

## Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report

Stefan LENDECKEL<sup>1</sup>, Andreas JÖDICKE<sup>2</sup>, Petros CHRISTOPHIS<sup>2</sup>, Kathrin HEIDINGER<sup>3</sup>, Jan WOLFF<sup>4</sup>, John K. FRASER<sup>5</sup>, Marc H. HEDRICK<sup>5</sup>, Lars BERTHOLD<sup>6</sup>, Hans-Peter HOWALDT<sup>1</sup>

<sup>1</sup>Department of Maxillofacial and Facial Plastic Surgery (Head: Prof. Dr. Dr. Hans-Peter Howaldt), Justus-Liebig-University Medical School, Giessen, Germany; <sup>2</sup>Department of Neurosurgery (Head: Prof. Dr. Dieter-Karsten Böker), Justus-Liebig-University Medical School, Giessen, Germany; <sup>3</sup>Department of Clinical Immunology and Transfusion Medicine (Head: Prof. Dr. Gregor Bein), Justus-Liebig-University Medical School, Giessen, Germany; <sup>4</sup>MacroPore Biosurgery Comp., Königstein, Germany; <sup>5</sup>Department of Surgery, Division of Plastic Surgery, University of California, Los Angeles, USA; <sup>6</sup>Department of Paediatric Radiology (Head: Prof. Dr. Gerhard Alzen), Justus-Liebig-University Medical School, Giessen, Germany

---

**SUMMARY.** This is a report of a 7-year-old girl suffering from widespread calvarial defects after severe head injury with multifragment calvarial fractures, decompressive craniectomy for refractory intracranial hypertension and replantation of cryopreserved skull fragments. Chronic infection resulted in an unstable skull with marked bony defects. Two years after the initial injury the calvarial defects were repaired. Due to the limited amount of autologous cancellous bone available from the iliac crest, autologous adipose derived stem cells were processed simultaneously and applied to the calvarial defects in a single operative procedure. The stem cells were kept in place using autologous fibrin glue. Mechanical fixation was achieved by two large, resorbable macroporous sheets acting as a soft tissue barrier at the same time. The postoperative course was uneventful and CT-scans showed new bone formation and near complete calvarial continuity three months after the reconstruction. © 2004 European Association for Cranio-Maxillofacial Surgery

**Keywords:** Skull defects; Stem cells; Fibrin glue; Autologous bone graft

---

### INTRODUCTION

Several procedures have been described for reconstructing the skull (bones). Most commonly, split thickness calvarial bone grafts have been used with good success (Goodrich et al., 1992; Inoue et al., 1995; Barone and Jimenez, 1997). Bone substitutes such as hydroxyapatite cement (e.g. BoneSource<sup>TM</sup>) or methylmethacrylates (e.g. Palacos<sup>TM</sup>) have also been recommended (Lew et al., 1997; Friedman et al., 2000; Eppley et al., 2003; Chiarini et al., 2004). Individual prefabricated titanium implants using CAD/CAM techniques are also described for reconstructing the skull (Joffe et al., 1999; Eufinger and Wehmöller, 2002). Nevertheless, it is widely accepted that autogenous bone grafts currently are the best material for bony reconstruction in craniofacial surgery, especially in children (Tatum and Kellmann, 1998; Bussieres and Tatum, 2000; Lenz et al., 2003).

However, in children, successful repair of large skull defects is often difficult because of the limited amount of autogenous bone available. Accordingly, bone regeneration augmented by stem cells or osteoinductive proteins have been suggested (Langer and Vacanti, 1993; Boo et al., 2002; Park et al., 2003; Yamada et al., 2003). Most adult stem cell work has

focused on mesenchymal stem cells from bone marrow. These have shown the potential to differentiate into adipocytes, chondrocytes, osteoblasts and myoblasts (Haynesworth et al., 1992; Bruder et al., 1997; Pittenger et al., 1999, 2000; Boo et al., 2002). However, harvesting adult stem cells from bone marrow yields low numbers of cells and may cause donor site morbidity (Ringe et al., 2002; Yamada et al., 2003).

Typically, cell culture is required to increase the number of stem cells. Zuk et al., (2002) recently reported that adipose tissue is an alternative source of multipotent stem cells. The stem cells are isolated from adipose tissue in large quantities and have shown stable growth and proliferation in vitro. They are also reported to be multipotent and can differentiate into various mesodermal tissues (e.g. fat, bone, cartilage and muscle) in vitro (Mizuno et al., 2002; Zuk et al., 2002; Dragoo et al., 2003; Lee et al., 2003).

