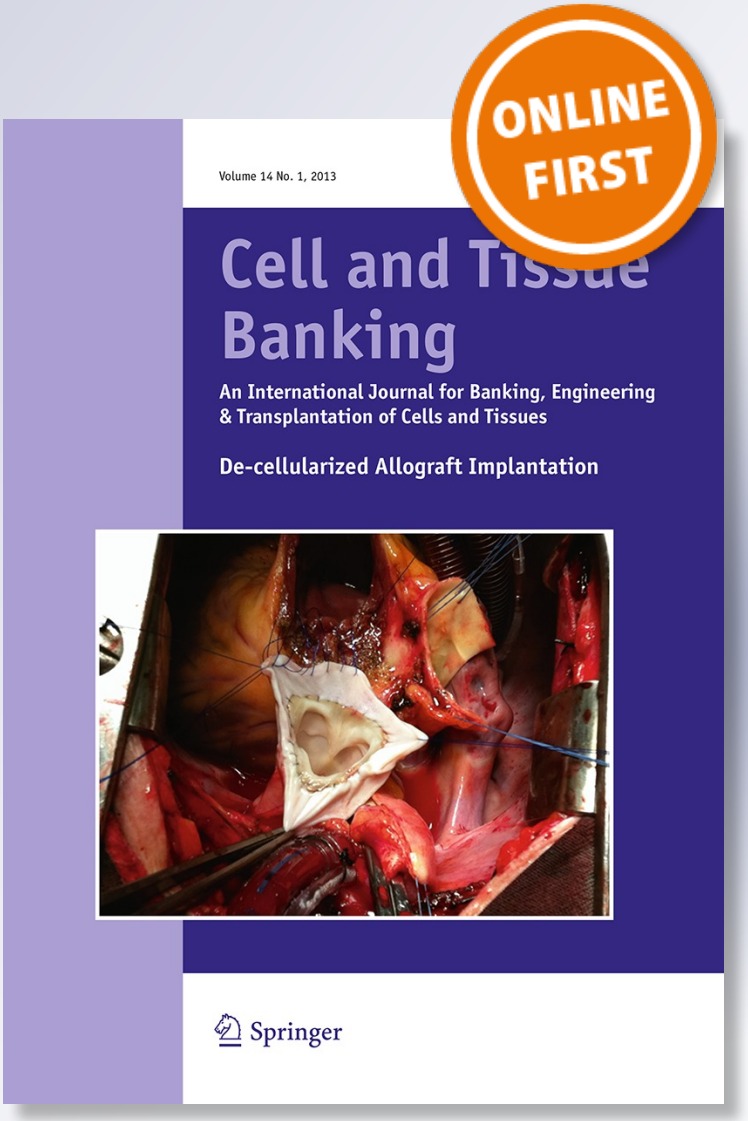
Albumin-coated structural lyophilized bone allografts: a clinical report of 10 cases

Tamás Klára, Lajos Csönge, Gábor Janositz, Zoltán Csernátony & Zsombor Lacza

Cell and Tissue Banking
International Journal for Banking, Engineering and Transplantation of Cells and Tissues Incorporating Advances in Tissue Banking

ISSN 1389-9333

Cell Tissue Bank
DOI 10.1007/s10561-013-9379-8



Springer

Cell Tissue Bank
DOI 10.1007/s10561-013-9379-8

ORIGINAL PAPER

Albumin-coated structural lyophilized bone allografts: a clinical report of 10 cases

Tamás Klára · Lajos Csönge · Gábor Janositz · Zoltán Csernátony · Zsombor Lacza

Received: 3 February 2013 / Accepted: 8 May 2013

Abstract Bone replacement and the use of bone supplementary biological substances have become widespread in clinical practice. Although autografts have excellent properties, their limited availability, difficulties with shaping and donor site morbidity have made allografts a viable and increasingly preferred alternative. The main drawback of allografts is that the preparation destroys osteogenic cells and results in denaturation of osteoinductive proteins. Serum albumin is a well-known constituent of stem cell culture media and we found that lyophilizing albumin onto bone allografts markedly improves stem-cell attachment and bone healing in animal models thus replacing some of the osteoinductive potential. As a first step in the clinical introduction of albumin coated grafts, we aimed to test surgical handling and early incorporation in aseptic revision arthroplasty in humans. We selected patients who needed large structural allografts and the current operation was the last attempt at preserving a moving joint. In a series of 10 cases of hip and knee revision surgery we did not experience any drawbacks of the albumin-coated grafts during handling and implantation. Twelve months radiographic and SPECT-CT follow-up showed that the graft was well received by the host and active remodelling was observed. The lack of graft-related complications and the good 1-year results indicate that controlled trials may be initiated in more common bone grafting indications where long-term effectiveness can be evaluated.

