The treatment of weightbearingarticulardefectswithfreshosteochondralallografttransplantation

Ph. D. Thesis

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Introduction

Over the past 25 years, the surgical treatment of cartilage damage was a revolutionary time period. Various cartilage resurfacing techniques were established and have evolved considerably. The increasing number and better results of endoprosthetic implantation are postponed to a later age due to the development of modern cartilage resurfacing techniques, such as chondrocyte transplantation, osteochondral autograft transplantation, and biodegradable matrix implantation. These previously mentioned techniques may be the answer for the treatment of focal cartilage lesions, but in the case of large and deep osteochondral defects, none of those methods seem to be an appropriate solution. For these massive defects, the transplantation of osteochondral allografts can provide a therapeutic option.

The long-term results from international literature published over the past decade prove the long term success of fresh osteochondral allograft transplantations, when the grafts are stored in hypothermic conditions. These grafts are capable of surviving and functioning for as long as 25 years due to their stable osseous foundation and the survival of the hyaline cartilage on the surface. The grafts contain a high quantity of living chondrocytes, which help maintain the mechanical properties of the extracellular matrix, even many years after the transplantation.

In the United States of America, several decades of successful clinical experiences support the efficiency of this method, which is so well established, that the fresh osteochondral allografts are not only used for the treatment of large, deep defects, but they are also used for superficial lesions in the younger population.

Until now, relatively few places in the world dealt with fresh allograft implantation, only a few transplant centers in North America have gathered substantial clinical experience in regards to this method. These centers collaborated with appropriately equipped tissue banks which have strict protocols. The grafts were obtained from donors aged between 15 and 40 years, whose cartilage surface was macroscopically intact. Graft harvesting takes place under aseptic conditions, while minimizing the warm ischemic period. The storage temperature of the harvested grafts is 4 degrees Celsius according to the current protocol; however there are some authors who have recorded better chondrocyte survival rates when stored at 37 degrees Celsius.

Regarding allograft transplantation, one of the most important factors affecting the long-term success of the graft is the time elapsed between the harvesting and implantation of the graft. At first, the goal was to perform the transplantation as soon as possible - within a few days. However, in overseas centers, the concern of the transmission of pathogenic agents demanded the introduction of a minimum 14-day screening period. This time is necessary to allow the tissue bank to identify aerobic, anaerobic and spore-forming bacteria, as well as viruses, before the graft were to be implanted into the donor.Taking this into account, even though there is no unified position regarding the survival time of fresh allografts, these transplant centers began

the practice of storing the allografts in hypothermic conditions for up to 24-28 days. In literature, allografts stored for so long are regardedas “prolonged fresh” or“delayed fresh” instead of using the adjective "fresh". The aforementioned relatively long time interval between harvesting and transplantation may result in a decrease in the number of surviving chondrocytes.

Objectives

1) At the Department of Orthopedics-Traumatology, Uzsoki Hospital, we started to perform fresh osteochondral allograft transplantation in 2008, and this was also the beginning of the application of the method in Hungary, so we had to create the conditions necessary of this technique at a national level. The greatest barrier of this technique is the lack of tissue banks in which fresh osteochondral allografts are available domestically. As an alternative, we performed experiments with grafts obtained from so-called living donors. Our aim was to confirm the application of these types of transplant grafts by histological examination.

2) Our goal was to apply the fresh osteochondral allografts from living donors in clinical practice also, if the histological results were encouraging. In addition, our aim was to obtain objective information about the clinical experience of this method when performed with an appropriate indication using a clinical scoring system with modern imaging techniques.

3) Until now, only certain clinical centers in the United States of America and Canada have significant clinical experiencein fresh osteochondral allograft transplantation. In these centers, a tissue bank provides the background for the storage of the allografts, and although there is no consensus regarding the shelf life of grafts, they are implanted within 24-28 days. Previous experimental and clinical experience has shown that chondrocyte survival correlates with the time elapsed between the harvesting and implantation of the graft. For this reason, our objective was to significantly shorten this time period, and to provide the conditions necessary to perform "ultra-fresh" transplantations - within 12-24 hours.