### CASE REPORT

A 7-year-old girl sustained severe head injury resulting in a closed multifragment calvarial fracture

after a fall. Because of refractory intracranial hypertension, a bilateral decompressive craniectomy had to be performed. Calvarial fragments were stored at  $-70^{\circ}\text{C}$  (cryopreservation) for three weeks until secondary replantation and fixation was performed with titanium miniplates. Thereafter, progressive and disseminated calvarial bone resorption occurred over several months probably due to insufficient fixation. Almost all osteosynthesis plates loosened and ossification failed to appear at the sites of the former osteotomies. Subsequently, chronic infection with accompanying significant bone resorption resulted in an unstable skull. The skull defects (Fig. 1) with the potential risk of secondary brain injury were the indications for another operation to reconstruct the calvaria.

In an interdisciplinary surgical procedure, all osteosynthesis material and intervening scar tissue were removed and an imprint with Palacos<sup>TM</sup> (as a template for resorbable sheet contouring) was created. Two resorbable macroporous sheets (Macropore PS Protective Sheet, Macropore, San Diego, USA), each measuring  $10 \times 20$  cm and being 0.5 mm thick, were moulded in hot water to fit the Palacos<sup>TM</sup> imprint. Approximately 15 ml cancellous bone was taken from the ilium, milled and evenly applied to all bony defects (approximately  $120\text{ cm}^2$ ). Thereafter, the premoulded protective sheets were fixed with resorbable tacks.

To enhance the regeneration process that was limited by the restricted amount of cancellous bone, autologous stem cells derived from fat were applied as well. These cells were extracted from adipose tissue obtained from the gluteal region during harvesting the bone graft from the dorsal iliac crest. Cell processing required two hours during the ongoing surgical procedure. Subsequently, 10 ml of the prepared solution of adipose stem cells were evenly injected through the holes of the protective sheets to soak the milled bone grafts. To keep the cells in place, 8 ml of autologous fibrin glue was applied using a spray adapter.

Postoperative healing was uneventful. Clinical follow-up has shown symmetrical calvaria contour. There were no neurological deficits. Ultrasound examination 2 and 6 weeks postoperatively proved the cancellous bone and the macropore sheets to be in correct and stable position without pathological findings. Thereafter, it was decided that the child no

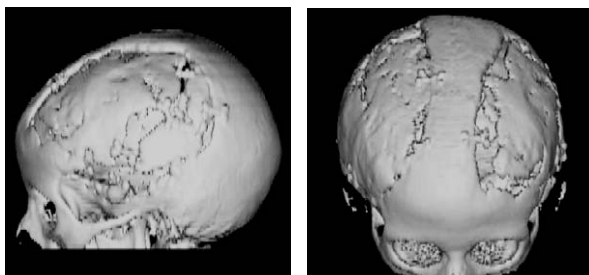


Fig. 1 – 7 year-old girl; 3-D-CT scans: Two views of widespread calvarial bone defects preoperatively.

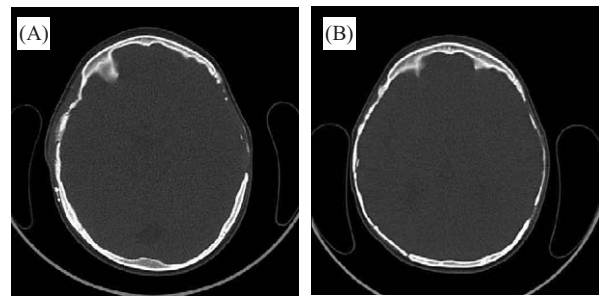


Fig. 2 – Same patient; axial CT scans: (A) calvarial bone defects preoperatively and (B) three months postoperatively.

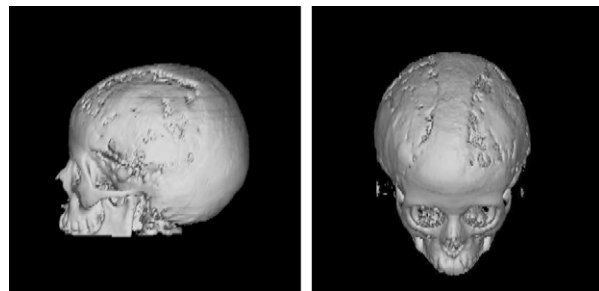


Fig. 3 – Same patient; 3-D-CT scans: two views of widespread calvarial bone defects three months postoperatively.

longer needed to wear the protective helmet she had worn for the previous year. CT scans 3 months postoperatively showed a marked ossification in the defect areas (Figs 2 and 3).