Keywords Albumin coated allograft · Freeze-dried allograft · Allograft-prosthesis composite · Total prosthesis revision

T. Klára (✉)
Trauma Department, Erzsébet Teaching Hospital, Győri út 15, Sopron 9400, Hungary
e-mail: klartam@t-online.hu

L. Csönge
Aladár Petz County Teaching Hospital, Western Hungarian Regional Tissue Bank Győr, Vasvári Pál street 2-4, Győr 9023, Hungary
e-mail: luisbathhelena@gmail.com

G. Janositz
Orthopedic Department, County Hospital Kecskemét, Nyíri street 38, Kecskemét 6000, Hungary
e-mail: gabor.janisitz@gmail.com

Z. Csernátony
Department of Orthopedic Surgery, University of Debrecen, Medical and Health Science Center, Nagyerdei street 98, Debrecen 4032, Hungary
e-mail: csz@dote.hu

Z. Lacza
Department of Orthopedics, Semmelweis University, Karolina út 27, Budapest 1113, Hungary
e-mail: zlacza@mac.com

Z. Lacza
Department of Human Physiology and Clinical Experimental Research, Semmelweis University, Tűzoltó u. 37-47, Budapest 1094, Hungary

Published online: 16 May 2013

Introduction

There are two main trends in regenerative medicine, which apply different planning criteria to development. The first involves in vitro tissue production in the course of which live tissues—even organs—are created in laboratories and subsequently grafted (Atala 2012). The second is a less complicated approach involving the grafting of a cell-free matrix that attracts host stem cells, which in turn colonize the graft and build the tissue in situ (Rust et al. 2007). While the former approach will eventually produce better results, currently the latter simplified, cell-free grafting is more widespread and this is the method that may become a routine procedure in the near future. Implanting allografts in order to provide a scaffold for new tissue formation is one such tissue-engineering approach.

Preserved bones are commonly used in prosthesis allograft composites. Used for many decades, the method involves structural allografts developed for the replacement of the proximal femur in the course of revision hip replacement surgery (Lee et al. 2011; Blackley et al. 2001; Head et al. 1999) or the proximal tibia in knee arthroplasty (Richards et al. 2011). These are typically complex clinical cases, where the patient have already undergone several operations and the revision is the last attempt for creating a moving, weight-bearing joint. In spite of the high rate of complication largely due to the reduced remodelling of the grafts, the clinical results are better with grafts than without (Richards et al. 2011; Enneking and Mindell 1991). Thus, there is an increasing demand from clinicians towards tissue banks for bone grafts with better biological properties.

Several previous protocols were introduced in order to improve the remodelling capacity of allografts. Enneking observed in allografts retrieved from patients that markedly better allograft remodelling can be achieved when the graft is mixed with autologous materials such as bone or marrow (Enneking and Mindell 1991; Enneking and Campanacci 2001). The use of autologous growth factors separated from blood in the form of platelet rich plasma (PRP) is routinely used in dentistry, however, without solid proof of efficacy (Intini 2009). Restoring bone structure proteins such as collagen or fibronectin has been shown to moderately increase the stem cell adherent properties of the scaffold but human albumin coating not only increased seeding efficiency, but also cell proliferation in vitro (Weszl et al. 2012). Since serum albumin in high concentrations is a standard constituent of mesenchymal stem cell media, one may assume that local delivery of serum in the form of coating the allograft surface may have beneficial effects as well (Ryser et al. 2009). In an animal model of bone loss, albumin coating improved the ingrowth of the host bone into the graft, even without adding any exogenous stem cells (Weszl et al. 2012).

In the current study we hypothesized that human albumin coated bone allografts can be used for structural bone replacement in revision arthoplasty procedures.

Materials and methods

Patients

Under the approval of the ethical committee of the Győr-Moson-Sopron County Government Offices (registration number: 2287-6/2010) patients were selected to participate in the study. The selection criteria were: (1), aseptic loosening of hip or knee total endoprosthesis which were scheduled for revision surgery, (2), critical bone loss in the peri-articular area, (3), no signs of bacterial infection of the implant. Due to the heterogeneous nature of this patient cohort no standardization was possible. The clinical parameters of each patient is summarized in Table 1.

Examinations

Every patient was examined and followed-up according to the same protocol. The first test is an X-ray performed at the 6th, 12th, 24th and 48th weeks following the operation. We additionally performed single-photon emission computed tomography (SPECT-CT) scans at the 10–12th month in order to observe signs of osteoblast activity in the periprosthetic region. In each patient 700 MBq Technetium-99m-coupled hydroxymethylene diphosphonate was administered iv. and 2 SPECT images were taken at the early and 3 h late perfusion phases, together with a low-dose CT image 130 kV and 19 m As with a Symbia T2 (Siemens AG, Münich, Germany) device.