4) Our goal was to gain experience in the clinical effectiveness and the survival of chondrocytes, which are transplanted within the aforementioned shorter time period elapsed.

5) In order to have access to a large number of donors on a regular basis as well as to promote the fresh osteochondral allograft

transplantation program, we set out a goal to organize a Hungarian fresh osteochondral allograft transplantation institutional system.

6) To ensure graft quality, we aimed to work out a graft harvesting criteria system and we created our own team, who harvest the grafts from the cadaver donors according to our criteria and arrives to the scene within a few hours after being notified.

7) It is known that one of the major problems of fresh osteochondral allograft transplantations are sizing and fitting discrepancies. This can be resolved by donor - recipient matching based on proper sizing and fitting using quantitative radiographic parameters. Our target was to establish such a waiting list, which makes use of a data system matching the best possible donor to the recipient for the transplantation.

8) Our goal was to develop rehabilitation protocols following fresh osteochondral allograft transplantations, and to perfect these requirements based on the information gained by modern imaging techniques.

Methods

EXPERIMENTAL EXAMINATIONS

Histological analysis of osteochondral samples from living donors

At our primary clinical research site, the Department of Orthopedics and Traumatology of Uzsoki Hospital, a large number of knee and hip replacement surgeries are performed; therefore in this research, it seemed evident to examine the osteochondral tissues removed from the patients during these endoprostetic replacement surgeries. In some cases, these tissues are only partially damaged and contain sections, which seem to have intact cartilage on the preoperative radiographs and upon intraoperative inspection. Even these intact regions need to be resected in order to be able to implant the endoprosthetic replacement. For this reason, we can acquire osteochondral tissue during these endoprosthetic replacement surgeries, which may be suitable for homologous transplantation.

Typically two anatomical regions were potential allograft sources: 1) in hip endoprosthetic replacement surgery due to hip arthritis, the intact cartilage surface of the caudal part of the femoral headbased on the preoperative radiological image, 2) in total knee endoprosthetic replacement surgery,in the case of varus type arthritis of the knee, the resected the intact cartilage

surface of the lateral femur condyle (LFC) based on the preoperative radiological image.

The tissue blocks sent for histological examination were prepared using a thin oscillating saw. The blocks measured average *3-4 cm* in length– (*0.6-0.8 cm* in the case of the femoral head) and *2-3cm*in width (in the case of LFC), and were of total thickness (containing articular cartilage, subchondral cortical bone, as well as cancellous bone tissue). The preparations were fixed in Sainte Marie fixative (4:1 mixture of alcohol-formalin), and were sent for histological analysis.

The portion of the femoral head or femur condyle containing a cartilage surface was decalcinated in 25% formic acid, and embedded in paraffin. Five-seven micrometer thickness sections were sliced perpendicularly to the articular surface of the paraffin embedded blocks. The sections were processed with dimethylmethylene blue and picrosirius red staining.

The microscopic images were taken with a NIKON ECLIPSE 800 Research Microscope equipped with a SPOT JUNIOR digital camera. The examiner performing the histological evaluation had no information of the radiologic and macroscopic results.

CLINICAL EXAMINATIONS

Knee joint transplantation analysis

A total of 9 knee transplantations were performed, of which two cases were from living donors, while in seven cases, cadaver donors provided the allograft. Based on previous national and international research, experience, and pre-clinical studies, the clinical research plan was approved by the Scientific and Research Ethics Committee of the Medical Research Council in Hungary (file number: 2237-0/2011-EKU (62/PI/11)) and the operations were started accordingly. Based on previous experience and pre-clinical results, the new technology of "ultra fresh implantation" seemed to be justified.Our patients were informed before surgery and consented to participation in the study. The operations were performed by a surgeon who had extensive experience in musculoskeletal surgeriesand in a variety of methods cartilage replacement procedures.The prospective follow up of the patients were conducted by means of physical examinations, imaging (radiographs, MRI) and by an independent reviewervia clinical scores (IKDC score, Hospital for Special Surgery score, Cincinnati score).

