The treatment of deep and large-sized osteochondral defects still remains a challenge in orthopedic surgery worldwide. [1] Fresh osteochondral allograft (OCA) transplantation is an increasingly and widely used technique and is currently almost the only option for treating massive osteochondral lesions, particularly in young patients. Long-term follow-up published in the international literature reports a success rate of 50 to 89% 10 years after implantation for this method. [2-7]

The basic concept behind fresh OCA transplantation is to transplant a mature, bone-based hyaline cartilage that survives hypothermic (or isothermal) storage, while retaining its metabolic activity and collagen matrix. Hyalin cartilage is a tissue with properties that are ideal for transplantation. Primarily, given that it is an avascular tissue, it does not require a blood supply and receives its metabolic uptake from the synovial fluid by diffusion. Secondly, due to its aneural structure, innervation is not required for its function. Finally, articular cartilage can be considered

**Objectives:** This study aims to investigate whether ultra-fresh osteochondral allograft (OCA) transplantation was a good therapeutic alternative for the treatment of otherwise challenging, massive osteochondral defects in the knee joint.

**Patients and methods:** Between April 2011 and July 2022, a total of 16 ultra-fresh knee transplantations (9 males, 7 females; median age: 30.2 years; range, 14 to 62 years) having large osteochondral defects on femoral condyles were included. The operations were performed by two surgeons. The condition of the patients were evaluated based on regular follow-up physical examinations, imaging studies and by recording and evaluating clinical scores (modified Cincinnati scores, and 2000 International Knee Documentation Committee [IKDC] scores).

**Results:** The median follow-up was 65±48 (range, 6 months to 12 years). At two years after transplantation, there was a significant improvement in the modified Cincinnati scores (preoperative score of 35.75 increased to 83.75; p<0.001) and also to the IKDC scores (preoperative score of 28.7 increased to 76.3; p<0.001). One patient developed an early septic complication, and another three patients underwent reoperation after the OCA transplantations for non-septic reasons.

**Conclusion:** Ultra-fresh OCA transplantation is a good therapeutic alternative for the treatment of otherwise challenging, massive osteochondral defects in the knee joint. Such a shortening of the transplantation time and its positive effect on the better long-term survival of transplanted chondrocytes has not yet been proven; however, the minimizing of transplantation time may create the conditions necessary for successful OCA transplantations many years after the implantation.

**Keywords:** Allograft transplantation, cartilage repair, chondrocyte survival, massive osteochondral defect.
immunologically neutral, as chondrocytes embedded in the cell-free matrix remain almost completely hidden from the recipient's immune system.\[19\] The other major component of OCAs is the bone base. This ensures that the graft is fixed and incorporated into the receiving side. During integration, this frame structure functions as a scaffold. Given that it is originally a vascularized tissue, the remaining blood cells can elicit a recipient immune response.\[9\]

The effectiveness of fresh OCA transplantation in an appropriately-chosen patient population is well documented in the international literature. Many authors have reported successful mid- and long-term outcomes for the use of these grafts.\[10-13\] These reports suggest that the gold standard for long-term successful OCA transplantation is the transplantation of fresh osteochondral tissues within 28 days, stored at 4°C, in a nutritious medium. These grafts are able to survive and function for up to 25 years due to the hyaline cartilage viability, which depends on a stable osseous graft base.\[14\] These grafts contain a large proportion of living chondrocytes capable of maintaining the mechanical properties of the extracellular matrix, even many years after implantation.\[15,16\] Regarding graft storage conditions, some studies have reported successful transplantation of grafts stored at 37°C; however, currently it appears to decrease chondrocyte survival compared to 4°C storage. The use of different nutrient solutions improves the shelf life of grafts, but their ideal composition still remains unclear.\[17\]

Tissue banks providing fresh OCAs are not yet available in many countries around the world. We, as the authors of this study, in the absence of a tissue bank, established their own donor surgery team, who procured fresh osteochondral donor tissue during planned donations. Transplantation of allografts from living donors in the absence of a tissue bank is also a possible alternative; however, the possibilities of this method are limited. Allografts from cadavers are superior in both quantity and quality compared to grafts obtained from a living donor and, therefore, widespread use of this living donor allografts is not expected.\[18\] In the present study, we aimed to investigate whether ultra-fresh OCA transplantation was a good therapeutic alternative for the treatment of otherwise challenging, massive osteochondral defects in the knee joint.