Table 1 Clinical data of the patients involved in the study

Patient initials	Sex	BMI	Age (years)	First implantation	Number of revisions	Region	Co-morbidity	Graft size
1	Male	27	43	2002	4	Proximal femur	Hip dysplasia smoking	12 cm
2	Female	34	55	2001	4	Proximal femur	Hip dysplasia hypertension hypothyroidism	10 cm
3	Female	27	80	1990	2	Acetabulum	Hypertension NIDDM coronary artery disease	6 cm
4	Female	30	58	1995	4	Acetabulum	Hip dysplasia hypertension hyperlipidemia	8 cm
5	Female	23	69	1998	1	Acetabulum	Hypertension	5 cm
6	Female	30	75	1999	2	Proximal tibia	Hypertension stroke atrial fibrillation	5 cm
7	Female	29	76	2000	4	Proximal tibia	Hypertension coronary artery disease	4 cm
8	Female	43	50	2009	1	Proximal tibia	None	"Z" form graft, 4 cm medial, 2 cm lateral
9	Female	28	57	2010	1	Proximal tibia	Hypertension	3 cm
10	Female	32	57	2003	1	Proximal femur + acetabulum	Hypertension coronary artery disease	Femur 8 cm

Allografts

The bone allograft was processed according to Urist's method as an autolysed, antigen-extracted allogeneic bone (Urist 1965). Bone was harvested from cadavers under the operational license of the West-Hungarian Tissue Bank and processed immediately. The preservation method was freeze drying. After freeze-drying the bone graft was submerged in sterile 10 % albumin solution for 1 min under aseptic conditions (low-salt-content Biotest human albumin infusion, Biotest Pharma GmbH, Dreieich, Germany). After albumin treatment, a second freeze-drying was performed with the same parameters like the first one packaged in double sterile wrapping and stored at room temperature until use, which was always less then 3 months. Earlier studies have shown that freeze-dried human serum albumin can retain its biological characteristics under these storing conditions for at least 2 years (Hawe and Friess 2006). It is important to note that the coated graft is indistinguishable by the naked eye from an uncoated one.

Surgery

The indication for surgery was aseptic endoprosthesis loosening and critical extent of bone loss. Over the past year we performed revision hip and knee revisions, involving the implantation of human albumin-treated freeze-dried structural allografts, on 10 occasions. In 4 cases we replaced the proximal tibia, in 2 the proximal femur, in 1 case the proximal femur and the acetabulum, and in 3 cases the entire acetabulum. Each revision surgery was preceded by aseptic loosening confirmed by negative bacterial culture taken at the time of operation. The type and duration of postoperative fixation of the limb, as well as mobilization protocols were adjusted to the needs and capabilities of each patient, details are shown in Table 2.

Results

We have not observed any major complications with lyophilised structural allograft prosthesis composites treated with human albumin during the first year after implantation. In the early postoperative period in one case we experienced prolonged wound drainage that ceased spontaneously. Treatment with albumin did not alter the mechanical properties of the allografts to any extent, and surgeons were able to handle it just like the classical uncoated allografts. In the surrounding tissue medium around the grafts we did not experience foaming, stickiness, or discoloration or any other sign that may be attributable to the albumin coating.

During knee revision arthroplasty the bone replacement was called for due to bone loss affecting the tibia (Fig. 1). The tibia plateau grafts were cemented to the

Table 2 Results of joint replacement augmented with bone allografts after 12 months

Patient initials	Preoperative mobility	Postoperative mobility	Preoperative function	Postoperative function	Postoperative X-ray	Postoperative SPECT
1	100 m walking distance with 2 crutches	1,000 m walking with 1 crutch	Harris Hip Score: 26.3	Harris hip score: 55	Marked callus formation can be seen bridging the host and the graft bone.	High activity around the allograft, and the bone graft junction. The CT scans confirm excellent bone quality.
2	200 m walking distance with 1 crutch	1,000–1,500 m walking distance with 1 stick	Harris Hip Score: 27.8	Harris Hip Score: 56	Moderate callus formation can be seen bridging the host and the graft bone.	Moderate activity around the allograft, but high activity in the bone graft junction. The CT scans confirm good bone quality.
3	50 m walking distance with walking frame	500–100 m walking distance with 1 crutch	Harris Hip Score: 20.90	Harris Hip Score: 53	Excellent callus under the acetabular component	High activity in the graft host bone junction. Inside the graft isotope accumulation is not visible.
4	100 m walking distance with 2 crutches	1,000 m walking distance with 1 crutch	Harris Hip Score: 25.8	Harris Hip Score: 51	Large-scale callus under the acetabular component	High activity in the graft host bone junction. Inside the graft isotope accumulation is visible
5	10 m walking distance with 2 crutches	500–1,000 m walking distance with 1 crutch	Harris Hip Score: 22.8	Harris Hip Score: 52,7	Large-scale callus under the acetabular component	High activity in the graft host bone junction, and inside the graft as well
6	Unable to walk (wheelchair)	500–800 m walking distance with 2 crutches	Oxford Knee Score:6	Oxford Knee Score: 26	There are signs of calcified tissues filling the gap, With external callus	The host bone and the graft junction are very active, but inside the allograft isotope accumulation is not visible.
7	5–10 m walking distance with walking frame	800 m walking distance with 2 crutches	Oxford Knee Score: 8	Oxford Knee Score: 27	The gap had become filled and although it is still visible, no external callus	The host bone and the graft junction are very active, in the edge of allograft isotope accumulation is visible.
8	100 m walking distance with 2 crutches	1,500–2,000 m walking distance with 1 stick	Oxford Knee Score: 11	Oxford Knee Score: 32	The gap had become filled, and excellent external callus visiable	Extensive activity is observed in the lateral part of the allograft. The host bone and the graft junction are very active on the medial side, but inside the allograft isotope accumulation is not visible.
9	10 m walking distance with 2 crutches	800–1,000 m walking distance with 2 sticks	Oxford Knee Score: 12	Oxford Knee Score: 30	The gap had become filled, and moderate external callus visiable	The host bone and the graft junction are moderate active, in the edge of allograft isotope accumulation is visible.
10	50 m walking distance with 2 crutches	500–600 m walking distance with 2 crutches	Harris Hip Score: 18	Harris Hip Score: 35,30	Moderate callus in the acetabulum, radiolucent line under the femoral graft without callus	Moderate activity around the femoral allograft, in the bone graft junction. High activity in the acetabular graft host bone junction.