In one patient, follow up arthroscopy was performed during the 34^{th} postoperative week, and we took advantage of this situation to obtain tissue samples for histological analysis and to cell counting. The obtained tissue cylinder was fixed using the Sainte Marie fixation technique - 4:1 mixture of alcohol-formalin, decalcification 25% formic acid, and embedded in paraffin. Five-seven micrometer thickness sections were sliced perpendicularly to the articular surface of the paraffin embedded blocks. The sections were processed with hematoxylin-eozin, dimethylmethylene blue and picrosirius red staining. The picrosirius red-stained sections were investigated under a

normal light microscopy as well as a polarized microscope. The microscopic images were taken with a NIKON ECLIPSE 800 Research Microscope equipped with an OLYMPUS DT 72 digital camera.

For cell count analysis, the cartilage was aseptically washed from the bone, and while immerged in PBS, mincing was performed using a scalpel. Then, the PBS was removed from the cartilage pieces and 1 ml of sterile trypsin-EDTA solution (Sigma-Aldrich Co, St. Louis, MO, USA) was added to the minced cartilage in both the graft biopsy group and the control group. The samples underwent dissociation at 37°C for 30 minutes using trypsin-EDTA solution (DMEM containing 100units of penicillin +streptomycin/ml).Subsequently, the DMEM containing trypsin-EDTA was carefully removed from the cartilage pieces, and was further digested in a DMEM solution containing 1 ml of 2 mg/ml collagenase D enzyme (Roche Applied Science, Indianapolis, IN, USA) for a further 3 hours. After 3 hours, the cartilage pieces remained together, but a portion of the DMEM containing the enzyme was removed and 300g 7'centrifuged. The residue was stained with 100 μl 1:10 diluted 0.4% trypan blue solution (Life Technologies). The cell suspension was dropped onto a slide and covered with a coverslip, and then was examined under x60 and x100 magnification of a NIKON ECLIPSE Research Microscope.

The cartilage samples were further digested and after 48 hours, the entire matrix was fully digested. The sample then underwent 300g 10' centrifugation, the residue was stained with 1:10 diluted 0.4% trypan blue solution, and the living and stained (dead) chondrocytes were counted in a Burker chamber.

Ankle joint transplantation results

Due to the favorable results of the fresh osteochondral allografts in the knee joint, we started to apply the method to other joints as well. In a 67 year-old male patient – who suffered from severe ankle joint pain due to arthritis, but had a good range of motion – we performed fresh allograft transplantation in the ankle joint using the unilateral talocrural joint from the donor. The follow up of the patient was conducted by means of follow up physical examinations, imaging (radiographs, MRI) and via clinical scores (Hannover score and AOFAS ankle – hind foot scale).

THE ORGANIZATION OF AN INSTITIONAL SYSTEM FOR FRESH OSTEOCHONDRAL ALLOGRAFT TRANSPLANTATIONS IN HUNGARY

In order to have access to a large number of donors on a regular basis as well as to promote fresh osteochondral allograft transplantation on a program basis, we contacted the Hungarian National Blood Transfusion Service and Organ Coordination Office, which are the domestic institutions responsible for organ and tissue transplantations. Our goal was to join into the Hungarian transplantation practice -in respect of fresh allograft transplantations, taking into account the specificity of the method – as a national center.For the quickest possible accessibility, we wanted to participate in the

transplantations in the central region of Hungary.On behalf of this collaboration, we established our own donor surgery team, who obtain the grafts from the cadaver donors arriving to the scene, and are capable of removing the distal epiphysis of the femur, the proximal end of the tibia, or the talocrural joint. This team is comprised of three orthopedic-traumatologist specialists, who are available every day of the week based on the preliminary roster, via telephone hotline,ready to acquire the necessary graft from a donor. To optimize the work of the donor surgery team, we worked out a criteria system for graft removal from the donor.

THE ORGANIZATION OF FRESH OSTEOCHONDRAL ALLOGRAFT TRANSPLANTATION WITHIN A 24 HOUR PERIOD

Taking into account the practice of foreign centers performing allograft transplantations, we believed that by further shortening the time elapsed between procurement and implantation, we can increase the survival rate of the chondrocytes in the transplanted tissue and therefore this may improve the long-term outcome of the transplant. A greater number of chondrocytes would guarantee good quality cartilage in the long run, thereby ensuring graft survival, and the success of the transplant, even after several years.

