Clinical experience with lyophilized structural allografts treated with human albumin, obtained in the course of revisions knee and hip replacement surgery

Doctoral thesis

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1. Introduction

1.1. The origins of bone transplantation

The first instance of bone transplantation was reported in 1682 by Meekren who used the skull bone of a dog to mend the skull fracture of a Russian soldier. The next creditable accounts date from 1810 and 1820 when Merrem and P. Von Walther respectively published reports of human autologous bone transplantations. In 1920 Axhausen and Albee published results of periosteum and bone marrow transplantations and of the successful clinical use of bones prepared by various methods.

Key discoveries with respect to biologically active substances are linked with the name of Marshall Uris. In 1965, following the intramuscular implantation of demineralised bone matrix (DBM), he observed the formation of a new bone. He proceeded to describe the bone morphogenetic protein (BMP) and launched molecular-level research. The development of the technology of lyophilisation is also linked with his name.

In the 1980s and 90s numerous growth factors in various bone cultures have been demonstrated and reported in a great many publications. Various growth factors have different biological activities, their common feature being the fact that they enhance osteoblast proliferation and increase the biosynthetic activity of the bone matrix. The production of certain growth factors by means of recombinant molecular techniques has led to the successful introduction to the therapeutic arsenal of some highly effective new bone-growth drugs with a new mode of action (BMP-2 (INFUSE) and BMP-7 (OP-1)). In the meantime, the use of various types of bone graft is growing; currently up to 2 million implantations are performed annually around the globe. Nowadays bone is the second most frequently transplanted human tissue after blood products. The development of the protocol for preparing allografts has increased their quality, and they are becoming more widespread than autografts and artificial bone replacements.

1.2. Bone replacement in clinical practice

Performed on a daily basis, the most widespread type of surgery in clinical practice is bone replacement and the use of biologically active substances. While the latter are showing increasingly convincing results, they are exceedingly costly and in the case of substantial

bone loss they will not suffice alone. There are numerous ways to replace extensive bone loss. It can be accomplished with various types of metal implants or autografts. Autografts have excellent properties, but limited availability, difficulties of sculpting and donor site morbidity have increasing led to the alternative use of allografts. Allografts are increasingly being used – particularly in the context of large-joint prosthetics and prosthesis revisions – as an alternative to the surgical treatment of fractures involving significant loss of bone, pseudarthrosis, bone cysts and tumours. BMPs have a role to play in the form of substances injected locally, as well as in the early treatment of osteonecrosis, in complementing the surgical treatment of pseudarthrosis; also, BMP might be injected in the course of vertebral fusion surgery.

Cellular-level therapy is developing at a rapid pace. Currently gene therapy is in an experimental phase. While the clinical use of recombinant proteins and other growth factors is in principle possible, it will require more experience. Implants of adequate osteoconductive grafts supplemented with efficient osteoinductive substances remains the only proven, good solution for the time being. In terms of cost and efficiency, they are the most widely used techniques.

The research into ways of replacing bone was considerably fuelled by the demand for bone replacement, and consequently we have a number of substances to choose from. The best solution would be the use of a so-called ideal graft that possessed three fundamental characteristics: it would consist of an osteoconductive base with osteoinductive properties, or the graft contained other osteoinductive factors, and the system would also contain osteogenic cells. The traditional method of bone replacement is the use of some material of human bone origin. These bone grafts can be grouped according to several criteria.

An autograft is a bone tissue graft transplanted from one part of the patient’s body to another. When the bone tissue graft is transplanted between different individuals of the same species, it is called an allograft, and when it is transplanted from an individual of one species into an organism of another species, it is called a xenograft.

Also of human (but non-bone) origin, growth-factor type bone replacement substances have also recently become widespread in clinical practice. Others include cell-based substances, which include stem-cell derivatives; and ceramic-based bone graft substitutes (Table 1).