**PATIENTS AND METHODS**

This prospective study was conducted at Uzsoki Hospital, Departement of Orthopaedic & Trauma, Budapest, Hungary between April 2011 and July 2022. Most of our young patients were placed on a waiting list due to massive osteochondral lesions of their knees and they were, then, treated with ultra-fresh OCA transplantation. A total of 16 patients (9 males, 7 females; median age: 30.2 years; range, 14 to 62 years) were included. Indicative distribution of our 16 knee transplants were as follows: six cases of post-traumatic osteochondral defects with significant bone loss, five cases of large-sized osteochondritis dissecans, two cases of osteochondronecrosis due to steroid therapy, one case of a lesion developing due to chronic hemorrhagic synovitis, and two cases of osteochondral defects, which developed in conjunction with the absence of anterior cruciate ligaments (Table I).

Prior to surgery, all patients underwent a physical examination, a comparative X-ray was performed on both knees (in those patients for whom the X-ray did not provide sufficient information about the defect, and a preoperative magnetic resonance imaging [MRI] was also performed). We transplanted the OCAs from living donors in two cases, and from cadavers for the remaining 14 cases.

The dates of physical examinations were as follows: preoperatively, at Weeks 2, 6, 12, 24, and one year postoperatively, then annually. X-rays were performed on the following dates: preoperatively, at Weeks 6, 12, 24 and one year postoperatively, then annually. All MRI examinations were done accordingly: at six months and one year postoperatively, and at two years following surgery. Physical examination and imaging scans were evaluated by the operating physician and an independent radiologist.

All patients were followed prospectively. Physical examination, imaging (X-ray and MRI), and clinical scores (Cincinnati score, International Knee Documentation Committee [IKDC] score) were used to follow our patients. The patients received the questionnaires at the time of their follow-up examinations, and they returned them in person or by e-mail.

