Literature study

Summary of relevant clinical studies for the submission of the "Human lyophilized allografts" of the company Arthro Kinetics Biotechnology GmbH

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This literature study serves the summary of the published clinical studies of human allogeneic bone. The main focal point lies on the studies that were conducted with chemically cleaned, freeze-dried and γ-irradiated products. The allogeneic bone tissues processed like this are most similar respectively to be equated to those produced by Arthro Kinetics Biotechnology GmbH. However, also further articles are listed that are important for the evaluation and the clinical utilization of the human lyophilized allografts of the Arthro Kinetics Biotechnology GmbH.

1. INTRODUCTION

Bone defects are a frequent and still difficult therapy problem. It can be assumed that approx. 15% of all operations on the skeletal system cause the necessity of bone replacement in order to be able to recreate stability and shape of the bone section in question (v. Garrel T. et al. 1998). Autogenous and allogeneic bone, demineralised bone, ceramics, and combinations are in clinical use. For the material to be an ideal bone replacement, it should not trigger any immunological defence reactions, be free

of toxic or metagenous side effects and be sterile, allow complete installation and modification, show a high biological potency, meaning an osteogenetic effect through cellular bone formation, osteoinductive effect through the release of bone growth factors, osteoconductive effect through guide channel function and have a firmness corresponding to the requirements. Further, no quantity and storage problems should occur, there should be an optional selection of shapes and sizes, the material should possess an easy workability and be low-cost (v. Garrel T. et al. 1998). None of the applied replacement materials fulfils **all** characteristics of an ideal bone replacement. Autografts are the golden standard because they possess inherent osteogenetic, osteoinductive and osteoconductive characteristics (Galea G., et al., 2005). As one does the reach the boundaries of these transplants due to their limited availability, especially for larger defects, in children and in patients in a poor general condition, alternatives to the autografts are in demand.

Allogeneic bone is such an alternative due to its biological reliability and cost effectiveness (v. Garrel T. et al. 1998). These allografts are provided by bone banks that have the responsibility for the bone materials from the harvesting of tissue up to graft release. In order to guarantee highest possible safety of the grafts, national and international guidelines were released that determine the parameters of the donor selection and testing. Further measures in the direction of tissue safety are taken in the framework of production and sterilization.

Tissue can be sterilized in different ways. Irradiation is very widely spread as a technique. A γ-irradiation with 25kGy has a proven and scientifically recognized inactivated effect on bacteria with a SAL (sterility-assurance-level) of at least 10^{-6}. γ-irradiation also has a depleting effect on viruses and acc. to Galea et al. has no negative effects on the mechanical characteristics of the materials or the in vivo incorporation (Galea G., et al., 2005).

In spite of the primarily osteoconductive effect of the irradiated allograft, reconstruction into new bone takes place gradually (Galea G., et al., 2005). Thus, allogeneic bone is used in many indications in which especially osteoconductive characteristics are needed. These are osteosynthesis in fractures, oligodystrophic and atrophic pseudoarthroses, additive corrections of malpositions, endoprostheses defect filling of bone cysts, skeleton metastases and pathological fractures, primary bone tumors and bone replacement on the pelvis (v. Garrel T. et al. 1998).

2. HUMAN LYOPHILIZED ALLOGRAFTS OF THE ARTHRO KINETICS BIOTECHNOLOGY GmbH

The products of the Arthro Kinetics Biotechnology GmbH are gained from human bone tissue, cleaned mechanically and chemically, lyophilized, γ-irradiated and marketed in different assemblies. The applied process eliminates bothering tissue parts, but spares the natural content of collagen and minerals. This way the end product keeps structural characteristics comparable to natural conditions, as the porous architecture is maintained.

2.1. Type of application

The products that are processed with this method are used in bone defect coverage and/or filling. They are also used in places where access to autologous bone grafts is not possible, among others as bone filling material in orthopaedics, traumatology and trauma surgery.

2.2. Cooperation C^+TBA (Cells$^+$ Tissue Bank Austria) and Arthro Kinetics Biotechnology GmbH

Arthro Kinetics Biotechnology GmbH prepares the human bone tissue for the tissue bank C^+TBA under contract. The extracted tissue is handed over to Arthro Kinetics Biotechnology GmbH for processing after receipt, inspection, and approval by the responsible person of the C^+TBA. After preparation of the tissue and approval by the responsible person of Arthro Kinetics Biotechnology GmbH, C^+TBA is responsible for the marketing of the finished products and distributes them itself or over suppliers.

2.3. Specifications for manufacture

The process of manufacture of the human allografts starts with the tissue extraction. This is done in certified extraction locations through trained personnel, whereas the donor selection and testing is done according to national guidelines. The transport of the donor material from the extraction location to the tissue bank is performed at <5℃ and within 24h. After incoming goods inspectio n the material is immediately stored at ≤-70℃. After receiving the results of th e microbiological (germ presence max. 10^3 germs) and serological (HBV, HCV and HIV negative) examinations, the

responsible person of the C+TBA evaluates the material and approves of the release for processing by Arthro Kinetics Biotechnology GmbH if the results are adequate.