In order to be able to perform the transplantation within 24 hours of graft procurement when a donor is available, the recipient patients were put on a

waiting list, where we listed their address, telephone number, age, gender, body height and weight, foot size, the side of the affected joints, diagnosis, and previous interventions. The patients were informed that if a suitable donor were available, they would be informed by a phone call and askedto come in for surgery the next morning. This waiting list allowed us to best match the recipient to the available donor's physical parameters, especially according to gender, body weight and height, and foot size. This selection allowed for the best sizing and fitting of the graft, which was of assistance to the operating surgeon. On the other hand, it also ensured that if a donor is available, we were readily able to perform the transplantation to the recipient. For this reason, we had to organize and provide the conditions necessary for the non-stop availability of allotransplantations, independent of the elective or acute care surgical schedule.

THE DEVELOPMENT OF A REHABILITATION PROGRAM FOLLOWING FRESH OSTEOCHONDRAL ALLOGRAFT TRANSPLANTATIONS

Since the rehabilitation guidelines following fresh osteochondral allograft transplantations found in literature were only considered a foundation for our own transplantations, we had to develop an individual rehabilitation program for each patient following the transplantation. This was in some cases modified according to the feedback from the patient and the physical therapist, as well as according to the follow up imaging.

The key principles which we respected when planning the individual based rehabilitation program for the patients werethe importance of the nutrition and conditioning of the graft's cartilage layer, as well as the gradual angiogenesis of blood vessels into the bone. Therefore, we found it essential to introduce the appropriate load-adapted partial weight bearing of the joint. The amount and time period of partial weight bearing was determined by the mass of the graft as well as the vitality of the recipient bed. The surgical technique always supported the postoperative treatment required after cartilage replacement – immediate mobilization. However, the transplanted bone - mainly depending on the amount –required non weight bearing for a shorter or longer period of time.

The gradual increase in the tolerable limit ofweight bearing was reached by the end of the third postoperative month. The partial weight bearing periods (15-30-50 kg phases of weight bearing) played an important role in the rehabilitation.When the function of the extremity was fully restored, the patient was allowed to engage in recreational sports activities around the sixth postoperative month. To avoid the intensive strain of the implanted graft, we did not allow the return to pivoting sports or sport with sudden directional changes until one full year after the transplantation. The use of orthotics was deemed necessary in cases after unipolar femoral condyle replacement performed, and the "hinge" type of brace was modeled in a valgus or varus position in order to protect the graft from strain during the rehabilitation.

Results

EXPERIMENTAL EXAMINATIONS

Results of the histological analysis of osteochondral samples from living donors

Microscopic sections of a total of 27 patients underwent histological evaluation. Of these, 15 were potential allograft donors (the resected femoral head or femur condyle contained macroscopically intact surfaces), while 12 were of macroscopically destroyed surfaces, used as a control group. Of the 15 cases which were deemed macroscopically intact by the surgeon, 14 were histologically evaluated as good or acceptable, while only one case did not justify the operating surgeon's observation. The cases which were observed as macroscopically damaged proved to be histologically damaged also.

CLINICAL EXAMINATIONS

Knee joint transplantation results

The follow-up time of knee transplant patients was 3- 48 months. For quantitative comparative evaluation, the modified HSS score, the IKDC score, and the Cincinnati score was used. No septic or thromboembolic complications were observed. Of the nine knee transplantations, reoperation in two cases was necessary. In one patient, TKR implantation was performed 18 months following the allograft transplantation. In the other patient, the implanted allograft remained in place, but 6 months after the transplantation, partial necrosis of the graft was observed, and debridement of one third of the transplanted area was performed as well as microfracture and removal of the fixation screws. The protocol follow-up of the two patients was not feasible due to residence abroad, although they did appear for some follow-up examinations.

In the case of the patient who had a follow-up arthroscopy performed, we observed a good quality sliding surface of the transplanted medial femoral condyle, with the same quality of cartilage as of the patients own surrounding cartilage, and good consistency when examined with a probe. After obtaining a tissue sample from the previously transplanted allograft, we observed good bleeding from the biopsy area, which confirms the integration of the graft into the host area. The histological examination of the biopsy showed that mature hyaline cartilage was found on the articular surface in all sections. A live cell count was performed on 100-100 cells, showing that the living cell ratio (not stained by tryptan blue) was *68%* in the allograft sample, while it was *48%* in the control sample taken from the LFC.