**Preoperative preparation and donors**

Between 2011 and 2022, we performed ultra-fresh OCA transplantations on 16 knee joints in 16 patients at our hospital without a tissue bank background. Grafts were obtained in two cases from pre-screened living donors undergoing joint replacement surgeries, and in 14 cases from pre-screened cadavers with negative virus-serological results, obtaining the osteochondral tissue within 6 to 10 h after cardiac arrest, after the removal of
| Patients | Sex | Age (year) | Knee | Diagnosis | Part of the knee | Total graft area (cm²) | Graft technique | No. of grafts | Follow-up (years) | Improvement of modified Cincinnati score or outcome |
|----------|-----|------------|------|-----------|------------------|------------------------|------------------|--------------|------------------|-----------------------------------------------|
| 1        | Male | 29         | Right | Posttraumatic osteochondral necrosis | LFC              | 15                     | Structural graft  | 1            | 12               | 61†                                            |
| 2        | Female | 30       | Right | Osteochondral necrosis due to arthritis hemorrhagica | LFC              | 12                     | Shell            | 1            | 1.7†             | TKA                                           |
| 3        | Male | 32         | Right | Posttraumatic osteochondral necrosis | MFC              | 7                      | Plug             | 2            | 10.9             | 55†                                            |
| 4        | Female | 27       | Left  | Posttraumatic osteochondral necrosis | LFC              | 8                      | Shell            | 1            | 9.5                           | 63†                                            |
| 5        | Female | 6        | Left  | Osteoarthritis (15 years after ACL reconstruction) | MFC              | 10                     | Shell            | 1            | 0.6†             | Revision allograft                              |
| 6        | Male | 40         | Left  | Osteochondritis dissecans | MFC              | 8                      | Plug             | 2            | 9.1                           | 9†                                             |
| 7        | Female | 62       | Left  | Posttraumatic osteochondral necrosis | LFC              | 12                     | Structural graft  | 1            | 8.7                           | 38†                                            |
| 8        | Female | 23       | Left  | Osteochondritis dissecans | MFC              | 7                      | Plug             | 1            | 8.3                           | 22†                                            |
| 9        | Male | 15         | Left  | Osteochondritis dissecans | LFC              | 7                      | Plug             | 1            | 7.7                           | 15†                                            |
| 10       | Male | 17         | Left  | Osteochondral necrosis due to steroid use (RA) | MFC              | 10                     | Plug             | 2            | 2.6†             | Revision allograft                              |
| 11       | Male | 28         | Left  | Osteochondritis dissecans | LFC              | 14                     | Plug             | 2            | 6.6                           | 45†                                            |
| 12       | Male | 41         | Left  | Osteochondritis dissecans | LFC              | 12                     | Shell            | 1            | 5.5                           | 48†                                            |
| 13       | Male | 32         | Left  | Osteochondral necrosis due to steroid use (AML) | LFC+LTP          | 8                      | Shell            | 1            | 3                             | 53†                                            |
| 14       | Female | 29      | Right | Osteochondral necrosis due to IDDM | LFC              | 10                     | Structural graft + plug | 1+1 | 0.2              | Revision allograft                              |
| 15       | Female | 28       | Left  | Posttraumatic osteochondral necrosis | LFC              | 10                     | Shell            | 1            | 0.8                           | 36†*                                           |
| 16       | Male | 14         | Left  | Osteochondritis dissecans | LFC              | 12                     | Plug             | 1            | 0.5                           | 24†                                            |

LFC: Lateral femoral condyle; TKA: Total knee arthroplasty; MFC: Medial femoral condyle; ACL: Anterior cruciate ligament; LTP: Lateral tibial plateau; RA: Rheumatoid arthritis; AML: Acute myeloid leukemia; IDDM: Insulin dependent diabetes mellitus; t: Time to revision allograft/conversion to TKA; * Less than two years follow-up.
other organs. In the latter case, en bloc resection of the knee joint was performed, together with 5 to 8 cm of adjacent metaphysis bone, and it was cleaned of the soft tissue. It was, then, placed in gauze sheets soaked in physiological saline, then in double sterile packaging, into a refrigerated transport box, and transported to the implantation facility, where the grafts were refrigerated at 4°C until implantation. Viral serology screening was performed by a donor testing laboratory and included the following: human immunodeficiency virus (HIV) Ag/Ab, Anti-hepatitis C virus (HCV), hepatitis b surface antigen (HBsAg), anti-TP, anti-HBc, anti-HBs, anti-cytomegalovirus (CMV) immunoglobulin (Ig) M, anti-CMV IgG (every cadaver donor was admitted to the intensive care unit [ICU] more than two weeks before donation). The technique we used was considered a clinical trial; therefore, we priorly received permission from the national scientific research and ethics committee, and the patients treated in this way signed a statement of consent after being given detailed information about the procedure. The implantations were performed by two surgeons.

**Surgical technique**

All fresh OCA transplantations were performed no later than 36 h after donor tissue removal (ultra-fresh implantation). After transportation in an ice-cooled, insulated transport box, the donor tissue was stored at 4°C in a refrigerator until implantation.

Donor tissue removal, transport, storage, and implantation were performed under sterile conditions. The technique of implantation depended on where the defect was located on the weight-bearing surface and its size. In those knee joint transplantations, where the osteochondral defect did not affect the edge of the femoral condyle (contained defects), mega OCA Transfer System (OATS®) technique was used to implant the relatively large diameter mosaic-like grafts, which could be fixed in a press fit manner (Figure 1). For this technique, we used the Arthrex Allograft OATS® instrumentation or the BioUni™ OATS® instrumentation set. If the edge of the femur condyle was also affected (uncontained defects), the transplanted graft was fixed with hardware after free-hand preparation of the osteochondral graft to model the defect (Figure 2).