Table 1

Classification of bone graft substitutes by Laurencin

Classification of bone graft substitutes
A. Harvested bone grafts and graft substitutes
I. Bone grafts
1. Autologous bone grafts
2. Homologous bone grafts
3. Bone marrow
II. Demineralized bone matrix (DBM)
B. Growth factor-based bone graft substitutes
I. BMP and other growth factors
II. Platelet-rich plasma
C. Cell-based bone graft substitutes
I. Stem cell
II. Collagen
III. Gene therapy
D. Ceramic-based bone graft substitutes
I. Calcium hydroxyapatite (HAp)
II. Tri-calcium phosphate (TCP)
III. Bioactive glass
IV. Calcium sulphate
V. Calcium phosphate cement (CPC)
E. Polymer-based bone graft substitutes
I. Natural and synthetic polymers
II. Degradable and non-degradable polymers
F. Miscellaneous
I. Coral

1.3. The general characteristics of bone allografts

The application of bone grafts is greatly limited by their frequently unreliable incorporation. The ideal bone graft possesses good mechanical strength, as well as osteoconductive, osteoinductive and osteogenic capabilities. To the best of our knowledge, only the autologous

human bone graft (autograft) possesses all of the mentioned properties therefore it is the benchmark bone graft material. The unique bone healing potential of autograft stems from its three well-defined features: 1) osteoconductivity, provided by the native bone; 2) osteoinductivity, given by inbuilt bone morphogenetic proteins and other growth factors; and 3) osteogenic capacity, provided by osteoblasts, osteoclasts and mesenchymal stem cells which are present in a freshly harvested autograft. However, the complications associating with the harvest of autograft, like chronic pain at the site of extraction and its availability limits its use in several clinical applications. Unfortunately, autograft donor site morbidity and persistent complications can be as high as 20–25%, even exceeding the complication rate of the grafting itself.

Allogenic bone graft (allograft) which derives from donors can be the alternative of autograft. Fresh, frozen and freeze-dried allografts are used in well-established protocols, as a result of which implantation can be performed without complications. The freeze-dried allograft is the most widespread in clinical use. These can be structural grafts, small spongious bones or ground bone material.

Sterilisation kills osteogenic cells and denatures most of the osteoinductive proteins on the surface of allograft. Therefore, the allograft is characterised by good osteoconductive but low osteoinductive and osteogenic capacity. Numerous publications have reported good long-term results with the use of structural allografts, with a 70% success rate over a span of 5 years and 60% in 10.

2. Objectives

We are aware of almost every characteristic of previously used structural allografts. Research into how their modest remodelling capacity could be enhanced goes back many decades. Our objective is to achieve the clinical application of physiologically better-quality allografts by means of a specific procedure that enhances remodelling: surface treatment with human albumin. Why, of several options, did we elect to use human albumin? Previous in vitro examinations have confirmed that mesenchymal stem cells (MSCs) can only be propagated in vitro in a serum albumin solution; accordingly, MSCs adhere to, and proliferate on, lyophilised spongious bone treated with human albumin.

Whether or not previous promising in vitro and in vivo results will be confirmed by human implantations, is yet to be seen. Will bone remodelling occur in the deeper layers of lyophilised allografts treated with human albumin, and will the relatively modest previous

bone-remodelling rate of 10% improve? If that can be accomplished, how will it affect long-term results? Could the structural allograft become the number-one choice in replacing extensive bone loss in certain cases?