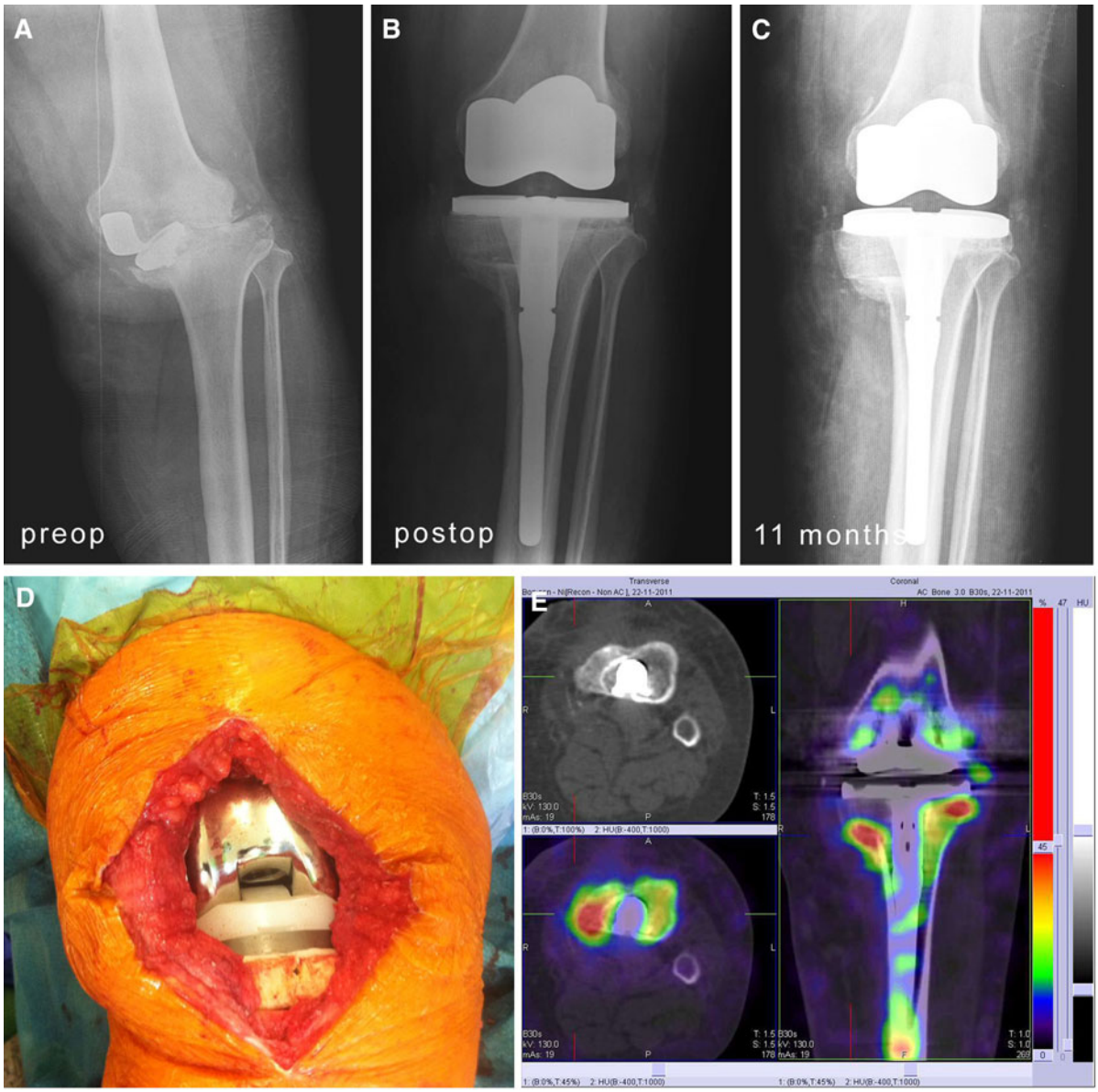


Fig. 1 Proximal tibia graft. **a** A preoperative X-ray of a Engh (AORI)type T2/b tibial component loosening. **b**, **c** Postoperative X-rays immediately after surgery or 12 months later. A "Z" form allograft was applied according to the extent of the bone loss and shaped to the contour of the revised tibial component. At 12 months the gap had become filled and although it is still visible, there are signs of calcified tissues filling the gap. **d** An intraoperative picture of the hinge-type prosthesis and allograft composite cemented into its place. **e** A SPECT-CT image of the revision knee prosthesis and tibia allograft 12 months after the surgery. Extensive activity is observed in the lateral part of the allograft. The host bone and the graft junction are very active on the medial side, but inside the allograft isotope accumulation is not visible

tray and implanted intoto without further fixation, however, this procedure adequately secured the graft as we did not observe any early loosening and joint instability did not develop during the first postoperative year. Calcification was observed in the radiolucent line between the graft and the host bone, and high osteoblast activity was observed even in the depth of the allograft indicating that remodelling was ongoing even several months after the implantation (Fig. 1.)

