABSTRACT

Objective: The present study aimed to report the results of the first series of cases of fresh ostechondral allografts in the knee joint in Brazil with a minimum follow-up of two years.
Methods: A protocol of procurement, harvesting, processing, and utilization of fresh osteochondral allografts in the knee joint was established, beginning with legislation modifications, graft harvesting techniques, immediate processing, storage of fresh grafts, and utilization of two surgical techniques of osteochondral transplantation. Eight patients were treated and followed-up for a minimum of two years.
Results: Patients were evaluated with subjective IKDC, KOOS, and modified Merle D'Aubigne and Postel questionnaires. Mean subjective IKDC score was 31.99 ± 13.4 preoperative and 81.26 ± 14.7 at the latest follow-up; preoperative KOOS score was 46.8 ± 20.9 and postoperative was 85.24 ± 13.9, indicating a significant improvement over time ($p < 0.01$). Mean modified Merle D'Aubigne-Postel score was 8.75 ± 2.25, preoperatively, and 16.1 ± 2.59 postoperatively. Friedman test for non-parametric samples demonstrated a significant improvement in postoperative scores ($p < 0.01$).
Conclusion: The use of fresh osteochondral allografts in Brazil is a safe procedure, with good clinical results in the short- and medium-term for the treatment of osteochondral lesions greater than $4\,cm^2$ in the knee joint.

☆ Study conducted at Universidade de São Paulo (USP), Faculdade de Medicina, Hospital das Clínicas, São Paulo, SP, Brazil.

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Transplante osteocondral a fresco no joelho no Brasil: mínimo de dois anos de seguimento

Palavras-chave:
Traumatismos do joelho
Cartilagem articular
Transplante homólogo
Ortopedia

RESUMO

Objetivo: Relatar os resultados dos primeiros casos de transplante osteocondral a fresco na articulação do joelho no Brasil com um mínimo de seguimento de dois anos.
Métodos: Foi feito um protocolo de captação, processamento e uso de transplantes osteocondrais a fresco na articulação do joelho. Iniciou-se com modificações na legislação vigente, técnicas de captação de enxertos, processamento imediato, armazenamento a fresco dos enxertos e uso de duas técnicas cirúrgicas de transplante osteocondral. Oito pacientes foram transplantados e acompanhados com mínimo de dois anos de seguimento.
Resultados: Os pacientes foram avaliados por meio dos questionários do *International Knee Documentation Committee* (IKDC) subjetivo, *Knee Injury and Osteoarthritis Outcome Score* (KOOS) e índice de Merle D'Aubigne e Postel modificado. A média da pontuação da escala IKDC subjetiva pré-operatória foi de 31,99 ± 13,4 e de 81,26 ± 14,7 no pós-operatório e da escala KOOS pré-operatória foi de 46,8 ± 20,9 e de 85,24 ± 13,9 no pós-operatório, com melhoria significativa ao longo do tempo ($p < 0,01$). A média da pontuação pelo índice de Merle D'Aubigne e Postel modificado foi de 8,75 ± 2,25 no pré-operatório e de 16,1 ± 2,59 no pós-operatório. O resultado do teste de Friedman para amostras não paramétricas demonstrou melhoria significativa ao longo do tempo ($p < 0,01$).
Conclusões: O transplante osteocondral a fresco no Brasil é um procedimento seguro, com bons resultados clínicos em curto e médio prazo para o tratamento de lesões osteocondrais maiores do que 4 cm^2 na articulação do joelho.

Introduction

Chondral lesions in the knee joint affect approximately 900,000 US citizens each year, resulting in over 200,000 surgical procedures for diagnosis and treatment.[1] There are no statistics on this disease in Brazil. The goal in the treatment of traumatic chondral and osteochondral lesions is to reestablish anatomy and function of the joint as well as eliminate pain.