## MATERIAL AND METHODS

### Production of autologous fibrin glue

Two days prior to surgery the patient underwent plasmapheresis to obtain 315 ml of plasma. From this, autologous fibrin glue was manufactured following a standard cryoprecipitation procedure (CryoSeal<sup>®</sup> FS Systems, Thermogenesis, USA). The CryoSeal<sup>®</sup> FS System produces concentrated thrombin and the same amount of cryoprecipitate containing highly concentrated factor VIII, factor XIII, and fibrinogen from a single unit of autologous plasma in about one hour (Gosselin et al., 1997; Rock et al., 2001). Two syringes (4 ml each), one filled with sterile thrombin and one with sterile fibrinogen, were assembled in a dual syringe system, frozen  $-70^{\circ}\text{C}$ , stored at  $-30^{\circ}\text{C}$  and defrosted immediately prior to surgery.

### Extraction of autologous adipose derived stem cells

A total of 42.3 g adipose tissue was excised from the left gluteal area (simultaneously with bone harvesting from the dorsal iliac crest). The adipose tissue was

dissected into small pieces, transferred into a sterile blood bag and processed according to a standardized protocol developed by MacroPore Biosurgery and the Giessen University according to *Zuk et al. (2001)*. The process took two hours including washing steps performed with phosphate-buffered saline (PBS), addition of 0.075% Liberase™ (Roche, Germany) for enzymatic digestion, and final washing steps with PBS and centrifugation. The harvested cells were suspended in 14 ml of saline. The total number of extracted cells was  $295 \times 10^6$  mononuclear cells. About 2–3% of these cells could be expected to be stem cells as was found by others (unpublished material, J. Fraser, MacroPore Biosurgery).

### Quality assurance of adipose tissue processing

In addition to the 7-yr-old girls treatment, adipose tissue was taken from three other patients, processed, and microbiological, viability and functional tests of the processed cells were performed. In microbiological testing of the cell suspensions, no bacterial contamination was found after processing. Vitality testing with trypan blue staining was also conducted to determine the total number of nucleated cells as well as to determine the viability of the cells within the suspension. Ninety-four percent of the cells were vital.

A limited panel of functional tests was performed to confirm the functional ability of these extracted cell fractions. These included a transmigration assay with stromal cell-derived factor-1 to test their migratory capacity, and a fibroblast colony-forming unit assay to test for clonal expansion capacity. The functional capabilities of the cells tested were compared with highly purified mesenchymal stem cells harvested from bone marrow of young healthy patients. In both assays, the adipose derived stem cells harvested from the three test persons showed results comparable with the bone marrow derived stem cells taken from the controls.

### DISCUSSION

In children, the repair of large skull defects may be difficult due to the limited amount of autogenous bone available. The use of alloplastic material is restricted to adults because of continuous calvarial growth in children. Hence the search for an alternative method of skull reconstruction in this patient.

Using autogenous stem cells for bone regeneration is a promising strategy (*Langer and Vacanti, 1993; Yamada et al. 2003; Park et al. 2003*). Until recently, only mesenchymal stem cells derived from bone marrow had been used experimentally to enhance bone regeneration (*Benayahu et al., 1989; Haynesworth et al., 1992; Bruder et al., 1997; Pittenger et al., 1999*). However, harvesting adult stem cells from bone marrow may only yield low numbers of stem

cells and cause donor site morbidity (*Ringe et al., 2002; Yamada et al., 2003*). As was recently shown, adult stem cells from adipose tissue can also differentiate into various mesodermal cells (fat, bone, cartilage and muscle) in vitro (*Zuk et al., 2001; Mizuno et al., 2002*). Adipose tissue may therefore serve as an easily available and abundant source of autologous stem cells for bone regeneration (*Zuk et al., 2002; Dragoo et al., 2003; Lee et al., 2003*).

This was supported by the results of functional tests (CFU-F assay and transmigration assay) performed in the patients reported here. The difference in functional capacity of stem cells from adipose tissue compared with those from bone marrow may be related to the differential purification rates between the two cell types. Improving the purification process of stem cells is the subject of further investigations.