In revision hip replacement surgery due to Paprosky IV type of femoral bone loss approximately 10 cm-long grafts were transplanted, mainly

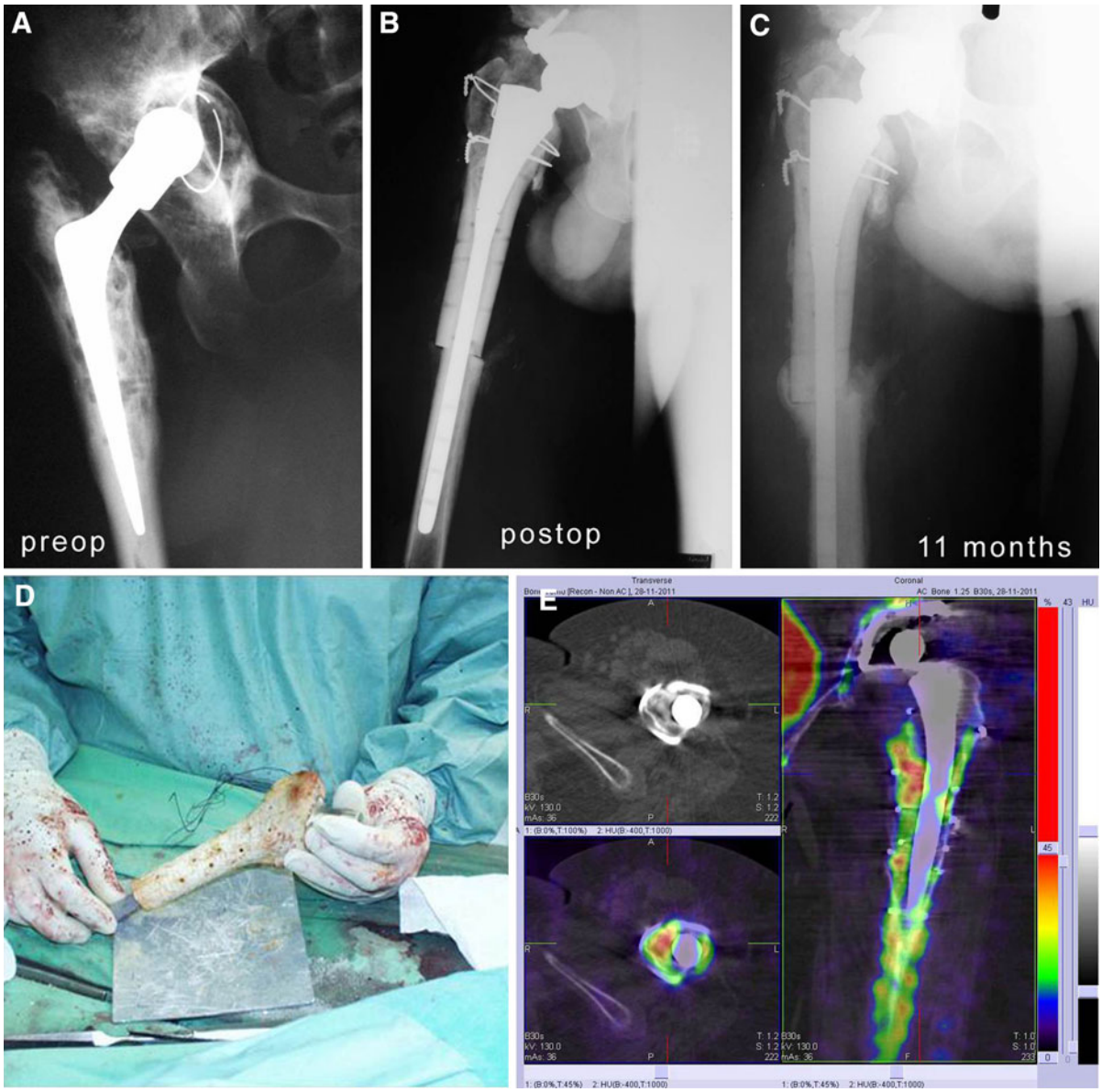


Fig. 2 Proximal femoral graft. **a** A preoperative X-ray of a Paprosky IV type femoral bone loss. **b** An immediate postoperative X-ray, a 10-cm-long allograft and cementless prosthesis composite is visible. At 12 months after surgery (**c**) marked callus formation can be seen bridging the host and the graft bone. **d** an intraoperative picture of the allograft-prosthesis composite on the sterile table. The allograft is prepared with a conventional rasp, before implanting the femoral component **e** shows the same SPECT-CT of the same case after 12 months. High activity can be found around the prosthesis stem in the allograft. The CT scans image confirmes excellent bone quality

consisting of cortical long-bone shafts (Fig. 2). Due to the limited volume of cancellous bone in this type of grafts, one would assume that the albumin effect is low, however, surprisingly strong callus formation and osteoblast activity was observed (Fig. 2.) In case of the acetabular grafts X-ray evaluation of the graft is unreliable, however, osteoblast activity was observed by SPECT-CT at the graft-host interface (Fig. 3).