The treatment of chondral lesions greater than 4 cm^2 by debridement or microfracture techniques does not promote good results, as it does not address the subchondral bone injury and promotes repair with fibrocartilaginous tissue instead of hyaline cartilage, therefore not being recommended for the treatment of these injuries.[2,3] Autologous osteochondral transplantation is a good treatment option, as it promotes repair with hyaline cartilage and grafts possible defects of the subchondral bone. However, it is limited by the morbidity of the donor site; it can be ideally used in injuries of up to 2.5 cm in diameter and up to 10 mm deep.[4–6]

Currently, treatment options for chondral and osteochondral knee lesions larger than 4 cm^2 are autologous chondrocyte implantation and fresh osteochondral allografts (FOA). Autologous chondrocyte transplantation is a complex technique that requires two operations for biopsy and cell transplantation, and has a very high cost.[7] The use of FOA for the treatment of large osteochondral lesions of the knee is a biological option in young patients; its main advantage is that it is a tissue with live hyaline cartilage, featuring chondrocytes in a chondral matrix with preserved collagen fiber architecture.[8,9]

In other countries, FOA have been used for decades.[10–14] This technique was first introduced to treat post-traumatic bone defects.[15,16] However, it is now used for the treatment of various disorders of the knee, such as osteochondritis dissecans (OD), secondary osteonecrosis, and degenerative disease of the knee, as well as in fracture sequelae.[17–20] The principle of FOA is to restore the biological structure of the joint, rebuild the articular hyaline cartilage surface, and provide an osteochondral tissue capable of supporting the mechanical load of the individual.[21,22]

To the best of the authors' knowledge, there are no studies or case reports on the use of the FOA technique in Brazil, because until 2009 the laws regulating tissue banks did not allow fresh tissues to be used for transplantation in time for the release of cultures; it was necessary to wait for the results of these tests before use.[23]

This study aimed to report the results of the first cases of FOA transplantation in the knee joint in Brazil, with a minimum follow-up of two years.

Methods

This study was conducted at the Institute of Orthopedics and Traumatology of this institution and was approved by the Ethics Committee for Research Project Analysis (CAPPesq).

The inclusion criteria comprised young patients, between 15 and 45 years of age, with traumatic or acquired osteochondral lesions in the knee, chondral or osteochondral lesions

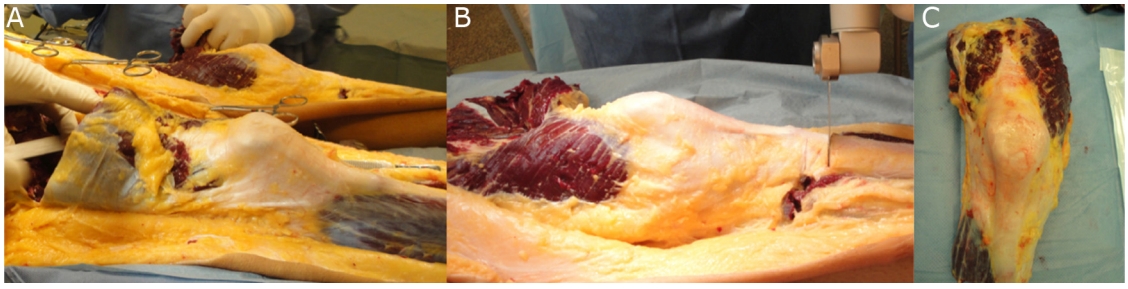

Fig. 1 – (A) Osteotomy of the femur 10 cm above the joint line without violation of the joint capsule; (B) Tibial osteotomy 2 cm below the ATT; (C) Final result of the piece before transportation to the Tissue Bank.

larger than 4 cm^2, and whose chondral or osteochondral lesions failed previous treatment for articular cartilage repair.

Patients with inflammatory arthropathy, with active infection in the knee or elsewhere in the body, and smokers were excluded.

For donor selection, the inclusion and exclusion criteria for musculoskeletal tissues set forth by the Brazilian Association of Organ Transplantation (Associação Brasileira de Transplante de Órgãos [ABTO]) was used, and individuals between 15 and 45 years were selected.

The sample consisted of five organ donors and eight recipients (eight knees), which were operated from March to October 2012.