In the next step the tissue released by the tissue bank is relocated to Arthro Kinetics Biotechnology GmbH, also subjected to an incoming goods control and stored at <-70℃. On the day prior to process commencement the tissue is removed from the freezer and stored in a thawing container overnight. The tissue is then cleaned of remaining soft tissue by a coarse cleaning, brought into the required shape through sawing and grinding, and cleaned in the ultrasound bath. This is done to reduce the protein and lipid load of the bone tissue, remove blood and marrow components and to destroy prokaryotic and eukaryotic cells. After this steps or an optional intermediate storage at <-70℃ the prepared materia l is degreased further in a vibrator bowl with diethyl ether that elutes the diethyl ether with decreasing concentrations of ethanol (96%→ 70% → 50%) and treats it with 3% hydrogen peroxide. All the chemicals have a germicide effect (bactericide, fungicide and virucide). After finishing this chemical cleaning the material is either packaged and stored at <-70℃, or immediately freeze-dried. Afte r lyophilization, which also possesses depleting potency towards certain virus groups (Uhlenhaut C., et al. 2005), the material is filled into the primary packaging. This is followed by secondary packaging and the final sterilization with γ-irradiation at 25-30,5kGy, which poses to be a further depletion towards bacteria and viruses.

After filling the tertiary packaging the final product is stored at room temperature up to release by the responsible person and handed over to the C+TBA.

3. SUMMARY OF THE PUBLISHED CLINICAL STUDIES

The search for published clinical studies of human allografts was conducted in the search engines „pubmed“ and „dimdi“ over a period of time from November 2009 to the beginning of March 2010. The used search criteria contain the following terms in different selections: „bone allografts“, „bone graft“, „bone transplants“, „clinical study“, „clinical effectiveness“, „therapeutic effect“, „application“, „lyophilized“, „freeze dried“. Articles relating to clinical studies with products by Tutogen Medical GmbH, Charité – University Medicine Berlin and DIZG – Deutsches Institut für Zell- und Gewebeersatz GmbH were included, as these products are comparable to the end products

manufactured by Arthro Kinetics Biotechnology GmbH. The relevant articles were extracted from the received hits, and from these then again the applicable quoted articles.

The selected 14 articles are hereafter summarized and evaluated by indication, whereas products that are most similar to those manufactured by Arthro Kinetics Biotechnology GmbH are listed at the beginning in the respective indication. Table 1 gives a short overview of the evaluated articles.

In order to be better able to compare the used products, Table 2 provides a summary of the preparation of the bone material.

Number	Authors	Indications	Used product	Conclusions
3.1.	Komender J. et al.	1. Bone filling after tumour removal, 2.Posttraumatic and degenerative cases, 3.Femoral head, arm, leg and underarm surgeries	Lyophilized allografts of the Central Tissue Bank Warsaw	Lyophilized and γ-sterilised allografts are adequate for the tested indications.
3.2.	Pruss A. et al.	1.Femoral head and knee revision 2.Bone filling 3.Posttraumatic defects 4.Reconstruction of the Acetabulum 5.Lumbar spine instability 6.Reconstruction of bone defects 7.Corrective osteotomy	Lyophilized allografts of the DIZG and Charité	The products from Charité and DIZG show a good to very good clinical effectiveness in all tested indications.
3.3.	Zasacki W. et al,	1.Posterior and anterior connections of the spine, 2.Joint arthrodesis, 3.Reconstructive surgeries, 4.Bone filling 5.Posttraumatic reconstructive lesions	Allografts of the Polish bone bank	Allograft transplantations allow successful results independent of the indication.
3.4.	Gambini A. et al.	Defect filling after removal of benign tumors	Tutoplast allografts	The chemical cleaning and the γ-irradiation have no negative influence on the stability of the graft, the healing success, or the graft integration.
3.5.	Rayesn G.P. et al	Instable radius fractures	Tutoplast allografts	No significant differences in the treatment of instable radius fractures between the allograft and autograft group.

Number	Authors	Indications	Used product	Conclusions
3.6.	Caltran M. et al.	Femoral head revision	Phoenix bone allografts, produced by TBF Laboratory	Lyophilized and γ –sterilized allografts are well tolerated and very adequate for this indication.
3.7.	Galia C.R. et al,	Femoral head revision	Lyophilized and sterilized product from the HCPA University Hospital Tissue Bank	Lyophilized and sterilized allografts are adequate for femoral head revisions.
3.8.	Cornu O.H. et al,	Defect of the Tibia plateaus	Allografts from own bone bank namely of the University Louvain, department for orthopaedics and traumatology, Brussels, Belgium	Lyophilized and sterilized allografts are reliable for this orthopaedic indication according to this article, clinically as well as radiologically.
3.9.	Müller H.P., Morach M.	Defect of the Tibia plateaus	Allografts of the bone bank of the Surgical Clinic in the Canton Hospital Aarau, Switzerland	Autologous and homologous cancellous bone plastics are equal.
3.10.	Lasanios N., et al.	Tibia plateau fractures	Lyophilized allograft of the Transplant Service Foundation, Barcelona	Lyophilized allografts is the bone replacement material of choice in this indication.
3.11.	Thalgott J. S. et al.	ALIF (anterior lumbar interbody fusion) Procedure	Allografts “Verti Graft” of the company Lifenet	Lyophilized and frozen allografts show little static significant differences in clinical results.
3.12.	Recht J. et al.	ALIF (anterior lumbar interbody fusion) Procedure	Allografts from own bone bank namely the University Louvain, department for orthopaedics and traumatology, Brussels, Belgium	The authors recommend the use of lyophilized allografts.

Number	Authors	Indications	Used product	Conclusions
3.13.	Putzier M. et al.	Spondylodesis of the lumbar spine	Lyophilized allograft of the Charité	In this study an equal clinical result is achieved with autograft and allograft treatment.
3.14.	Anderson W.J.	Wrist	Lyophilized, sterilized allografts of the University of Miami School of Medicine	Results of the allografts are comparable with those that are achieved by using autografts.