In one case the patient fractured the limb due to a fall, which required operative fixation. During this second procedure it was possible to observe the graft-host interface months after implantation and histology

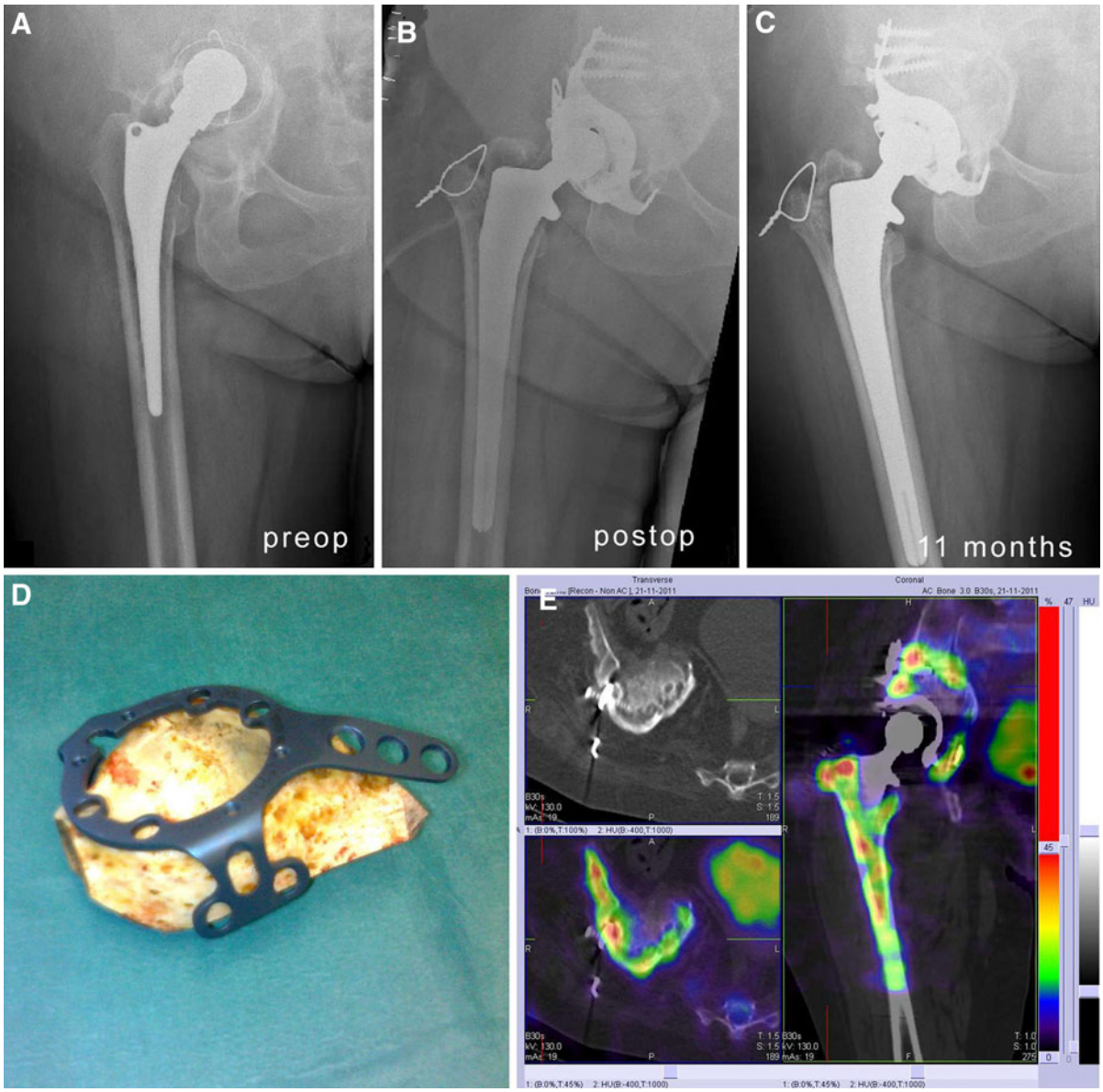


Fig. 3 Acetabular graft. **A**. a preoperative X-ray of a Paprosky type III/b osteolysis in the area of acetabulum. **b**. is An immediate postoperative X-ray of the same case. The allograft-prosthesis composite achieves a stabile position. **c.** An X-ray 12 months after surgery. The allograft in the bottom of the acetabulum is solid **d**. An intraoperative picture of the acetabular graft during preparation just before implantation. The graft is shaped to the desired size on a sterile table, and the acetabulum is prepared with a manual drill. **e**. The SPECT-CT images of the revision hip prosthesis and acetabulum allograft after 12 month of the surgery. High activity in the graft host bone junction. Inside the graft isotope accumulation is not visible

was performed from the discarded bone stock. Fortunately, the graft-host interface was clearly visible in some of the sections (Fig. 4). Extensive remodelling activity can be seen at the junction, osteoclasts are eating away the dead bone and newly formed bone is visible.