3. Methods

3.1 Sculpting the allograft

One of the most frequent areas of use of allografts is the prosthesis-allograft composite (PAC), which involves the implantation of so-called structural grafts to replace extensive bone loss caused by prosthesis loosening. Of many preservation methods we opted for freeze-drying, that is, lyophilisation. The Regional Tissue Bank of West Hungary at the Aladár Petz County Teaching Hospital produces the freeze-dried human allografts. The procedure involves the modification of the original preserved tissue in a way that at the end of the preservation process the surface of the bone is treated with 10% human albumin and lyophilised again under aseptic conditions. The surface of the bone is coated with an even layer of albumin, which does not have any adverse effects. In our procedure we do not decorticate the grafts treated with albumin, and perforation is performed prior to lyophilisation and surface treatment, so that the albumin solution can reach deeper layers. Replacement of the protein structure can improve the cell adhesion properties of the allograft. Several proteins have been used in the cell culture for increasing adhesion, among other ones, bone structure proteins such as fibronectin and collagen. It is not only necessary to induce adhesion, but also cell proliferation, to enable the stem cells to better cover the bone surface area and migrate to the deeper layers of the allograft. The main component of the culture medium is the serum which is required for proliferation; this way the albumin behaves as an adhesion-enhancing protein on the surface of the graft.

We perform PAC implantation in revisions knee and hip replacement surgery. In every case revision surgery was necessitated by sterile loosening. That is an important criterion because following up the remodelling process is extremely difficult for the want of accurately assessable, objective examination methods. In the case of prosthesis loosening of different etiologies (e.g. sceptic environment, tumour), the basic condition, as well as the state of the surrounding soft-tissue, etc. can substantially affect the fate of the allograft, which makes assessment more difficult. Consequently, we excluded these cases from the current

tests. In every case revision surgery involved thorough preoperative examinations as well as bacteriological testing and rapid intraoperative tests.

3.2 The technical details of PAC implantation surgery

3.2.1 Revision hip replacement surgery

Periprosthetic osteolysis is today considered to cause the greatest long-term complications, with a prevalence exceeding the total occurrence rate of all other prosthesis-related complications combined. Several publications consider the prevalence of osteolysis to be 30–60% in prostheses older than 10 years. The data in the Swedish Hip Arthroplasty Register reveal that 75% of revision surgery is indicated by osteolysis-related causes. Several authors have highlighted the connection between bone loss and the frequency of periprosthetic fracture. For decades now, structural allografts developed for the replacement of the proximal femur have been used in revision hip replacement surgery. Indications for implantation are based on bone loss that involves circular, segmental Paprosky type III and IV osteolysis and Vancouver B3 type fracture affecting the entire proximal femur, that is, Gruen zones 2, 3, 6 and 7. After the implant and the coat of cement have been removed, the extent of actual bone loss can be established. It is generally accepted that in the case of bone loss exceeding 5 cm, one surgical option is bone replacement by allograft. We proceed accordingly, and after removing the prosthesis shaft, we do not remove the extensively damaged and thinned cortical bone matter, but keep the muscle adhesions and fold them aside. The sculpting of the femur is accomplished in the usual way. Both the allograft and the autograft are dilated to the desired size by means of drills and rasps. We determine the length of the revision prosthesis shaft and use a test implant for repositioning, without the graft for the time being. When adequate stability has been achieved, we determine the exact length of the graft to be implanted, as well as the rotational position of the joint of the graft and the bone. This is particularly important when planning to perform an osteotomy. On a separate sterile operating table we assemble the allograft-prosthesis system. Where the quality of the receiving bone structure is inadequate, or where the graft is small, we believe it is important to perform Z osteotomy at the joint of the graft and the bone, to achieve rotational stability and a larger touching surface. Next, we place the adequately sculpted prosthesis allograft composite into the receiving bone. We use cementless implants which, where necessary and possible, distal cross-locking screws. We use cerclage wiring at the joint of the graft and the bone to improve stability. We fix the bone matter previously folded aside also by means of cerclage.