Table 1: Overview of the articles used in the literature study

Product	Donor selection	Anamnesis	Serological testing	Procedure
Allografts der Central Tissue Bank Warsaw	No statement	No statement	No statement	Lyophilisation gamma sterilization
DIZG	yes	yes	HIV 1/2 Antibodies Hepatitis C Hepatitis Bc Antibodies Hepatitis Bs Antigen Treponema Pallidum	Cleaning Cutting Degreasing Sterilization with paracetic acid/ethanol Lyophilisation
Charité	yes	yes	HIV 1/2 Antibodies Hepatitis C Hepatitis Bc Antibodies Hepatitis Bs Antigen Treponema Pallidum	Cleaning Cutting Degreasing with chloroform/methanol Sterilization with paracetic acid/ethanol Lyophilisation
Allografts of the Polish bone bank	No statement	No statement	No statement	Deep freezing Lyophilisation Packaging Gamma sterilization 35kGy
Tutoplast	yes	yes	HIV 1/2 Antibodies Hepatitis C Hepatitis Bc Antibodies Hepatitis Bs Antigen Treponema Pallidum	Pre-cutting Ultrasound degreasing Osmosis NaCl+H_2O Alkaline NaOH Oxidation H_2O_2 Acetone drying Cutting 1. Packaging Gamma sterilization <20kGy LAL Test 2. Packaging
Phoenix bone allografts by TBF Laboratory	yes	yes	No statement	Chemical cleaning Lyophilisation Gamma sterilization 25kGy

Product	Donor selection	Anamnesis	Serological testing	Procedure
Product der HCPA University Hospital Tissue Bank	no statement	no statement	no statement	Chemical cleaning (with chloroform/methanol, hydrogen peroxide) Lyophilisation Sterilization
Allografts of the University Louvain, department for orthopaedics and traumatology, Brussels, Belgium	yes	yes	no statement	Cutting Cleaning Chloroform/methanol solution Lyophilisation Gamma sterilization 25kGy
Allografts of the surgical Clinic in the Canton Hospital Aarau, Switzerland	no statement	no statement	no statement	Cutting Treatment with antibiotic solution Lyophilisation
Allografts of the Transplant Service Foundation, Barcelona	no statement	no statement	no statement	Lyophilisation
Allografts “Verti Graft” of the company Lifenet	yes	yes	HCV Antibodies HBV Surface Antigen HIV1/2 ab HTLV 1+2 Antibodies RPR for syphilis HIV Nat HCV Nat	Centrifugal drying Hydrogen peroxide Washing steps Hydrogen peroxide Gamma sterilization > 20 kGy
Allografts der University of Miami School of Medicine	yes	yes	no statement	Lyophilisation

Table 2: Summary of the processing of the used bone tissues

3.1. Komender J. et al., **Therapeutic Effects of Transplantation of lyophilized and radiation-sterilized, allogeneic bone.** Clin. Orthop. Rel. Res. 272: 38-49, Nov, 1991

In cooperation with three large orthopaedic institutions, this article summarizes information of patients who had lyophilized allograft transplants sterilized by irradiation transplanted between1965 and 1981. This is done for the following indications:

- Bone filling after tumour removal,
- Posttraumatic and degenerative cases,
- Femoral head, arm, leg and lower arm surgeries.

Using a special questionnaire that is answered by the patients two years after the surgery, an analysis of the therapeutic effect is to be made. 1014 of 1374 treated patients, which corresponds to approximately nearly 74%, whose average age is 14.4 years, filled out this questionnaire. The analysis of the distribution of age shows that allografts are used much more frequently on children and youth than on older patients. Further,

a **radiological examination** is done,

the **biostatic data** of the transplant is recorded,

the **results of the treatment** are considered and the

patient satisfaction is evaluated.

During these treatments allografts of the Central Tissue Bank Warsaw that are freeze dried and γ-irradiated are used.

In the radiological results 94% of the patients showed an improvement of the condition, 87% even had a complete reconstruction of the graft into a new bone. If you consider the biostatic data, the transplant is very good in 87% of the cases. The treatment with processed bone is not dependant on age. The results are different in different diagnostic groups. Better results are seen in congenital changes and treatments after benign tumors than in posttraumatic deformations and degenerative illnesses. But also in the latter case 41% achieve complete healing through the

application of allografts. Further the results are dependent on the localization of the defect and the monitoring period after the surgery. The treatment success decreases the longer the preoperative problem resp. the chronic illness has been in existence. Allografts and autografts are transplanted in 46 cases and here the autograft does not achieve improved results. In transplantation with allografts, further accompanying illnesses like for example tumors or degenerative changes on other places than the transplanted location are not a contraindication. In 90% of the patients there are no postoperative complications, in 7.3% of the cases a repeated surgery is necessary, in 2.8% of the cases the graft is removed. The patient satisfaction mirrors the success of the allograft: 37.1% rate the result as „very good“, 57.2% as „satisfactory“, and only for 9% it is „not satisfactory“. This article does not handle side effects that occurred during the treatment.

CONCLUSION:

A reconstruction of the transplant correlates with early treatment successes and positive end results. The rebuilding is the most important factor for a successful transplantation. As proven in this study, lyophilized and γ-sterilized allografts are adequate for the above mentioned orthopaedic indication, whereas better results are determined in bone filling after tumour removal than in the posttraumatic and degenerative cases. The results also show that the hip and arms are very good locations for allografts.