In the present case, the total calvarial defect was about 120 cm<sup>2</sup>. It was obvious that sufficient bone could not easily be harvested for a complete reconstruction of the calvaria.

The use of autologous stem cells for bone regeneration opened a potent new therapeutic option (*Langer and Vacanti, 1993; Yamada et al., 2003; Park et al., 2003*). It was decided to use adipose derived stem cells to augment the limited amount of bone available for calvarial reconstruction with the milled cancellous bone serving as an osteoconductive scaffold. Furthermore, fresh, autologous, milled, cancellous bone contains growth factors and other cells which may stimulate the stem cells to differentiate into osteoblasts and osteocytes (*Schmid et al., 1993*).

To keep the stem cells in place, autologous fibrin glue was used. It has been demonstrated that this showed no inhibition of stem cell proliferation (*Isogai et al., 2000*); it is highly biocompatible and biodegradable. Fibrin glue can also serve as a biological vehicle for cell transplantation (*Yamada et al., 2003*).

The simultaneous stem cell transplantation together with bone harvesting and skull reconstruction in one surgical procedure was only made possible by the innovative processing protocol which made the stem cells available within only 2 hours. In this case, CT scans showed marked ossification in the former defect after three months. Obviously, one cannot determine how much of the effect was due to the conventional bone grafting against that related to the combination of autologous adipose derived stem cell transplantation and autologous fibrin glue application.

### CONCLUSION

We believe that this is the first report of the use of adipose derived stem cells to augment cancellous bone for the treatment of a difficult reconstructive problem. Further studies, both in vitro and in vivo, are needed to turn this first case into a reproducible and reliable treatment regimen in craniofacial bone reconstruction.

## ACKNOWLEDGEMENTS

The authors wish to thank Joachim Misterek and Liesel Schindler-Wuepper from the Department of Clinical Immunology and Transfusion Medicine for their excellent technical assistance in processing the adipose derived stem cells. Furthermore, we are indebted to Dr. Detlef Kuhn, Department of Anaesthesiology, for his support in producing the autologous fibrin glue.