Harvesting

In the present study, all tissues for FOA were obtained from organ donors, harvested in an operating room with laminar airflow after the heart, liver and kidney had been harvested. The knees were harvested as a block; only the skin and subcutaneous tissue were dissected, and the joint capsule was kept intact. Osteotomy was performed on the distal femur 10 cm above the joint line and on the proximal tibia and fibula, 2 cm below the distal part of the anterior tibial tuberosity (ATT) (Fig. 1A–C).

The pieces, as a block, were placed in lactated Ringer's solution and transported at a temperature of 2°–8 °C. After harvesting, tissues were sent to the Tissue Bank for processing within 12 h of the harvest procedure.

Processing

The processing stage was performed in a proper operating room, classified as class 100 or ISO 5, and equipped with a laminar flow module. The articular capsule of the knee was opened through the medial parapatellar access route and structures were measured with a caliper to pair with the recipients in the FOA list. At this stage, the articular cartilage was analyzed and only pieces in which this structure was intact were used.

Pairing was performed by comparing the actual size of the proximal tibia of the affected knee at the level of the joint in the donor and in the recipient. This measurement was obtained by assessing this receptor segment through digital radiographs of the affected knee in anteroposterior view, discounting the magnification (Fig. 2). In the donor, this measurement was made using a caliper. For lesions of the proximal portion of the tibia, patella, femoral trochlea, and massive lesions of the femoral condyles, a difference of at most 5 mm between the donor and recipient was used as a parameter for matching. For focal lesions of the femoral condyle, a positive pairing was made when the donor condyle was equal to or larger than that of the receiver.

Once donor and recipient were matched, all analysis exams were performed. The selected tissues were packed in triple vacuum-sealed packages, containing a preservation medium with nutrients. It took a mean of 14 days for the tissue cultures to be released; during this period, the receiver was prepared

Fig. 2 – Measurement of the proximal tibia of the recipient for donor matching.

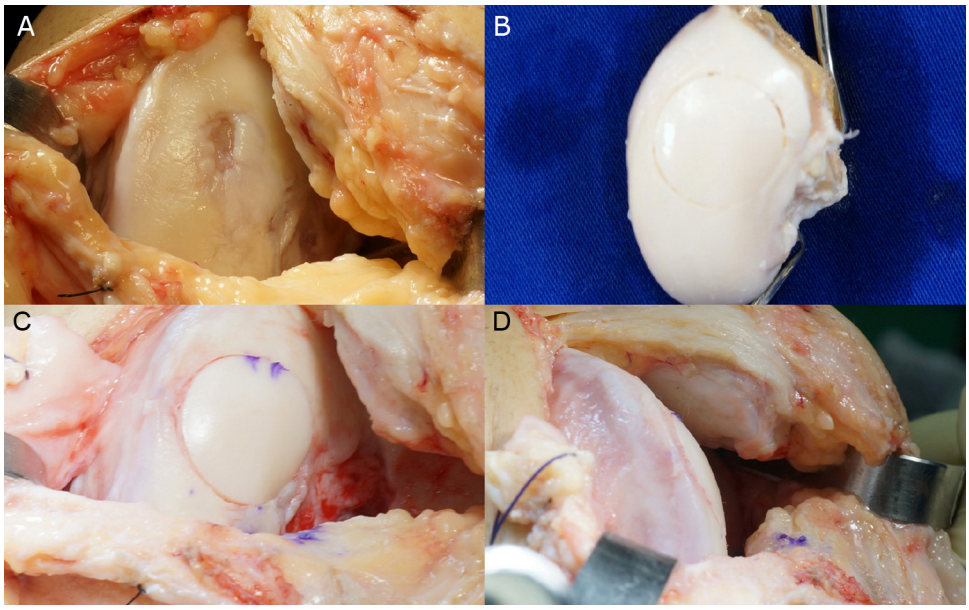

Fig. 3 – (A) Macroscopic appearance of osteochondritis dissecans lesion in the left medial femoral condyle; (B) Donor left medial femoral condyle with a cylinder prepared in the same anatomical site of the recipient's defect; (C) FOA restoring the articular surface of the medial femoral condyle; (D) Macroscopic lateral view of the transplant, the perfect congruence of the articular surface of the medial femoral condyle can be observed.

and the surgical procedure was scheduled to be performed as close as possible to the date of culture release.