The isolated destruction of the acetabulum can be caused, apart from the loss of femoral bone, by the loosening of the hip prosthesis, and the Paprosky classification is used establish the degree of this. Acetabulum structural graft implantation is performed in the case of 2A and 3B type loosenings. The structural graft comprises the entire acetabulum, the ileum (proximally), the pubis (distally) and part of the ischium. This is important because bone loss often goes beyond the acetabulum and its extent can be such that replacement of the acetabulum alone would be insufficient. After the removal of the implant and (where applicable) the cement, the extent of bone loss must be assessed with extreme precision to ensure the greatest possible adhesion of the graft in the area of defect. That can only be achieved with a structural graft that is adequate in size both proximally and distally, and after sculpting, it touches the remaining original bone matter in as many places as possible. Prior to constructing and implanting the PAC the defect is filled in with ground bone material. The acetabular allograft is prepared in the traditional way with manual milling instruments and insert it with the press-fit technique. Subsequently, we assemble the allograft revision acetabular allograft composite. Preoperative planning is particularly important with respect to the choice of the implant. Although the graft is secured in the recipient bone, full stability requires an adequate acetabulum. Consequently, only select implants can be considered; ones that bridge the graft and can be attached to the original bones in several points both proximally and distally.

3.2.2 Revision knee replacement surgery

The extent of the loosening of the knee prosthesis is performed according to the Engh classification. All of our cases of revisions knee surgery involved bone replacement due to tibial bone loss. These typically included T2B and T3 type loosenings. Exposure and the removal of the implant were performed according to the known principles. Extensive tibial bone loss caused damage to the ligaments in almost every case, which was why we used Legacy Constrained Condylar Knee (LCCK) or hinge type prostheses in the course of PAC implantation surgery. Naturally this involved the removal of the femoral component, whether or not it had come loose. Performing this circumspectly can prevent any damage to the femur. After removal of the implants, we assess the extent of bone loss. In every case we sought to preserve the tibial tuberosity, partly because of the extensor apparatus and partly because of the definition of the joint plain. Unfortunately, chances of reconstructing the soft tissues are slender in this procedure, but effort must nevertheless be made to do so. The sculpting of the graft and the recipient bone are performed together, in keeping with revision protocol. After

the test femur has been inserted, the exact size of the allograft can be determined. Due to the size of the allograft surface, the dimensions of the tibia are restricted, which can be problem because certain femur sizes only work with the right tibial component. On account of difficulties caused by size difference, we prepare allografts of different sizes, which enable us to choose the best one. The proximal tibia graft is always produced in a way that the cartilage surface is removed in the tissue bank. Naturally this does not conform to resection standards, so we perform it during surgery. In the course of implantation we only cement the tibial tray to the graft and no other fixing is applied.

There is considerable difference between bone loss that occasions surface replacement and bone loss that occasions total knee replacement surgery. In unicondylar knee arthroplasty replacement of the medial (or very rarely the lateral) compartment usually suffices. However, depending on the extent of bone loss, it can happen that different forms are sculpted from a single block, and allografts of different sizes are used in the medial and lateral compartments. In total knee prosthesis loosening a single block is usually used for replacement. The sculpting of the implanted grafts will yield countless variations.

4. Results

We have implanted lyophilised structural allografts treated with human albumin in 23 cases of revisions knee and hip replacement surgery since 1 January 2011. Sixteen prosthesis-allograft composites were implanted over a period of just over a year. With the exception of just one, we were able to perform postoperative follow-up examinations. The operations had involved 6 proximal tibia, 4 proximal femur, 5 acetabulum and 1 proximal femur + acetabulum replacements.

Only in one case involving lyophilised structural allograft-prosthesis composites treated with human albumin did we discover prolonged seroma drainage (in connection with a femoral allograft) which required medicinal treatment. In one case, septic complication occurred after the first year of tibial allograft implantation surgery, due to which the PAC was removed. Following removal of the components, arthrodesis was performed as a final solution. Naturally this case could not go to the 12^{th} month postoperative examination, meaning that our assessment is based on 15 cases. Treatment with albumin did not alter the mechanical properties of the allografts. The allografts were not absorbed and no fractions or pathological changes affecting the bone structure occurred. No lytic processes developed around the implants in either the cemented or the cementless grafts. We found no tissue

reaction related to the albumin after implantation. Postoperative follow-up is an on-going process, and we examine every patient according to the same protocol. Due to the known rules of ethics our chances for assessment are highly limited. An X-ray test is performed in the direct postoperative period, 6 and 12 weeks after surgery, and 6 12 months after. Isotope and SPECT/CT tests are performed in the 12th month of the postoperative period. We did experiment with a bone densitometry test, but since the test serves to determine the bone mineral density, it yielded little information of worth with respect to the allograft. Obviously, a histological test would provide the most information about cellular activity within the graft. However, the rules of ethic state that the test cannot be performed except during surgery performed to treat a complication.