3.2. Pruss A. et al., **Clinical efficacy and compatibility of allogeneic avital tissue transplants sterilized with a peracetic acid/ethanol mixture.** Cell and Tissue Banking 3: 235-243, 2002

Based on clinical reports, this retrospective study summarizes the results from the years 1997-2001 that were achieved with allografts manufactured from the University Hospital Charité and the DIZG (Deutsches Institut für Zell- und Gewebeersatz). By using a questionnaire, the

- **number** of treated patients,
- **main indications for cancellous bone** like,

    - hip and knee endoprostheses
    - Bone filling
    - Bone cysts
    - tumour-similar lesions
    - Reconstruction of the acetabulum
    - Dislocation of the Tibia and the femur
    - Fusion of the lumbar spine
- **Main indications for cortical bone** like,
    - Reconstruction of bone defects
    - Stabilization in case of scoliosis
    - Vertebra replacement after inflammation or tumour
    - Corrective osteotomy
- **Surgery technique**,
- **Handling**, but especially
- clinical **effectiveness** (integration, defect filling, new formation of tissue) and
- **side effects** are recorded.

The used products by Charité and DIZG are cleaned, in case of cancellous bone also degreased after extraction of the bone material and testing for HIV, HBV and HCV. This is followed by a treatment with paracetic acid and the lyophilisation or cryopreservation.

In the examinations in the follow-up period of 6 months to 5 years cancellous bone (90% of the patients), as well as cortical bone (98% of the patients) provide a good to very good spacer function. Also the integration rates, 82% for cancellous bone and 96% for cortical bone, are satisfactory. When using cancellous bone-chips good effectiveness is achieved in the surgery for total hip endoprothesis. No complications are found.

CONCLUSION:

Allograft products are well available and their use prevents complications that may occur through the cancellous bone extraction from the iliac crest. The products by

Charité and DIZG applied here show a good to very good clinical effectiveness in all indications, adequate handling and no clinically relevant side effects.

3.3. Zasacki W. et al, **The efficacy of application of lyophilized, radiation-sterilized bone graft in orthopaedic surgery.** Clin. Orthop. Rel. Res. 272: 82-87, November 1991

In this article, Zasacki et al. conducts a retrospective analysis of the clinical usage of lyophilized allografts sterilized by irradiation in different orthopaedic indications. This examination is done between 1963-1981 on 435 patients at ages from 3 to 74 years. The indications are classified into the following five groups:

- 224 patients with posterior and anterior connections of the vertebrae,
- 36 cases with joint arthrodesis,
- 83 reconstructive surgeries,
- Bone fillings in 59 cases and
- 35 patients with posttraumatic reconstructive lesions.

Target of this study are recordings about the effectiveness of the allografts, which is collected based on the data of the physical examinations, and radiological examinations in which the success of the treatment is equalled with the integration of the transplant.

The allografts, cancellous bone, cortical bones, and cancellous bone blocks used in this study come from a Polish bone bank. The bone material that is extracted after the death of the donor is deep-frozen, lyophilized and sterilized at 35kGy with γ-irradiation.

In general the results of the incorporation of the transplant and its reconstruction into natural bone is satisfactory. A therapeutic effect occurs in 91% of the cases (394 patients). These results are similar in all examined groups, or indications. Complications occurred in 17 cases as postoperative infections.

CONCLUSION:

As already mentioned, 91% of the cases are treated with successful results with the allograft transplants independent of the indication. There is not one case of microbial contamination through the implantation of lyophilized and sterilized allografts.

Lyophilized transplants show a low osteoinductivity and changed mechanical characteristics, like less flexibility and brittle behaviour. However, rebuilding also takes place here, even if slower than on allografts processed differently. However, this can also be of advantage for some indications, for example in arthrodesis of joints. Lyophilized allografts are only contraindicated only in application areas that require a high osteoinductivity, like for example congenital pseudoarthrosis, atrophic hypovasculary posttraumatic pseudoarthrosis and large gaps in bones after infections. Freeze-dried and sterilized products also have the advantage that no cooling chain is necessary, which facilitates the transport, storage, and distribution of this bone tissue.

3.4. Gambini A. et al, **Rehabitation of allograft with bone dehydrated with solvents in reconstruction after removal of bone tumors: MRI evaluation.** Chir. Organi Mov. LXXXIV: 359-366, 1999

This study analyses the behaviour of Tutoplast bone allografts for the filling of defects that remain after the removal of benign tumors, 15 patients in the years 1994-1998. Seven of the 15 patients are male, eight female, the age is 8 to 50 years. The healing process is evaluated via periodic X-ray and MRI examinations, whereas this is based on the % of the hollow space filled with allograft:

- >75% is evaluated as healed,
- 25-75% as partially healed and
- <25% as not healed.

The used bone allograft Tutoplast of the company Tutogen is produced as follows: After removal of the bone material and testing for HIV, HBV and HCV the material is treated with 26% NaCl, which removes the cells. This is followed by a treatment with 3% H_2O_2 and then with 1N NaOH to guarantee the inactivation of HIV-viruses and

hepatitis. After dehydration with propanon the bone material is sterilized with γ-irradiation at 15kGy.

Based on the radiological analysis after 6-13 months, 13 of the 15 patients can be evaluated as completely healed, the remaining 2 patients are regarded as partially healed, whereas the last radiograph is done 8 months after the surgery. A reconstruction of the transplant into newly formed bone is clearly visible on the X-rays. An MRI-examination already one month after the surgery showed necrotic tissue in the transplant. During the follow-ups however, cellular repopulation is visible in the transplanted area, which can hardly be distinguished from the healthy area. This process of the transplant integration starts at the peripheral area of the transplant and continues into the centre over time up to complete reconstruction.

No infections and no returning of the cystic changes are recorded.

CONCLUSION:

As the lesions treated here are relatively small (<10 cm), a small transplant is used. This fact, as well as the preservation of the periosteum and part of the cortex, and the good stability of the transplant without using further tools provides good conditions for the transplant integration and only a small risk of complications.