Ankle joint transplantation results

The follow-up time of the talocrural transplant patient was 8 months. For quantitative comparative evaluation, the Hanover score AOFAS ankle-hind foot scale was used. The preoperative Hannover score of the patient was 44 points, while 6 months following the surgery was 76 points. The AOFAS score during these same time periods were 21 points and 80 points consecutively. During the course of the rehabilitation, the patient performed continuous physical therapy without weight bearing for 6 weeks, after 6 weeks was allowed partial weight bearing of 10kg, after 10 weeks 30 kg, and after 14 weeks 45 kg. In addition, the patient participated in subaquatic physiotherapyduring the rehabilitation period. The patient was able to bear full weight without the use of crutches in the 17^{th} postoperative week, at which time proprioceptive exercises were also completed.No septic nor thromboembolic complications were observed in this case neither.

RESULTS OF THE AFTER TREATMENT IN TRANPLANTED PATIENTS

The rehabilitation of each transplanted patient was individualized, and as time progressed, some changes needed to be made based on the feedback from the patients and physiotherapists. The more massive the transplanted graft, the longer the time the rehabilitation was necessary. In the knee

transplant case where reoperation (debridement, microfracture and screw removal) was necessary due to partial graft necrosis, we suspect that the transition of partial weight bearing to full weight bearing was premature in time. A similar problem occurred in the talocrural transplant patient, who had an excellent range of motion and was bearing full weight, but in the 20th postoperative week, we observed on the radiologic examinations a minimal compression of the ventral side of tibia. In all other cases, no signs of compression, graft rejection, or graft necrosis were observed on the radiographic images.We observed progressive angiogenesis and improvement of circulation of the implanted grafts on the MRI examinations, but edema of the bone and some impairment of the circulation were still observed in some cases, even over one year following the surgery. The MRI examinations however, did not show any signs of total cartilage surface detachment in any case – but in one case we observed partial chondral delaminating on the ventral part of the graft and partial subchondral necrosis, in which we performed a reoperation, removing the detached portion and performing microfracture of the bone foundation.

Conclusions

- With the histological analysis, we have proven that fresh osteochondral allograft transplantation can be performed from the

macroscopically intact cartilage of the resected donor tissue from the properly selected patients undergoing endoprosthesis implantation ("living donors")

- Through our collaboration with the Organ Coordination Office, we made it possible in the musculoskeletal surgery of Hungary to perform fresh osteochondral transplantation from a cadaver donor, without the need for an allograft tissue bank.

- We established a waiting list, which registered the data of recipient patients waiting for osteochondral allografts transplantations. Based on these data (gender, age, weight, height, foot size), we were able to match the physical parameters of the awaiting recipient to resemble that of the available donor. The selected patients were informed of the possibility of surgery through this waiting list – in the case of a cadaver donor. The transplantation operations we performed demonstrated that the notification of patients through our waiting list works in practice.

- We developed the conditions necessary for the implementation of fresh allografts within 24 hours, from grafts harvested from both living donors, as well as from cadavers. These "ultra-fresh" grafts allowed for the better survival rate of chondrocytes, which has considerable significancein the long-term results of the transplantations.

- We created an autonomic graft harvesting team, which was notified through the Organ Coordination Office (when alarmed that an organ donation was to be performed), and this team performed the independent, professional procurement and transportation of the necessary osteochondral allografts to the center where the transplantation was to take place. We also developed a criteria system of osteochondral allograft harvesting, which the graft harvesting team could abide by.

- The experience from the rehabilitation of the transplant patients have confirmed the initial approach that a relatively longer non-weight bearing time period– depending on the mass of the graft – is necessary to protect the bone portion of the graft. This however needs to be followed by an adapted partial weight bearing period in order to allow proper nutrition of the cartilage and angiogenesis of blood vessels into the transplanted bone.

Author's publications

Author's publications related to the topic of the dissertation:

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