4.1.1 Radiological follow-up

Acetabulum replacements yield the best results for the simple reason that the size of the allograft implant is ideal, the soft tissue cover is considerable, the recipient medium has an ample blood supply and the graft is set in a layer of ground bone material. The coexistence of these conditions led to excellent results with every implant. The 12-month X-ray revealed perfectly remodelled grafts.

The radiological results of the first-year control of femoral replacements have been less successful. The graft size was the largest in these cases, since often the bone loss had been equivalent to the primer prosthesis shaft, or even in excess of that. On the basis of the X-ray tests it can be established that graft size and bone remodelling are inversely proportional. The incorporation of smaller-sized grafts is considerably better. The environment (blood supply and soft tissues) is equally good, and contact with surviving bone parts of no mechanical value but providing good muscle adhesion also contributed to the incorporation of the graft. In the case of large-sized allografts intensive remodelling was usually evident mostly at the joint of the graft and the bone; callus-like structures were often visible in these areas, but osteoblast activity was evident on the surface of the graft too.

Tibia replacement proved to be the most difficult area. The graft is often a large-sized block, while in terms of blood supply and soft tissue covering the environment is very scanty. Accordingly, the X-ray images of large-sized grafts did not reveal considerable change compared with their postoperative state. Only at the joint of the graft and the bone could radiological signs be picked up that were relevant to the assessment of bone remodelling.

4.1.2 Isotope and SPECT/CT tests

One ubiquitous method of examining pathological bone processes is three-phase bone scintigraphy, which relies on the Tc-99m phosphonates' adherence to hydroxyapatite crystals in the bone. Adherence is influenced by the bone's blood supply and metabolic activity. In certain bone processes the metabolic activity of the osteoblasts increases, which in turn leads to increased radiopharmaceutical activity. The method is readily available, it is not invasive, it involves lower radiation exposure, metal structures do not impede the test, it can reveal pathological processes earlier than X-rays; however, it is less specific. Specificity can be enhanced with an additional SPECT/CT scan. The method is highly sensitive and is based on the fact that the SPECT scan is equivalent to a 3D isotope test, the CT to a 3D X-ray; and the two combined produce SPECT/CT images. The results can be processed in many ways. Qualitative and semi-quantitative assessments are performed. The counts per second (CPS) of the regions of interest (ROI) on the allograft can be compared to the CPS in the corresponding ROI on the contralateral side. Another possibility is comparison with the early SPECT scan results of grafts not treated with albumin. Previously in Hungary, in the course of processing graft implants, very few assessments of – exclusively femoral – bone replacements were performed with this method. On the basis of available test results it can be established that in the case of untreated grafts, activity is only evident at the joint of the graft and the bone. Consequently, we assessed the scans of the ROI by means of semi-quantitative calculations rather then with the comparative method. It is a know fact that increased activity in the third phase is typical of both prosthesis loosening and osteoblast activity. Owing to its high sensitivity, the method allows for the differentiated diagnostics of the two processes. The CPS of the intact ROI is regarded as the unit. There is a slight increase in osteoblast activity; typically, however, the reference value does not exceed double the CPS. A larger CPS would indicate loosening. Generally it can be said that in the examined cases, no signs indicating loosening or infection were evident in the environment of the human serum albumin-coated allografts. In the third phase, a moderate intensification of activity of varying scope was evident in the lateral parts of the allograft, occasionally at deeper levels, as well as at the joint of the bone and the graft. Accordingly, the native, low-dose CT revealed sclerotisation.