Storage and preservation

The medium used for tissue preservation was the commercial Ham F-12 – GIBCO with glutamax medium (Invitrogen, Life Technologies, USA), which contains amino acids, vitamins, and minerals. To the medium, amphotericin B (12.5 mg/500 mL), streptomycin (50 mg/500 mL), gentamicin (25 mg/500 mL), and penicillin G (5,000,000 UI/500 mL) were added as prophylaxis against microorganisms. Tissues were stored in a refrigerator below 4 °C while awaiting culture results.

Surgical technique

Surgery was scheduled for the day after cultures release, in order to minimize the time between collection and transplantation.

The knees were approached by medial or lateral parapatellar arthrotomy depending on the site of the lesion to be transplanted. For cases of multiple lesions, a large arthrotomy was made, similar to the incision for total knee arthroplasty, which facilitated the access to all structures and preserved the meniscal insertions during the access route. In lesions of the posterior condyle, in which the approach is difficult, the anterior horn of the meniscus was cut radially; the meniscus was shifted for better access to injury, with subsequent suture.

Two types of surgical techniques for FAO were used: the osteochondral cylinder technique, in which a specific instrument was used to prepare the recipient bed and the donor graft (Biotechnology Ortopedia Importação e Exportação Ltd.; Fig. 3); and the surface technique, in which both the receiver and the donor were prepared manually with the aid of chisels, curettes, and a bone saw (Fig. 4).

The donor graft was taken from the same anatomical location as the lesion in the recipient. For this, the tissue bank was asked for a donor graft that corresponded to the lesion of the recipient.

Functional assessment

Patients were evaluated preoperatively, intraoperatively, and postoperatively through the International Knee Documentation Committee (IKDC) 2000 Subjective Knee Evaluation Form,[24] the Knee Injury and Osteoarthritis Outcome Score (KOOS),[25] and the Merle D'Aubigne and Postel Score, modified for the knee[26,27] for a detailed assessment of the lesion and of limb function.

Statistical analysis

Continuous and discrete data, such as subjective IKDC, KOOS, and modified Merle D'Albigne and Postel Score, were described as means and standard deviations. The Kolmogorov–Smirnov test was used to test the distribution of all data. For inferential statistics, the Subjective IKDC and the KOOS presented normal distribution; for the comparison over time, the one-way ANOVA for repeated measures and the *post hoc* Bonferroni test were used. To test the improvement by modified Merle D'Albigne and Postel Score, which did not present normal distribution, the Friedman test for related measures was used, as well as the Wilcoxon *post hoc* test to compare pairs of related measures with their respective corrections.