Discussion

Solid proof of efficacy in orthopaedic procedures can only be gained from years or even decades of widespread use, thus a stepwise introduction of innovations is promoted (Malchau et al. 2011). As the first clinical step

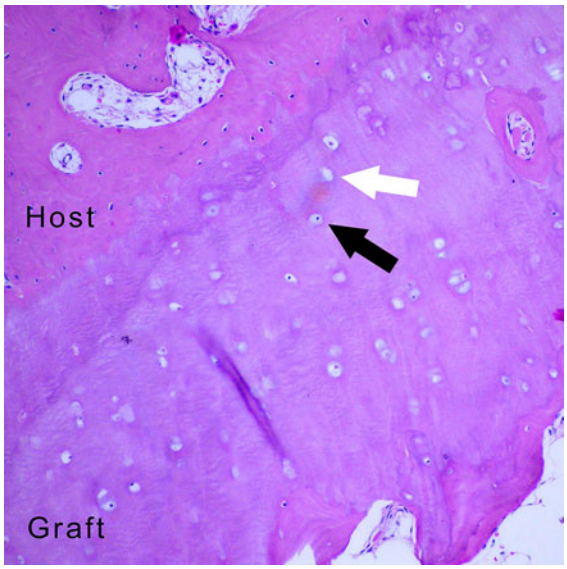


Fig. 4 Histology of the graft-host interface. A representative heamtoxylin-eosine stained histological image of a sample 12 months after grafting the acetabulum. The host can be easily distinguished from the graft by its lighter *pink* color. The host bone is intertwined with the graft, and the joining surface is uninterrupted, indicating continuous remodeling. The lacunae in the graft are mainly empty as expected (*white arrows*), but those closer to the host are filled with cell nuclei (*black arrows*), which is another sign of remodeling. (Color figure online)

in this process, we aimed to test the surgical handling and early biological compatibility of albumin-enhanced bone allografts in a small clinical series of revision arthroplasty. Results from the present study showed, that simply covering the allograft surfaces with a concentrated layer of human albumin supports bone remodelling, while did not require any specific new procedures or adjustments from the operating surgeon. The preparation of the graft is simplified in a way that it can easily be performed in any bone bank, which routinely produces lyophilized allografts.

Nowadays periprosthetic osteolysis and aseptic loosening are considered to be one of the significant challenges in revision arthroplasties (Harris 2001; Lindahl 2007). The goal of the surgical act is to eliminate the causes of bone resorption (elimination of the worn polyethylene, removal of the interface membrane), to rebuild solid bone structure and to implant a stabile prosthesis. Numerous publications have reported long-term results with the use of structural allografts (Haddad et al. 2000; Rust et al. 2007), with around 70 % success rate over 5 years and 60 % in 10 years (Babis et al. 2010; Blackley et al. 2001; Enneking and Mindell 1991). Previously, in an effort to achieve better results, the decortication and perforation of the graft was considered to be important, as is covering by remaining thin autografts. However, follow-ups showed that even in so-called successful cases bone restructuring was minimal. The callus developing at the bone graft interface is visible, but in every case the ossification is only sparsely present in a strip of just a few millimetres, which corresponds approximately to 10 % of the entire graft. In none of the cases could the development of a new osteon be shown in the depth of the allograft, indicating there is significant room for improvement in the biological properties of allograft technology (Enneking and Campanacci 2001).

Several approaches have already been proposed as a solution for this problem. Mixing allografts with autografts has advantages in long-term results as it was shown retrospectively (Enneking and Campanacci 2001), however, this procedure is not surgically feasible in most cases of bone grafting either because large structural grafts are needed, or just the opposite, very small pieces are needed which does not justify a harvesting procedure. One way to overcome this problem is to use autologous blood or bone marrow fractions such as bone marrow derived stem cells (BMSCs), activated conditioned serum (ACS), platelet rich plasma, or as we did in the present study serum albumin as an add-on to allografts (Intini 2009; Wehling et al. 2007; Rust et al. 2007). The exact mode of action of these biological enhancers is uncertain, however, it is widely accepted that blood serum and growth factors are responsible for the proliferation of stem cells (Brindley et al. 2012). Overwhelming in vitro scientific evidence supports this idea, however, clinical translation of this technique is more complex than it seems (Brindley et al. 2012). It is unquestionable that blood-derived growth factors induce BMDSC proliferation in cell culture. However, one overlooked feature is that blood serum itself, even without enrichment with growth factors (sometimes referred to as platelet-poor-plasma) is a potent stem cell proliferating agent. So much so, that 10 % serum albumin is a standard constituent of cell culture media. After finishing several in vitro human and in vivo animal experiments with albumin-coated allografts, the present study is the first step in the bench-to-bedside translation of this idea (Horváthy et al. 2012; Weszl et al. 2012).