Results by ROI:

1. Acetabulum graft: 5 patients were scanned. Relative CPS-s:

Patient	Patient 1.	Patient 2.	Patient 3.	Patient 4.	Patient 5.	**Average:**
CPS	1.44	1.96	1.70	1.13	1.60	**1.56**

SPECT/CT images reveal good activity in the allograft ROI.

2. Femur graft: 4 patients were scanned. Relative CPS

Patient	Patient 1.	Patient 2.	Patient 3.	Patient 4.	**Average**
CPS	1.45	1.78	1.51	1.14	**1.47**

In the case of large-sized grafts, the SPECT/CT images reveal intensification in activity at the join of the graft and the bone and on the surface; in the case of smaller grafts, on the surface and occasionally at deeper levels.

3. Tibia graft: 5 patients were scanned, Relative CPS

Patient	Patient 1.	Patient 2.	Patient 3.	Patient 4.	Patient 5.	**Average**
CPS	1.00	2.00	1.70	1.38	1.27	**1.34**

The SPECT/CT images reveal intensification in activity only laterally at the join of the graft and the bone. Only once did we find isotope accumulation in the allograft.

4. Acetabulum+femur graft: 1 patient was scanned. Relative CPS: **1.7**

SPECT/CT scans show different intensity in activity in the ROI of the two grafts. The result is comparable to results gained from the assessment of the same ROI of allografts where isotope accumulation was more intensive in the acetabulum area, and to a lesser extent in the area of the femur.

4.1.3 Histology after reoperation

Reoperation was necessary in 4 cases. Performed with haematoxylin eosin and trichrome stains, histological tests were possible in 3 of the 4 cases. In one case the PAC was removed after a sceptic complication following the implantation of a tibial allograft, and histology was excluded due to the above-mentioned criteria. In two cases reoperation was occasioned by trauma-related complications, and in the fourth case reoperation was indicated by chronic pain and difficulty of movement due to an incorrect choice of implant.

Fourteen months following revision hip replacement surgery and the implantation of an acetabulum graft, an otherwise complaint-free patient fell on some stairs, dislocating the hip prosthesis. Attempts at a closed repositioning being unsuccessful, the prosthesis was eventually surgically repositioned, and histological sample gained from the environment of the acetabulum. Vigorous osteoblast activity was evident in the area of the graft, as well as on the lateral sections of the necrotic areas.

Twenty-eight months after the revision knee surgery and the implantation of the tibial PAC, this patient fell again on the stairs and suffered a leg and implant fracture. Revision surgery confirmed that the allograft had not been damaged and the bone-graft remodelling was so successful that the fracture had occurred below this region in the upper third of the diaphysis and in the patient's own bone. This was particularly important to us in that scan images had suggested that this region possessed the least favourable remodelling capabilities. The condition of the graft was as hard as the original bone; bone-graft remodelling was outstandingly firm; and the surface, which at the time of implantation was completely smooth, had become uneven due to surface osteoblast activity. The histological test of the specimen gained form the graft yielded more modest results, but it nevertheless confirmed osteoblast activity between the necrotic tissues, in particular on the surface, and to a lesser extent at deeper levels.

Sixteen months after revision knee prosthesis surgery and PAC implantation, the patient had complaints due to spatial micro-movement of the tibia shaft caused by an incorrect choice of implant, indicating reoperation. Performed in the usual way with an oscillating saw and blade chisel, the removal of the tibial component did not cause further bone loss in the allograft. Like before, we found an outstanding-quality structure and the remodelling of the bone and graft was completely sound. The surface, too, was characterised by osteoblast activity, and we found a bleeding spongious surface. We were able to implant a new prosthesis whilst completely preserving the allograft. This raises the possibility of

applying albumin-coated grafts in two-part operations. Following replacement of the components, the complaints almost entirely subsided.

The histology was similar to the previous case, with that necrotic areas, cell-free bone trabeculae and moderate osteoblast activity in the allograft.