The usage of bone replacement material made of carcasses for filling defects that remain after the removal of benign tumors is a valid alternative to autografts, as fewer complications are to be expected, the surgery duration and the hospital stay is shorter, and a more simple rehabilitation is possible. Neither the chemical cleaning nor the γ-irradiation has a negative influence on the stability of the transplant, the healing success, or the transplant integration.

3.5. Rayesn G.P. et al, **Cancellous Allograft versus autologous bone grafting for repair of comminuted distal radius fractures: a prospective, randomized trial.** J. Trauma. 2006;60:1322-1329

This prospective, randomized trial tests the reliability of dehydrated, sterilized, allogeneic bone grafts compared to autologous bone grafts from the iliac crests. In the period from January 2000 and January 2003 90 patients, 73 female, 17 male with

an instable radius fracture are supplied in the framework of study, 44 with allografts and 26 with autografts. Inclusion criteria stated are

- Instability of fracture,
- Existence of a dorsal, metaphyseal hollow space after reposition and
- No massive damages of the surrounding tissue.

Patients that are immunocomprised, suffer from malignant, rheumatoid illnesses and infections or have suffered multiple injuries of the upper extremities are excluded from this study. A follow up is conducted three and twelve months after surgery, which includes a clinical and radiologic evaluation of the transplants.

The cancellous bone-chips Tutoplast of the company Tutogen that are used are manufactured as follows: After extraction of the bone material and testing for HIV, HBV and HCV, the material is treated with 26% NaCl that removes the cells. This is followed by treatment with 3% H_2O_2 and then with 1N NaOH in order to guarantee the inactivation of HIV-viruses and Hepatitis. After dehydration with propanon the bone material is sterilized by γ-irradiation at 15kGy.

Globally seen, the results are equivalent for the allografts and autograft group. Looking at the allograft group, a remodelling of the transplant is radiologically visible after 12 months. In the clinical parameters, 87% of the patients regain the grip strength and 89% have none to slight limitations in everyday life. 81% of the participants of this group are pain free. If autologous iliac crest cancellous bone is used, radiologic examinations show the following improvements: in 96% the grip is regained, 87% have none to slight limitations in everyday life and 85% are pain free. Comparing the results of the two groups, no significant differences in the examined parameters can be determined.

However, when looking at surgery duration and complications, differences are by all means visible. The duration of the surgery in the group receiving a Tutoplast-allograft-transplant is significantly shorter than that of the group receiving an autologous iliac crest cancellous bone-transplant. Further, no local or systemic immune reactions occur when using the allografts, while complications occur in the autograft group that are caused by the withdrawal of the cancellous bone from the iliac crest. These complications include great pain, hematomas, infections, in some

cases paratheresis, loss of sensitivity of the lateral upper thigh and pain up to one year later.

CONCLUSION:

No significant differences are determined between the allograft and autograft group in the treatment of radius fractures in the connection of the fracture. Advantages of the allograft group are the shorter duration of surgery and the rarer occurrence of short term and longer term complications. The usage of Tutoplast cancellous bone chips eliminates the risk of complications that would arise from the withdrawal of cancellous bone from the iliac crest, decreases the duration of the surgery and thus of the anaesthesia, which is in further consequence important for the postoperative mortality and rehabilitation of older patients.

3.6. Caltran M. et al., **Use of freeze-dried bone allografts in revision total hip arthroplasty.** Eur. J. Orthop. Surg. Traumatol. (2002) 12: 186-191

This prospective phase II study contains a clinical validation of bone allografts for the treatment of acetabular bone defects in femoral head revisions. 44 of these surgeries are conducted on persons at the age of 18-80 years between March 1996 and September 1997. The radiological quality of the bone reconstruction, and transplant integration and migration are examined in the following control appointments.
The Phoenix bone allografts transplanted in this study, manufactured by TBF Laboratory, are made of femoral heads that are chemically cleaned, lyophilized and irradiated with γ-irradiation (25 kGy according to EN552).
The number of acetabular, involved segments are different in this study. In 43% of the patients one segment is involved, in 26% two segments, in 24% three segments and in 7% four segments. 1-6 allograft transplants are used per patient, for example chips, blocks, half or whole femoral heads, whereas cement is used in 48% of the cases. 35 of the 44 patients are analysed in the follow-up; clinical improvement was determined in all cases. Five patients from the group in which one segment is involved are rated with „good“. Of the 16 patients in which two segments are involved, 15 are rated as „good to satisfactory“, one as „poor“ and on two, dislocations are determined. In the group with three involved segments all of the examinations were rated „good to satisfactory“, but also here two dislocations were

found. If four segments are involved, three of four patients are rated with „good to satisfactory“, one as „poor“, and again there were two dislocations. Groupwide, a very good incorporation of the transplant (in 97% of the patients) can be determined. No side effects are recorded in association with the transplants, and no secondary infections.

CONCLUSION:

Good incorporation of allografts in acetabular bone defects can be recorded. The usage of cement, good for supplying large defects, does not have any negative effects on the transplants. Allografts meet two requirements that make it possible to use them in this indication: first, they allow a reconstruction of the bone, and allow good fixation of the acetabular component. As proven with this study, lyophilized and γ –sterilized allografts are well tolerable and very adequate for this indication.

3.7. Galia C.R. et al, **Femoral and acetabular revision using impacted nondemineralized freeze-dried bone allografts.** J. Orthop. Sci. (2002) 14: 259-265

This article summarizes the clinical results and the radiologically examined bone incorporation of lyophilized allografts in 42 femoral head revisions. These are the results at the middle term of the study. The analysis is done after 63-127 months, 31% of the patients are male and 69% are female. In 12 cases the femoral component is replaced, in 20 cases the acetabular component is replaced, and in 10 cases both components. The clinical examination is conducted according to the D`Aubigne-Postel score. The radiological examinations in which antero-posterior images are created are performed right after the surgery and after 1, 3, 6 and 12 months. The monitored criteria for the acetabular component are resorption and the equal density of transplant and the surrounding bone, for the femoral component loosening, changing of position and integration of the transplant.