**Rehabilitation protocol**

Individually-tailored rehabilitation was used for our transplanted patients due to the different localizations and sizes of the implanted grafts. Two main considerations shaped the rehabilitation plan: a time period of non-weight bearing for the appropriate
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Time (but not for too long), and immediate range of motion exercises. The more massive the repaired defect, the longer the non-weight bearing time (the total non-weight bearing time varied between six weeks and three months). However, immediate movement of the operated knee joint was also endorsed after surgery, thereby stimulating the integration of the transplanted osseous tissue into the recipient site.

Statistical analysis

Statistical analysis was performed using the SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data were presented in median (min-max) or number and frequency, where applicable. Preoperative and two-year postoperative IKDC and modified Cincinnati scores were compared using a two-sample paired t-test (there was a significant improvement in the Modified Cincinnati Score [preoperative score of 35.75 increased to 83.75 (p<0.001)] and also to the IKDC score [increased to 76.3 from the preoperative score of 28.7 (p<0.001)]. A p value of <0.05 was considered statistically significant.

RESULTS

Following our 16 knee allograft transplantations, one patient developed an early septic complication. The median follow-up was 65±48 (range, 6 months to 12 years).

Due to medial femoral condyle necrosis, an ultra-fresh allograft (structural graft) was implanted in a 29-year-old female patient with type 1 diabetes mellitus. In this case, due to purulent arthritis, we removed the transplanted structural allograft along with the fixing Herbert screws eight weeks post-implantation. Another three of our transplanted patients underwent reoperation after fresh OCA transplantation for non-septic reasons. In one of these cases, a 30-year-old female patient with chronic hemorrhagic synovitis of autoimmune origin needed total knee arthroplasty 19 months after the allograft implantation due to necrosis of the transplanted graft.

In two additional cases (17-year-old male and 36-year-old female patients) arthroscopic

FIGURE 2. Free-hand carved and Herbert screw-fixed grafts (a and c) in a 36-year-old female patient in the medial femur condyle, (b) in a 27-year-old female patient in the lateral femur condyle.
Debridement, free body removal, partial hardware removal due to sequestration or delamination of part of the implanted graft were performed between six months and 2.6 years following the transplantation.

However, at least 60% of the cartilage surface of the transplanted grafts of both patients survived after the reoperation. During these interventions, microfracture was simultaneously performed on the affected weight bearing surfaces at the site of the removed grafts.

Due to another indication (medial meniscus tear), one patient underwent arthroscopy of the affected knee joint 34 weeks after the implantation. We observed the integration of the previously implanted allograft, and a congruent articular surface with good hardness was found using an arthroscopic probe (Figure 3). Two osteochondral cylinder biopsies with a diameter of 2 mm and a length of 1 cm were obtained from the transplanted area and sent for histological analysis (Figure 4a, b).

No thromboembolic complications or immunological rejections were observed. No transplantation-related viral transmission was confirmed in any of the cases.

**Scores**

Regarding the IKDC scores, the patients had a median preoperative score of 28.7 (range, 13.8 to 43.7), which increased to 76.3 (range, 26 to 94.3) points at the postoperative second year (p<0.001).

For the Cincinnati scores, the patients had a median preoperative score of 35.75 (range, 14 to 81), which increased to 83.75 (range, 74 to 100) points at the postoperative second year (p<0.001) (Figure 5).

Data were collected at the postoperative second year from patients, who at that time, had a functioning transplanted allograft in the recipient area; i.e., these patients did not undergo graft removal or conversion to total knee arthroplasty. In the postoperative period, these patients either had no surgical procedure performed on their transplanted knee joint, or had arthroscopic debridement and/or hardware removal.