4.2 A summary of clinical data

In our assessment we only examined implants older than 12 months. The first operation in this group was a 32-month revision knee replacement with tibial PAC implantation. The last was a 14-month revision hip surgery with femoral PAC implantation. Generally speaking, preoperative pain and the ability to walk improved in every case, leading to a significant improvement in life quality. Considerable improvement occurred in terms of the use of mobility aids too. Previously unable to walk, the patient is currently ambulant with the help of two crutches; 4 patients are able to walk without any aid. Pain had reduced in all cases, and 6 patients consider their condition to be painless.

The assessment of the operations was group according to body regions.

In the revision hip surgeries femoral allografts were implanted in 4 cases. The structural graft sizes varied greatly in size, from 7–16-cm. This corresponds to the literature, which does not recommend femoral bone replacement shorter than 5 cm; however, the top of the range is 25 cm. In the Hungarian literature Csernátony et al. report 16 cases where femoral grafts of 11–25 cm were used.

The acetabulum was replaced with structural allografts in 5 cases. Bone replacement in this region is based on the extent of damage to the acetabular ring and the size of the remaining, intact bones. The acetabular allograft is developed in a way that it can be implanted by means of the press-fit technique. The size of the acetabular allograft is crucial. Donor bones were 6–8 cm, into which we inserted size 46–50 acetabula.

We assessed revision hip surgeries in the preoperative stage and in the 12th month according to the Harris Hip Score. This was performed in 10 patients (5 acetabulum, 4 femur, 1 femur + acetabulum replacements). Patient scores differed according to the region.

Acetabular allografts

	Preoperative H. H. S.	**1-year postoperative H. H. S.**	**Preoperative support**	**1-year postoperative support**	**Graft size + size of acetabulum**
Patient 1	25.9	70.9	Walking frame	One crutch	6 cm + 46 acet.

Patient 2	26.1	70.1	Two crutches	_____	8 cm + 50 acet.
Patient 3	26.8	72.2	Two crutches	_____	7 cm + 48 acet.
Patient 4	27.1	71.9	Two crutches	One walking stick	8 cm + 50 acet.
Patient 5	27.4	70.8	Two crutches	One crutch	6 cm + 46 acet.
Average	26.66	71.18			**7 cm + 48** acet.

Femoral allografts

	Preoperative H. H. S.	**1-year postoperative H. H. S.**	**Preoperative support**	**1-year postoperative support**	**Graft size**
Patient 1	23.9	60.3	Walking frame	One cane	7 cm
Patient 2	24.8	62.2	Walking frame	One walking stick	9 cm
Patient 3	27.2	60.4	Two crutches	One crutch	11 cm
Patient 4	26.3	61.9	Walking frame	One crutch	16 cm
Average	25.55	61.2			**10.75 cm**

Acetabular+femoral allografts

	Preoperative O. K. S.	**1-year postoperative H. H. S.**	**Preoperative support**	**1-year postoperative support**	**Graft size**
Patient 1	20.9	51.3	Walking frame	Two crutches	Femur 9 cm acetabulum 6 cm Acet. 48

In revision knee surgeries, bone replacement due to the loss of the proximal tibia was performed in 6 cases. We performed replacements of tibial bone loss caused by total endoprosthesis loosening in 4 cases, with allograft blocks of 4–6 cm. Due to late sceptic complications, 3 patients were involved in revision surgery. There were 2 cases of bone loss caused by medial surface replacement prosthesis loosening. In one of the cases, medial replacement was performed with a 3-cm graft. In the other case an asymmetrical block (medially 3 cm, laterally 2 cm) was used as a replacement. Patients were evaluated on the

basis of the Oxford Knee Score. Due to a sceptic complication, the PAC was removed in one patient, so assessment was performed in 5 patients before and 12 months after surgery.