## References

- Barone CM, Jimenez DF: Split-thickness calvarial grafts in young children. *J Craniofac Surg* 8: 43–47, 1997
- Benayahu D, Kletter Y, Zipori D, Weintraub S: Bone-marrow derived stroma cell line expressing osteoblast phenotype in vitro and osteogenic capacity in vivo. *J Cell Physiol* 140: 1–7, 1989
- Boo JS, Yamada Y, Okazaki Y, Hibino Y, Okada K, Hata K, Yoshikawa T, Sugiura Y, Ueda M: Tissue-engineered bone using mesenchymal stem cells and a biodegradable scaffold. *J Craniofac Surg* 13: 231–239, 2002
- Bruder SP, Jaiswal N, Haynesworth SE: Growth kinetics self-renewal and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem* 64: 278–294, 1997
- Bussieres M, Tatum SA: Secondary craniofacial surgery for trauma. *Facial Plast Surg* 16: 135–152, 2000
- Chiarini L, Figurelli S, Pollastri G, Torcia E, Ferrari F, Albanese M, Nocini PF: Cranioplasty using acrylic material: a new technical procedure. *J Cranio-Maxillofac Surg* 32: 5–9, 2004
- Dragoo JL, Choi JY, Lieberman JR, Huang J, Zuk PA, Zhang J, Hedrick MH, Benhaim P: Bone induction by BMP-2 transduced stem cells derived from human fat. *J Orthop Res* 21: 622–629, 2003
- Eppley BL, Hollier L, Stal S: Hydroxyapatite cranioplasty: 2. Clinical experience with a new quick-setting material. *J Craniofac Surg* 14: 209–214, 2003
- Eufinger H, Wehmöller M: Microsurgical tissue transfer and individual computer-aided designed and manufactured prefabricated titanium implants for complex craniofacial reconstruction. *Scand J Plast Reconstr Surg Hand Surg* 36: 326–331, 2002
- Friedman CD, Costantino PD, Synderman CH, Chow LC, Takagi S: Reconstruction of the frontal sinus and frontofacial skeleton with hydroxyapatite cement. *Arch Facial Plast Surg* 2: 124–129, 2000
- Goodrich JT, Argamaso R, Hall CD: Split-thickness bone grafts in complex craniofacial reconstructions. *Pediatr Neurosurg* 18: 195–201, 1992
- Gosselin RC, Larkin E, Owings JT, Coehlo P: CryoSeal system, a new device for generating cryoprecipitate from plasma. *Clin Chem* 43: 1782–1783, 1997
- Haynesworth SE, Goshima J, Goldberg VM, Caplan AI: Characterization of cells with osteogenic potential from human marrow. *Bone* 13: 81–88, 1992
- Inoue A, Satoh S, Sekiguchi K, Ibuchi Y, Katoh S, Ota K, Fujimori S: Cranioplasty with split-thickness calvarial bone. *Neurol Med Chir* 35: 804–807, 1995
- Isogai N, Landis WJ, Mori R, Gotoh Y, Gerstenfeld LC, Upton J, Vacanti JP: Experimental use of fibrin glue to induce site-directed osteogenesis from cultured periosteal cells. *Plast Reconstr Surg* 105: 953–963, 2000
- Joffe JM, Nicoll SR, Richards R, Linney AD, Harris M: Validation of computer-assisted manufacture of titanium plates for cranioplasty. *Int J Oral Maxillofac Surg* 28: 309–313, 1999
- Langer R, Vacanti JP: Tissue engineering. *Science* 260: 920–926, 1993
- Lee JA, Parrett BM, Conejero JA, Laser J, Chen J, Kogon AJ, Nanda D, Grant RT, Breitbart AS: Biological alchemy: engineering bone and fat from fat-derived stem cells. *Ann Plast Surg* 50: 610–617, 2003
- Lenz JH, Henkel KO, Hingst V, von Versen R, Gundlach KKH: Reconstruction of the frontal calvarian continuity in a child using a freeze-preserved autogenous bone graft. *J CranioMaxillofac Surg* 31: 154–158, 2003
- Lew D, Farrell B, Bardach J, Keller J: Repair of craniofacial defects with hydroxyapatite cement. *J Oral Maxillofac Surg* 55: 1441–1449, 1997
- Mizuno H, Zuk PA, Zhu M, Lorenz HP, Benhaim P, Hedrick MH: Myogenic differentiation by human processed lipoaspirate cells. *Plast Reconstr Surg* 109: 199–209, 2002
- Park J, Ries J, Gelse K, Kloss F, vonderMark K, Wiltfang J, Neukam FW, Schneider H: Bone regeneration in critical size defects by cell-mediated BMP-2 gene transfer: a comparison of adenoviral vectors and liposomes. *Gene Ther* 10: 1089–1098, 2003
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR: Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143–147, 1999
- Pittenger MF, Mosca JD, McIntosh KR: Human mesenchymal stem cells: progenitor cells for cartilage bone fat and stroma. *Curr Top Microbiol Immunol* 251: 3–11, 2000
- Ringe J, Kaps C, Burmester GR, Sittinger M: Stem cells for regenerative medicine: advances in the engineering of tissues and organs. *Naturwissenschaften* 89: 338–351, 2002
- Rock G, Berger R, Lange J, Tokessy M, Palmer DS, Giulivi A: A novel, automated method of temperature cycling to produce cryoprecipitate. *Transfusion* 41: 232–235, 2001
- Schmid D, Thielemann F, Holz U, Herr G: Osteoinduction with the dog tibial defect model. *Unfallchirurgie* 19: 1–8, 1993
- Tatum SA, Kellmann RM: Cranial bone grafting in maxillofacial trauma and reconstruction. *Facial Plast Surg* 14: 117–129, 1998
- Yamada Y, Boo JS, Ozawa R, Nagasaka T, Okazaki Y, Hata K, Ueda M: Bone regeneration following injection of mesenchymal stem cells and fibrin glue with a biodegradable scaffold. *J CranioMaxillofac Surg* 31: 27–33, 2003
- Zuk PA, Zhu M, Mizuno H, Huang JI, Futrell WJ, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH: Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 7: 211–227, 2001
- Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH: Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 13: 4279–4295, 2002

## Dr. S. LENDECKEL, MD

Department of Maxillofacial and Facial Plastic Surgery  
Justus-Liebig-University Medical School  
Giessen 35385  
Germany

Tel.: +49 641 99 46271

Fax: +49 641 99 46279.

E-mail: stefan.lendeckel@chiru.med.uni-giessen.de

Paper received 28 October 2003

Accepted 21 June 2004