The product used is lyophilized and sterilized, manufactured by the HCPA University Hospital Tissue Bank, where it is chemically cleaned, among other things with chloroform, methanol and hydrogen peroxide, freeze-dried and sterilized with ethylene oxide.

40 patients end the follow-up, the other two pass away, however not in association with the hip revision. 90% show a satisfactory result in the clinical evaluation. In the acetabular X-ray two years after the surgery 93% show a remodelling and good incorporation, in the femoral X-ray 86,5% show remodelling and good incorporation after an equal time. No displacement can be determined in the femoral location. There are no massive postoperative side effects, one patient requires a re-revision after 3 years.

CONCLUSION:

Lyophilized and sterilized allografts are adequate for femoral head revisions. The usage of allografts shows excellent clinical and radiological results in the presented study. The results are equal to those of the frozen allografts. However, a longer postoperative follow-up will be conducted yet.

3.8. Cornu O.H. et al, **Tibial tubercle elevation with bone grafts – A comparative study of autograft and allograft.** Arch. Orthop. Trauma Surg. (1995) 114: 324-329

In a comparing, retrospective study, patients with a tibial tubercle elevation are treated with an autograft or allograft transplant. 64 patients are treated between 1980 and 1989. They are divided into two equally large groups, whereas one half is treated with autografts and the other half is treated with allografts. As inclusion criteria, the knee to be treated must not have had any previous transplants. A postoperative and radiologic follow-up is conducted after 6 months according to the following criteria:

- fusion,
- resorption,
- loss of elevation,
- first standing up after the surgery,
- pain,
- duration of the hospital stay and
- patient satisfaction.

The used autografts are made of material from the iliac crest. The bone material for the allografts comes from the own bone bank, namely of the University Louvain,

department for orthopaedics and traumatology, Brussels, Belgium. After donation, it is shaped, chemically cleaned in order to remove blood cells, marrow and lipids, lyophilized and irradiated at 25kGy with γ-irradiation.

The radiologic results in the follow-up examinations for the autografts are „good“ for 42%, for the allografts 31%. No significant differences were visible in the rating or in one of the examined parameters between the two transplant types. In the examination of the clinical parameters the duration of the hospital stay for the allografts is 9.3 days, shorter than the group that was treated with autografts, who stay in the hospital for 12.1 days. The time of the first standing up after surgery is 4.4 (autograft group) resp. 3.4 (allograft group). No infections occur, however, pain is still reported in the autograft group at the withdrawal location of the transplant 6 months after the surgery. The patient satisfaction is statistically not influenced by the type of transplant.

CONCLUSION:

Lyophilized allografts treated with γ-irradiation have osteoconductive characteristics. According to this article, these allografts are reliable for orthopaedic indications, clinically, as well as radiologically. They prevent side effects that would occur through the withdrawal of autologous bone material and shorten the hospital stay.

3.9. Müller H.P., Morach M. **Über die Verwendung lyophilisierter, homologer Spongiosa bei der Tibiakopffraktur.** Hel. Chir. Acta 45: 43-47 (1978)

This article summarizes a retrospective study that targets a comparison of homologous and autologous cancellous bone in mechanical support of the elevated tibial plateau. In the period from 1968 and 1974, 30 patients are treated with a cancellous bone graft, 19 of them with an autologous and 11 with a homologous graft. Six months to seven years later, follow-up examinations are conducted in which 27 patients are examined clinically, and 26 are examined radiologically. Target criteria are

- postoperative complications,
- subjective discomforts,

- limitation of the mobility of the knee joint and a
- radiologic evaluation,

which examines the joint surface, the level of arthrosis, the level of osteoporosis and the cancellous bone healing.

The used homologous allografts are manufactured in the bone bank of the Surgical Clinic on the Canton Hospital Aarau, Switzerland. In this case the material is cut into blocks after sterile extraction from younger fatalities, submerged in antibiotic solution, filled, deep-frozen, and lyophilized. Subsequently, the allografts are stored at room temperature.

The results of this study show that in this indication, homologous cancellous bone is absolutely equal to autologous cancellous bone in quality. Also the number of postoperative infections (2 patients with autografts, 4 with allografts) shows no significant differences.

CONCLUSION:

In spite of the small number of examined patients it can be shown that autologous cancellous bone grafting is equal, especially in the mechanical supporting function.

3.10. Lasanios N., et al. **The use of freeze-dried cancelous allograft in the management of impacted tibial plateau fractures.** Injury, Int. J. Care Injured (2008) 39: 1106-1112

The use of freeze-dried cancelous allograft in the management of impacted tibial plateau fractures is examined. 25 patients with impacted tibial plateau fractures are included in this study, patients without impacted fracture, with an open fracture or hypersensitivity towards allografts are excluded. The follow-ups are conducted 10-20 months after surgery on 23 patients, 15 males and 8 female. A clinical examination that tests pain, walking ability, mobility, and stability is conducted, as well as antero-posterior and lateral X-rays that checks joint suppression, chondylene widening and osteoarthrosis.

Lyophilized cancellous bone chips of the Transplant Service Foundation from Barcelona, Spain are used.

In the last follow-up examination, 21 of 23 patients show excellent or good clinical and radiological results. Two patients require a long antibiotic therapy after the surgery, one suffers a loss of the anatomic axiality of the joint surface, as it was subjected to full strain too early. The lyophilized allografts are fully incorporated within a short time (8-12 weeks) and are structurally strong enough to support the reconstructed joint surface. No complications occur in association of the usage of the allografts.