**DISCUSSION**

Regarding our ultra-fresh OCA implants, we used the North American practice, with one significant difference: while the graft source for the American fresh OCA transplants is a certified tissue bank with strict protocols, in our own practice, with the collaboration of the National Organ Coordination Office, the fresh grafts are harvested by our own team from donor cadavers and are prepared for implantation. Our transplantations over the past 12 years demonstrate that ultra-fresh OCA transplantation is an appropriate therapeutic option.
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for the treatment of massive osteochondral defects, particularly at a young age.[19]

For our first two knee implantations, in the absence of a cadaver donors at the time, the transplanted tissue came from a living donor. This refers that we obtained osteochondral tissue for the transplantation from patients undergoing arthroplasty surgery, where we found the supporting tissue to be intact based on the preoperative X-ray examination and intraoperative macroscopic findings. These patients underwent preoperative virus screening tests. Due to the fact that OCA from cadaver is more favorable both in quantity and quality (in case of living donors, the transplanted tissue was obtained from a joint more or less affected by degeneration), we do not perform living-donor transplantations anymore.[18]

According to the North American practice, fresh allograft implantations are performed in centers that collaborate with tissue banks, which are properly equipped and work with strict protocols. Grafts are obtained from donors between the ages of 15 and 40 years, in whom the cartilage surface appears macroscopically intact.[20] Graft harvesting is performed under aseptic conditions while minimizing the warm ischemic period. The harvested grafts are usually stored refrigerated at 4°C (reports of storage at 37°C have also been published.[17] Several studies have reported the storage of tempered grafts in a variety of media containing amino acids, glucose, and inorganic salts, which has a beneficial effect on chondrocyte survival and structural integrity. These studies have shown that cell density, viability, and metabolic

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**FIGURE 4.** (a-d) Microscopic image of impeccable hyaline cartilage from the transplanted allograft: DMMK - dimethylmethylene blue staining (a) (200 micrometers magnification) and (b) (500 micrometers magnification). (c) H&E - hematoxylin-eosin staining (200 micrometers magnification) and (d) (500 micrometers magnification). (e, f) Microscopic images of the articular cartilage from the implanted graft, photographed in both (e) normal and (f) polarized light (PS - picrosirius staining). In normal light, collagen-containing structures are red. The microscopic images under polarized light show that the collagen fibers are organized in the birefringent (glossy) structures (the brighter the structure, the more organized its structure).

**FIGURE 5.** Improvements observed in the modified Cincinnati score and IKDC score. Preoperative values were compared to two-year postoperative values. The differences were found to be significant (p<0.001).
activity can be considered unaffected for a period of 14 days from the start of storage, while the hyaline matrix remains more or less intact for 28 days, after which degeneration becomes significant.\cite{21,22} These reports suggest that fresh OCAs could be stored for up to four weeks, but empirical practice overseas remains that these grafts are mostly used within 14 to 19 days.\cite{13,23}

In our own practice, we attempted to minimize the transplantation time, to achieve an even shorter transplantation time than in the North American practice. Therefore, we performed ultra-fresh (within 24 to 36 h) transplantations with the aim of ensuring the survival of as many chondrocytes as possible in the transplanted tissue. As mentioned previously, in contrast to the donor availability in the United States practice, no tissue bank was available in our own practice to provide the fresh allografts required for transplantation; within the framework of the donation collaboration, our own team obtained and, then, implanted the osteochondral tissue after minimal storage time. The necessary pre-screening and infectious agent testing of the cadaver donors, which is necessary for the transplantation of organs other than osteochondral tissues, was performed by the National Organ Coordination Office. Donation and transplantation of organs or tissues were only performed, if the investigated donor had negative virus screening results.