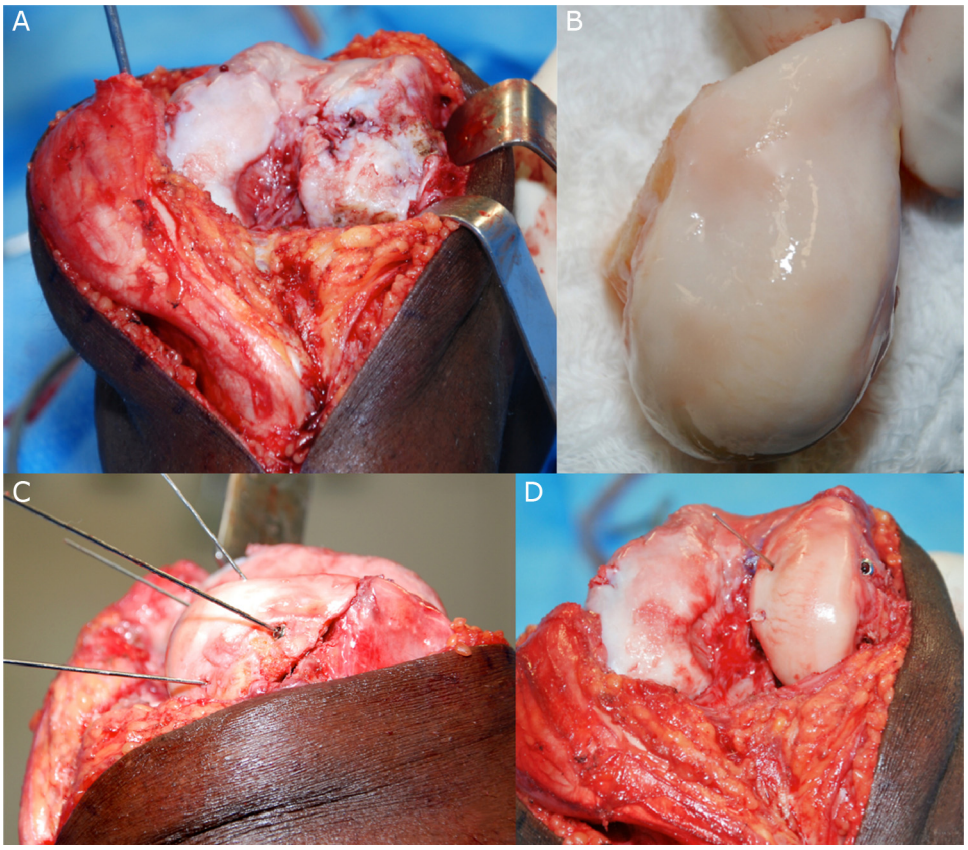

Fig. 4 – (A) Osteonecrosis sequelae in the lateral femoral condyle (LFC) with lateral parapatellar access route and medial patellar luxation; (B) LFC of the donor during surgery; (C) Aspect of the provisional fixation of the graft showing the congruence of the articular surface; (D) Final fixation of the LFC that restores the anatomy of the joint.

For statistically significant differences, a type I error equal to or lower than 5% was adopted. SPSS v. 20.0 software for Mac was used in data analysis.

Results

Eight FOA were conducted from March 2012 to October 2012, from five donors and eight operated knees. Patients were followed-up for at least two years after surgery (30–37 months).

Five patients had an initial diagnosis of OD, one patient had post-chemotherapy femoral condyle necrosis, and two had post-traumatic sequelae. The mean age of the transplanted patients was 30.1 years (17–44) and the mean transplanted area was 10.6 cm^2 (4.6–22.4 cm^2). Mean number of days between harvesting and transplantation was 15.3 (14–16) and mean number of surgeries prior to FOA was two (0–4) (Table 1). Six transplants were performed in the femoral condyle, one in the tibial plateau with the meniscus, and one in the patella.

One patient was lost to follow-up at six months (patient 5); in this case, all data of the scores were replaced by the worst value among all patients, which characterized the use of the worst case scenario and analysis by intention to treat by not excluding this patient from the study.

Mean preoperative IKDC subjective score was 31.99 ± 13.4 and 81.26 ± 14.7 postoperatively. Mean preoperative KOOS was 46.8 ± 20.9 and 85.24 ± 13.9 postoperatively. ANOVA indicated that patients showed significant improvement over time, when comparing preoperative and postoperative results ($p < 0.01$).

The mean modified Merle D'Aubigne and Postel Score for the knee was 8.75 ± 2.25 preoperatively and 16.1 ± 2.59 postoperatively. The Friedman test for nonparametric samples indicated that the patients showed significant improvement over time, when comparing preoperative and postoperative results ($p < 0.01$).

Discussion

FOA transplantation in the knee joint were not performed until 2009 in Brazil, as the legislation in the country did not allow the storage of fresh tissues long enough for the procedure to be performed safely.[28] This study is the first reported use of FOA in South America.