CONCLUSION:

Lyophilized allografts are the bone replacement material of choice for this indication, as they are easy to use, transport and storage is simple and they show good results in treatment .

3.11. Thalgott J. S. et al, **A Prospective, randomized, blinded, single-site study to evaluate the clinical and radiographic differences between frozen and freeze-dried allograft when used as part of a circumferential anterior lumbar interbody fusion procedure.** Spine Vol. 34, Number 12: 1251-1256 (2009)

This article compares the results and fusion rate of an ALIF (anterior lumbar interbody fusion) procedure with lyophilized and deep-frozen allografts in a prospective, randomized clinical examination. Between September 2000 and October 2002, 50 ALIF patients are supplied with one of the two transplants. Monitoring is conducted for at least 24 months after surgery.

The following are examined

- Fusion status,
- Intactness of the transplant,
- pain,
- complications,
- Owestry Disability Index and
- SF-36 value.

The used bone materials „Verti Graft“ are manufactured by the company Lifenet in both cases, meaning the lyophilized and the frozen materials. After extraction, the

bone material is treated with detergents, hydrogen peroxide and isopropyl alcohol to remove bone specific cells like marrow and to disinfect it. This is followed by sterilization with γ-irradiation at a low radiation dosage and temperature. Then the frozen allografts are stored at -70℃, the others a re lyophilized and then stored at room temperature.

40 of the 50 patients absolved the complete follow-up, whereas 19 received the freeze-dried transplant, and 21 received the frozen transplant. No significant clinical effect depending on the allograft type is determined, also no difference in the number of complications (re-revision) or side effects. However the freeze dried allografts are slightly more difficult to handle, as they have a higher probability of breaking during surgery. A fusion is determined in 71.4% of the cases.

CONCLUSION:

These two methods of bone replacement hardly show significant differences in the clinical results. Only the probability of breaking during the surgery seems to be higher with the freeze-dried transplants.

3.12. Recht J. et al, **Freeze-dried allograft versus autograft bone in scoliosis surgery.** Eur. Spine J. (1993) 2: 235-238

As autografts, in spite of generally excellent results, are not without complications, like increased surgery duration and blood loss, and pain at the location of withdrawal, alternatives to this therapy form for scoliosis are high in demand. Two groups with 36 patients each that are treated against scoliosis with a posterior vertebral fusion are compared retrospectively. The postoperative follow-up is done after years. After this period, the loss of correction (Cobb angle) is examined with help of X-rays.

The first group receives a transplant from the own iliac crest, the second group receives an allograft from the bone bank of the University Louvain, department for orthopaedics and traumatology, Brussels, Belgium. For the allografts, the bone material is extracted from a donor, and the bone marrow is removed by washing with demineralized water. The material is chemically cleaned for the extraction of the lipids, cell membrane, and antigens. This is followed by the lyophilisation and an

irradiation with γ-irradiation at 25 kGy. The end product is stored at room temperature.

Twelve months after surgery the extent of the bone fusion with allografts can be compared to that the autografts, in both cases the correction is >50%. After 24 months a loss of correction is visible in both groups, approximately to an equal extent. No infections are recorded. In this study, no difference in the duration of surgery or the blood loss between the two groups is determined.

CONCLUSION:

A longer follow-up to exclude risks and the confirmation of the results is still necessary for the allografts. However, the authors recommend the use of lyophilized allografts.

3.13. Putzier M. et al, **Allogenic versus autologous cancellous bone in lumbar segmental spondylodesis: a randomized prospective study.**

Eur. Spine J. (2009) 18: 687-695

In order to cover the need for alternative transplants with comparable results like autologous bone in spondylodesis of the lumbar spine, this prospective, randomized, non-blind study is conducted with allografts. 40 patients with degenerative spine illnesses are divided into two groups, one is treated with cancellous bone of the iliac crest and the other group is treated with allografts of cancellous bone. The study is conducted between September 2003 and July 2004. Patients with a degeneration of the adjoining segments are excluded from this study. Exanimation is conducted after 3, 6, 9 and 12 months by the Oswestry Low Back Pain disability questionnaire and in addition, the patient satisfaction, the willingness of the patient to conduct the surgery again was examined, along with pain occurrence and radiologic results.

Cancellous bone from the iliac crest is used, also an autograft, and on the other hand allograft cancellous bone manufactured by the Charité. These allografts are cleaned mechanically and chemically after withdrawal of the bone material from the donor, sterilized with PES and lyophilized.

Looking at the results six months later, the allografts show a lower fusion rate than the autografts. After 12 months however, no significant difference can be seen between the two groups anymore.

CONCLUSION:

In this study an equal clinical result is achieved with autograft and allograft treatment. After 12 months analogous fusion rates are determined.

3.14. Anderson W.J. **Allograft bone for arthrodesis and repair of skeletal hand problems.** J. of Hand Surgery (1989) 14B: 332-335

Between November 1984 and December 1987,16 patients receive allografts for three different types of wrist surgeries, in detail a carpo-metacarpal arthrodesis, modified Russe procedure and inter-carpal arthrodesis. Subject of examination are subjective, objective, X-ray graphical and economic criteria, whereas also the pain status and the stiffness are rates. Especially pain the surgery location, clinical signs for an infection and rejection of the transplant are monitored. The follow-up examinations are conducted between 12 and 38 weeks after surgery.

Allografts are manufactures at the University of Miami School of Medicine. The protocol of this bone bank intends a lyophilisation and sterilization of the material.