The limited nature of this first-in-human study does not allow far-reaching conclusion about the efficacy of albumin coating. Undoubtedly our series is a collection of case reports, and each procedure was unique which together represent almost all suitable cases for such a procedure in a period of 1 year and a population of 10 million. Surgical technique and time frame varied somewhat between surgeons, and even changed regarding the same surgeon along different cases. Given the complexity and poor prognoses of these cases, however, the observed promising early findings in this human experiment are of great value and warrant a future controlled study into both standardization of the procedures and a wider indication of albumin-coated grafts.

Acknowledgments We are thankful for Lacerta Technologies Inc. for providing the albumin coating technology. The authors wish to thank Dr. Andrea Radácsi for her expert advice with SPECT-CT images. The present work was funded by Grants from TÉT-SIN-CELLTHER, TÁMOP-4.2.1/B09/1/KMR-2010-0001, OTKA 83803.

References

Atala A (2012) Regenerative medicine strategies. J Pediatr Surg 47:17–28

Babis GC, Sakellariou VI, O'Connor MI, Hanssen AD, Sim FH (2010) Proximal femoral allograft-prosthesis composites in revision hip replacement: a 12-year follow-up study. J Bone Joint Surg Br 92:349–355

Blackley HR, Davis AM, Hutchison CR, Gross AE (2001) Proximal femoral allografts for reconstruction of bone stock in revision arthroplasty of the hip. A nine to fifteen-year follow-up. J Bone Joint Surg Am 83(A):346–354

Brindley DA, Davie NL, Culme-Seymour EJ, Mason C, Smith DW, Rowley JA (2012) Peak serum: implications of serum supply for cell therapy manufacturing. Regen Med 7:7–13

Enneking WF, Campanacci DA (2001) Retrieved human allografts: a clinicopathological study. J Bone Joint Surg Am 83(A):971–986

Enneking WF, Mindell ER (1991) Observations on massive retrieved human allografts. J Bone Joint Surg Am 73:1123–1142

Haddad FS, Spangehl MJ, Masri BA, Garbuz DS, Duncan CP (2000) Circumferential allograft replacement of the proximal femur. A critical analysis. Clin Orthop Relat Res 371:98–107

Harris WH (2001) Wear and periprosthetic osteolysis: the problem. Clin Orthop Relat Res 393:66–70

Hawe A, Friess W (2006) Physico-chemical lyophilization behavior of mannitol, human serum albumin formulations. Eur J Pharm Sci 28:224–232

Head WC, Emerson RHJ, Malinin TI (1999) Structural bone grafting for femoral reconstruction. Clin Orthop Relat Res 369:223–229

Horváthy DB, Vácz G, Cselenyák A, Weszl M, Kiss L, Lacza Z (2012) Albumin-coated bioactive suture for cell transplantation. Surg Innov. doi:10.1177/1553350612451353

Intini G (2009) The use of platelet-rich plasma in bone reconstruction therapy. Biomaterials 30:4956–4966

Lee PT, Clayton RA, Safir OA, Backstein DJ, Gross AE (2011) Structural allograft as an option for treating infected hip arthroplasty with massive bone loss. Clin Orthop Relat Res 469:1016–1023

Lindahl H (2007) Epidemiology of periprosthetic femur fracture around a total hip arthroplasty. Injury 38:651–654

Malchau H, Bragdon CR, Muratoglu OK (2011) The stepwise introduction of innovation into orthopedic surgery: the next level of dilemmas. J Arthroplast 26:825–831

Richards CJ, Garbuz DS, Pugh L, Masri BA (2011) Revision total knee arthroplasty: clinical outcome comparison with and without the use of femoral head structural allograft. J Arthroplast 26:1299–1304

Rust PA, Kalsi P, Briggs TW, Cannon SR, Blunn GW (2007) Will mesenchymal stem cells differentiate into osteoblasts on allograft? Clin Orthop Relat Res 457:220–226

Ryser MF, Thieme S, Bornhauser M, Lehmann R, Brenner S (2009) Serum albumin strongly influences SDF-1 dependent migration. Int J Hematol 89:269–275

Urist MR (1965) Bone: formation by autoinduction. Science 150:893–899

Wehling P, Moser C, Frisbie D, McIlwraith CW, Kawcak CE, Krauspe R, Reinecke JA (2007) Autologous conditioned serum in the treatment of orthopedic diseases: the orthokine therapy. BioDrugs 21:323–332

Weszl M, Skaliczki G, Cselenyak A, Kiss L, Major T, Schandl K, Bognar E, Stadler G, Peterbauer A, Csonge L, Lacza Z (2012) Freeze-dried human serum albumin improves the adherence and proliferation of mesenchymal stem cells on mineralized human bone allografts. J Orthop Res 30:489–496