As the first study of FOA in Brazil, only patients between 15 and 45 years old with a history of traumatic or acquired lesions of the knee greater than 4 cm^2 were included, and patients with degenerative lesions were excluded.

All grafts were obtained from organ donors harvested in the operating room, after the heart, liver, and kidneys were collected, unlike the study by Vangsness et al.[29] in the United States, where only 33% of the harvest procedures occurred in an operating room, while the remaining occurred in morgues

Table 1 – Transplant characteristics: transplant site, lesion size in cm^2, time between harvesting and transplantation in days, type of surgical technique, age, diagnosis, and number of previous surgeries.

Patient	Transplant site	Lesion size	Time interval Har – Tx (days)	Surgical technique	Age	Diagnosis	Prior surgeries
1	MFC	4.6	14	Cylinder	44	OD	3
2	LFC	12.96	15	Surface	27	LFC necrosis post-chem	0
3	Patella	13.3	16	Surface	43	Patellar Fx sequelae	2
4	MFC	8.75	15	Cylinder	25	OD	1
5	Medial plateau	22.4	15	Surface	29	Plateau Fx sequelae	4
6	LFC	5.2	15	Cylinder	17	OD	2
7	MFC	4.8	16	Cylinder	18	OD	2
8	MFC	13	16	Cylinder	38	OD	2

Har, harvest; LFC, lateral femoral condyle; MFC, medial femoral condyle; Fx, fracture; OD, osteochondritis dissecans; post-chem, post-chemotherapy; Tx, transplantation.

or mortuaries. To date, harvesting in these facilities in Brazil is not possible due to legal aspects, which limits the number of grafts available for transplantation.

After being harvested, grafts were sent immediately to the tissue bank for processing, which occurred within 12 h of the procedure. This agility between harvesting and processing allowed for a short interval between collection and transplantation (15.3 days), a fact that contributes to increased cell viability of the transplanted chondrocytes in cartilage grafts when compared with grafts stored for longer periods.[30] Another factor that contributed to the short time between harvesting and transplantation was the fact that all grafts were harvested within a 100 km radius from the city of São Paulo, with no need for air transportation, which decreased the time interval between harvesting and transplantation.

Transplants were performed using two surgical techniques: osteochondral cylinder and surface. The specific instruments for the osteochondral cylinder technique were not available in Brazil at the beginning of this study; therefore, an instrumental set was manufactured by a national company for the surgical procedure with this technique. In this technique, the diameter of the osteochondral cylinder of the donor must be equal to or 1 mm smaller than the recipient bed. However, in the instrument set used, this difference was slightly greater than the optimum; therefore, it was necessary to fixate some grafts with 3-mm cannulated compression screws, which were removed by arthroscopy 12 weeks after transplantation.

Clinical evaluations made through objective and subjective questionnaires (IKDC, KOOS, and modified Merle D'Aubigne and Postel) demonstrated a significant improvement between the preoperative period and last follow-up ($p < 0.01$). Only one patient had a postoperative complication at follow-up. This patient had a history of medial tibial plateau fracture that developed acute infection after fracture fixation; it was treated with serial surgical debridement and removal of any hardware material. This patient had a recurrence of the prior infection three months after the osteochondral transplantation (three years after the fracture), with graft failure. Radiographic images of all other patients showed incorporation of the grafts, without subchondral cyst formation or graft collapse. Patients returned to their daily activities of work and leisure, as well as to low-impact sports. The level of patient satisfaction with the procedure was considered high by all transplant recipients.

The present study has several limitations. It had a small sample of patients, with a short follow-up period, and without a control group for comparison of results. Another limitation is the fact that two surgical techniques were evaluated together, which may present different results due to the difference in the size of the grafts and surgical technical difficulties.

Conclusion

In Brazil, FOA is a safe procedure with good clinical results in the short and medium term for the treatment of osteochondral lesions of the knee joint larger than 4 cm^2. This is a complex procedure that relies on a database of specialized tissues and a surgical team trained in harvesting and processing the tissue.

Conflicts of interest

The authors declare no conflicts of interest.

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