All achieved results are satisfactory. The average immobilization period for all patients lies at 11.4 weeks. All patients achieve grip strength with maximum 20% difference to their other hand. One infection, but no rejection reaction is determined. In average, fusion takes place after 12.4 weeks, whereas the X-ray examination supplies no indications for a resorption or a fracture of the allograft. Thirteen of the treated patients are able to pursue the same career as before, one terminates the employment relationship due to other reasons, and two change to easier work. Allografts therefore meet the parameters important for acceptable results, filling or bridging of the defect, mechanical stability and stimulation of the bone formation. Further the usage of allografts reduces the donor location morbidity, the time and type of anaesthesia, and the risk of infection.

CONCLUSION:

The results of the allografts are comparable to those achieved by using autografts. Besides, allografts possess the necessary characteristics to achieve successful results, but also to minimize some unwanted complications of the autografts. Thus, they pose to be an effective alternative to autografts to achieve a fusion between the transplant and the natural tissue, as well as healing of the bone in the skeletal reconstruction of the hand. Beyond that, the usage of allografts reduces the morbidity, the duration of the surgery, and the complication rate.

4. DISCUSSION

The clinical success of human lyophilized allografts can be clearly determined based on the summarized articles. In these articles, products comparable to the product of Arthro Kinetics Biotechnology GmbH are used, which pose to be an efficient, easy to handle bone replacement in different orthopaedic indications with only few side effects.

Used as bone replacement materials, allografts fill bones after tumour or cyst removal. Komender et al., 1991 and Pruss et al., 2002 describe that cancellous bone and corticalis both have a good to very good effectiveness, and no clinically relevant side effects occur. Zasacki et al., 1991, and Gambini et al., 1999, also certify that allografts pose to be a valid alternative to autografts in this indication.

Allografts are also used in posttraumatic and degenerative cases. Zasacki et al., 1991, describes this as well as Komender et al., 1991. The latter determines that allogeneic material shows a good effectiveness in this indication, but the effectiveness is lower than when filling bones after tumour or cyst removal.

As one of the most important areas of application, studies of femoral head revisions are summarized in several articles. Komender et al., 1991, and Pruss et al., 2002, confirm that this is a very good indication for allografts. Further Caltran et al., 2002, certifies that allografts enable a reconstruction of the bone, and therefore, a good fixation of the acetabular component is possible. Galia et al., 2002, confirms that lyophilized and sterilized allografts show excellent clinical and radiological results. The results are equal to those of deep-frozen allografts.

No significant differences between the usage of allografts or autografts are determined in association with an instable radius fracture, as Rayesn et al., 2006, describes. Advantages in the application of allogeneic material become visible also in the shorter duration of the surgery and the rarer occurrence of short term and long term complications.

The application of allografts is also examined in the treatment of different tibial injuries. Cornu et al., 1995, Müller et al., 1978 and Lasanios et al., 2008, state in the articles that in this indication, allogeneic material is the bone replacement material of choice and that it shows good clinical and radiological results. It is completely incorporated and has a mechanical support function equal to autologous tissue.

Thalgott et al., 2009, und Putzier et al., 2009, describe the application of allografts in the anterior lumbar interbody fusion procedure and in the spondylodesis. In both indications, equal results and analogous fusion rates are achieved as with autografts in allogeneic material, in two different processing types. Also in scoliosis, as described by Recht et al., 1993, the extent of the bone fusion and the correction can be compared to those after autograft application. In the Smith-Robinson anterior cervical fusion, as stated in Zdeblick et al., 1991, allografts show acceptable clinical results, but significantly lower connection rates and more cracks in the allogeneic transplants were recorded especially in multi-level fusions than in the autogenous transplants. Therefore the application of allografts cannot be recommended in this indication.

According to Anderson et al., 1989, allografts pose to be a comparable alternative to autografts also in the skeletal reconstruction of the hand, in which a fusion between the transplant and the natural tissue and healing of the bone is targeted.

In all of these indications, allografts are a good alternative to the autografts. They show good incorporation and remodelling in the studies on hand. With similar effectives, the frequency of side effects is lower when using allogeneic material, and transport, storage, and handling is easier. In addition, the application of allografts eliminates the risk of complications that would be caused by withdrawal of autologous cancellous bone from the iliac crest, and larger defects can thus also be treated.

Looking at the different applied products, no significant difference in the effectiveness, handling, or frequency of side effects can be determined in association with a product. All applied products, for example by the company Tutogen, the Charité, the DIZG and the products from the bone banks of various show good clinical results, and, if tests were conducted, good radiological results.

Caltran et al., 2002, Komendet et al., 1991, and Cornu et al., 1995, state in their articles that lyophilized and irradiated allografts are adequate, well tolerable and clinically and radiologically reliable for different orthopaedic indications. Gambini et al., 1999, again shows that the irradiation has no negative effect on the stability of the transplant, the healing success or the transplant integration. Further, freeze-dried and irradiated products provide the advantage according to Zasacki et al., 1991, that no refrigeration chain is necessary, which facilitates the transport, storage and distribution of these products. No case of microbial contamination through the implantation of freeze dried and irradiated allografts was determined.

CONCLUSION:

According to the stated literature, lyophilized and irradiated allografts are a valid alternative to autografts in the following indications:

- Bone filling after cysts or tumors
- Posttraumatic and degenerative injuries
- Femoral head revisions
- Injuries of the radius, hand and tibia
- Problems of the lumbar spine and scoliosis

They show an equal effectiveness, good incorporation, no rejection, and good remodeling with a lower frequency of side effects. Irradiation has no negative effect on the stability and incorporation of the transplant and the healing success.

5. USED LITERATURE

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