Tibial allografts

	Preoperative O. K. S.	**1-year postoperative O. K. S.**	**Preoperative support**	**1-year postoperative support**	**Graft size**
Patient 1	3	20	Not able to walk	Two crutches	6 cm
Patient 2	6	24	Walking frame	One walking stick	4 cm
Patient 3	7	26	Two crutches	One walking stick	5 cm
Patient 4	8	29	Two crutches	_____	medial 3 cm
Patient 5	9	30	Two crutches	_____	3+2 cm
Average	6.6	25.8			**4.6 cm**

5. Conclusions

Treatment with albumin, as well double lyophilisation, did not adversely affect the mechanical properties of the allograft. Resorption, rejection, graft fracture did not occur. Surface treatment did not induce reactive processes in the surrounding soft tissues. The sceptic complication that occurred in one case was not demonstrably related to the allograft; more likely it came from some other centre of infection. Assessment of the results of isotope and SPECT/CT scans revealed osteoblast activity at the joint of the bone and the graft, and often at deeper levels. This was confirmed by the histological tests. Typically, but not surprisingly, remodelling capability was found to be considerably different depending on the region. Earlier observations were confirmed in that “sheathing” remodelling activity of varying extent occurs at the joint of the bone and the graft and on the surface of the allograft. The difference observed was that osteoblast activity was evident at deeper levels after surface treatment, the extent of which varied, depending on the region. The region of the acetabulum produced the most intensive activity; activity in the femoral grafts was lesser; and least in the tibial grafts. The clinical use of surface treatment – as one method of enhancing bone remodelling – confirmed previous results of in vitro and in vivo research in that it is suitable for the surgical replacement of bone loss. Small and medium-sized structural grafts – 5–10

cm and 3–4 cm in length for femoral and tibial grafts, respectively – possess more reliable remodelling capabilities, which is evident on the surface and in the graft matrix. A year after implantation, expansive osteoblast activity is evident in the majority of grafts, indicating that even in this patient population with a poor prognosis, the extent of the remodelling process is adequate. Histological tests, too, confirmed that in grafts treated with albumin, remodelling yielded bone tissue, and there was no fibrotic cell proliferation indicative of sequestration. Remodelling was less successful in grafts exceeding 10 cm (femur) and 4 cm (tibia). In these cases a moderate increase in the area affected by osteoblast activity was observed at the joint of the bone and the graft, as well as on the surface.

6. Publications

6.1 Publications related to the thesis

1. Denes B. Horvathy, Gabriella Vacz, Tamas Szabo, Ildiko Toro, Boglarka Vamos, Istvan Hornyak, Karoly Renner, Imola Cs. Szigyarto, Tamas Klara, Bence T.Szabo, Csaba Dobo-Nagy, Attila Doros, Zsombor Lacza,: Serum albumin coating of demineralized bone matrix results in stronger new bone formation. *Journal of Biomedical Materials Research* Part B-Applied Biomaterials, 2015; Article first published online: 10 Feb 2015. doi:10.1002/jbm.b.33359
2. Klára T., Csönge L., Janositz G., Csernátony Z., Lacza Zs.: “Albumin-coated structural lyophilized bone allografts: a clinical report of 10 cases” in *Cell and Tissue Banking*, March 2014, Vol. 15: pp. 89–97.
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6.2. Publications unrelated to the thesis

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Patents:

Hangody L., Klára T., Blaskovits F., Kotormán I.: “CLC” implant and instrumentation for spinal interbody fusion, Hungarian Patent: A61B 17/58, A61B 17/76

Hangody L., Klára T., Blaskovits F., Kotormán I.: “CLC” implant and instrumentation for spinal interbody fusion, German Patent Pending

Hangody L., Klára T., Blaskovits F., Kotormán I.: “Arthrofix” implant and instrumentation for minimal invasive arthrodesis technique on the ankle, Hungarian Patent Pending

Hangody L., Klára T., Blaskovits F., Kotormán I.: “Arthrofix” implant and instrumentation for minimal invasive arthrodesis technique on the ankle, German Patent Pending