Although this practice may have a beneficial effect on chondrocyte survival, it is also a major logistical challenge for both the tissue procurement team and the surgeon performing the implantation, as well as the patient awaiting transplantation. Our patients awaiting allograft transplantation were placed on a waiting list at the time the indication for transplantation was decided and they were informed that they would be notified by telephone of a possible donation alert and would, then, have to arrive to the hospital for transplantation within 24 to 36 h. Good compliance was observed with patients regarding this practice, with only a negligible number of patients who did not decide to undergo surgery at the time of telephone notification. In addition to the affected joint, home address, and telephone contact information of patients on our waiting list, we recorded the following: age, height, weight, foot (shoe) size, which provided approximate information about physical similarity to the actual donor, thereby aiding donor-recipient matching. In our practice, in case of a donation alert, an employee from the Organ Coordination Office provided us information about the donor's age and physical parameters by
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From an organizational point of view, our own practice required somewhat more of a difficult solution (inserting an “unexpected surgery” into the daily elective surgery program, possibly during on-call time, calling in a waiting patient living in a potentially remote part of the country for surgery within one to two days, examination by anesthesiologist, preparation for surgery, etc.) compared to the routine of American centers working with a tissue bank background and a longer time window. This difference is also reflected in the fact that in addition to the deep, large-sized osteochondral defects that represent the “classical indication,” North American centers implant fresh allografts for more superficial, smaller-scale lesions; i.e., the procedure is now used in extended indications.

Regarding the technique of implantation, it is advantageous to have instrumentation that helps the surgeon and, somewhat, standardizes the implantation. In our practice, for ultra-fresh OCA transplantsations, we used either Arthrex Allograft OATS® Set (Figure 1) or the BioUni™ OATS® (Figure 6) instrumentation, which both support the most accurate restoration of joint surfaces. Unfortunately, these techniques can be only used in knee joint implants where the defect is surrounded by an intact condyle (contained defects). If the defect also affects the edge of the femoral condyle (uncontained defect) to a large extent, the grafts need to be free-handedly, manually shaped, and fixed with hardware to the appropriate recipient area, without the help of an instrumentation kit. This requires a highly skilled surgical routine.

Regardless of the technique chosen, the bony bases of the grafts are washed thoroughly with high-pressure saline (pulsed-lavage or jet-lavage) prior to implantation. This is necessary to remove the blood or cells of the bone marrow of the donor that may remain between the bone matrix, which may elicit an immune response.

Nonetheless, this study has certain limitations. First, the sample size is relatively small. Second, the evaluation of the results is further complicated by the fact that the follow-up time significantly varied among the presented cases. This is because, beside the use of other cartilage resurfacing methods, ultra-fresh OCA transplantation is a rare indication in our own cartilage resurfacing practice (we perform one or two transplants per year on average); most osteochondral defects can be treated with a different, logistically and technically more simple procedure.

In conclusion, our experience over the past 12 years demonstrates that ultra-fresh OCA transplantation is an appropriate therapeutic option for the treatment of massive osteochondral defects, particularly at a young age. If the technical and logistical conditions of the method improve, next to the classical indications, more extensive clinical applications of ultra-fresh allografts may become possible in the future and even surgical treatment of more superficial or smaller defects can be achieved. However, the procedure involves major logistical challenges: the completion of the entire ultra-fresh transplantation process within 24 to 36 h is a serious burden for both the teams involved in donor graft procurement and transport as well as the implanting surgeon, in addition to the extra operation inserted into the elective surgery program for that day, and patient from the waiting list suddenly summoned for surgery. Furthermore, the procedure is currently very demanding from a surgical point of view which makes it difficult to spread the technique more widely.

**Ethics Committee Approval:** The study protocol was approved by the Hungarian Medical Research Council (ETT-TUKEB - 21st April 2011/2237-0/2011-EKU). The study was conducted in accordance with the principles of the Declaration of Helsinki.

**Patient Consent for Publication:** A written informed consent was obtained from each patient.

**Data Sharing Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Author Contributions:** Idea/concept, design: L.R.H.; Control/supervision, critical review: L.H.; Data collection and/or processing: G.H., G.V.; Analysis and/or interpretation: T.G., L.R.H.; Literature review: L.R.H., G.H.; Writing the article: L.H.; References and fundings: R.I.B.S.; Materials: G.V., T.G.

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