Bioceramic Implant Induces Bone Healing of Cranial Defects

Thomas Engstrand, MD,
PhD*
Lars Kihlström, MD†
Kalle Lundgren, MD, PhD*
Margarita Trobos, PhD‡§
Håkan Engqvist, PhD¶
Peter Thomsen, MD, PhD‡§

Summary: Autologous bone or inert alloplastic materials used in cranial reconstructions are techniques that are associated with resorption, infection, and implant exposure. As an alternative, a calcium phosphate–based implant was developed and previously shown to potentially stimulate bone growth. We here uncover evidence of induced bone formation in 2 patients. Histological examination 9 months postoperatively showed multinuclear cells in the central defect zone and bone ingrowth in the bone-implant border zone. An increased expression of bone-associated markers was detected. The other patient was investigated 50 months postoperatively. Histological examination revealed ceramic materials covered by vascularized compact bone. The bone regenerative effect induced by the implant may potentially improve long-term clinical outcome compared with conventional techniques, which needs to be verified in a clinical study. (*Plast Reconstr Surg Glob Open 2015;3:e491; doi: 10.1097/GOX.0000000000000467; Published online 25 August 2015.*)

Autologous bone, inert alloplastic materials, or combinations thereof are today used in cranial reconstructions. Materials used in conventional alloplastic cranial implants are plastics, such as polyether ether ketone, poly(methyl methacrylate), and polyethylene, and metals such as titanium. Although these materials are considered biocompatible, there are major risks for long-term complications, such as extrusion through the skin and infection.[1–3] Recent publications report complications requiring surgical intervention in 20–30% of the cases treated with these methods.[1–9] In this context, the development of alternative techniques appears essential, for example, bone regenerative materials. Osteoconductive implants are based on materials that allow ingrowth from adjacent host bone. Although favorable, osteoconductive properties may not be sufficient to heal large bone defects. Osteoinduction, the stimulation of resident or circulating mesenchymal stem cells to differentiate into osteoblasts, may be required to potentially heal segmental bone defects.[10] However, neither osteoinductive cytokines,

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such as bone morphogenetic protein-2, nor certain bioceramics, with proposed combined conductive and inductive properties, have successfully been used for large bone defect repair in a clinical setting.[11–15] We recently reported the development of a bioactive calcium phosphate–based cranial implant (OssDsign, Uppsala, Sweden) used in a therapy-resistant patient.[16] Bone growth was indicated by ^{18}F-fluoride positron emission tomography/computed tomography after 27 months. The aim of the present investigation was to show new bone formation induced by the ceramic implant. We report concluding results from 2 patients from whom the bioactive implants were either replaced due to aesthetical concerns or surgically exposed for the removal of fixating titanium plates, respectively. This gave an opportunity to inspect the reconstructed areas and obtain biopsies 9 and 50 months after surgery for gene expression analyses and histological examinations.

MATERIALS AND METHODS

Mosaic-designed calcium phosphate implants were manufactured with molding technique as described previously.[16] Both patients were given written information about the procedures. Informed consent was obtained with signed approval to take perioperative biopsies.

Patient 1 was a 41-year-old man who suffered from previously infected bone flap and failed polymethyl methacrylate implant after neurosurgical intervention for the treatment of chronic infection in the frontal sinus area. The frontal bone defect measured approximately $60 \, cm^2$. A customized calcium phosphate implant was manufactured and implanted. Because of aesthetical concerns with a flat contour of the forehead, the implant was surgically removed, and the ceramic implant was replaced after 9 months. Tissue samples for histology ($n = 3$) and gene expression ($n = 10$) were obtained. RNA was extracted and reversed transcribed according to manufacturer's instructions using TATAA GrandScriptTM kit (TATAA Biocenter, Gothenburg, Sweden). Samples were amplified on the LightCycler 480 System (Roche Applied Science, Germany). The genes of interest coded for osteopontin, osteocalcin, collagen 1, calcitonin receptor, and cathepsin K. Biopsies for histological analyses were fixated in formalin, decalcified in formic acid solution, and dehydrated before embedded in paraffin. Ten-micrometer sections were stained with hematoxylin eosin.

Patient 2 was 33 years old and had an approximately $35 \, cm^2$ parietal defect after trauma. He was primarily reconstructed with the ceramic implant 50 months earlier. Lately, the patient had complaints with local discomfort from fixating titanium plates, and indication for reentry was the removal of the plates. Tissue sample from a ceramic tile located at the central part of the implant was obtained by the use of bone nipper. Histological sections were prepared as described above.

RESULTS

Patient 1 (9 Months Postoperatively)

The implant was inspected and appeared without macroscopic evidence of bone deposition (Fig. 1). Histology revealed collagen fibers and blood vessels, whereas no bone was detected (data not shown). Occasional multinuclear cells were detected. In the border between the calvarial defect and the preexisting parietal bone, newly formed bone was in direct contact with the surface of the ceramic tiles.

Gene expression analysis demonstrated a markedly higher expression of osteopontin, osteocalcin, and collagen 1 in the central defect and border sites compared with the parietal bone and soft tissues (Fig. 2). The calcitonin receptor and cathepsin K showed higher expression in the bone defect and in the border to parietal bone than in the parietal bone itself and soft tissue, irrespective of location (data
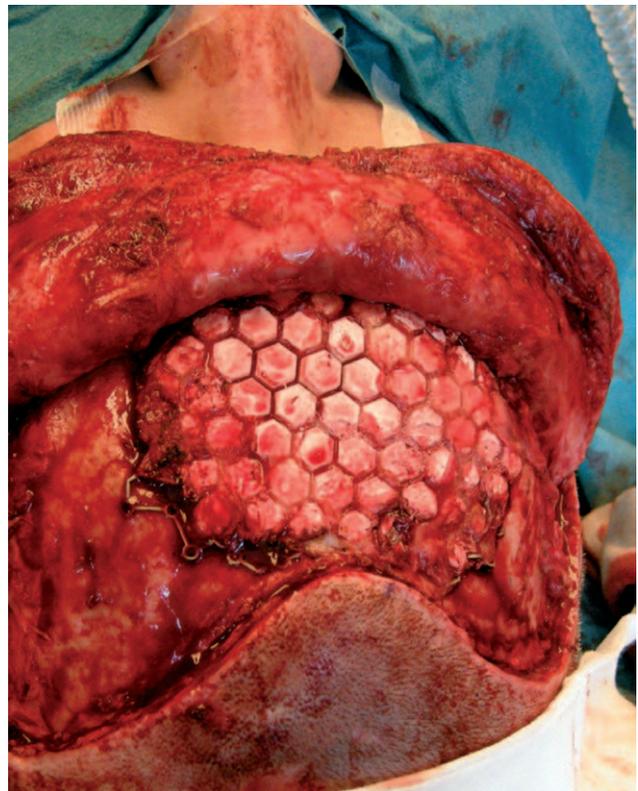

Fig. 1. In patient 1, the reconstructed frontal bone was surgically exposed 9 months after surgery. No macroscopic evidence of bone formation was present in the central part of the mosaic-designed implant. Ceramic tiles located in the cranial-implant border zone appeared integrated with host bone.
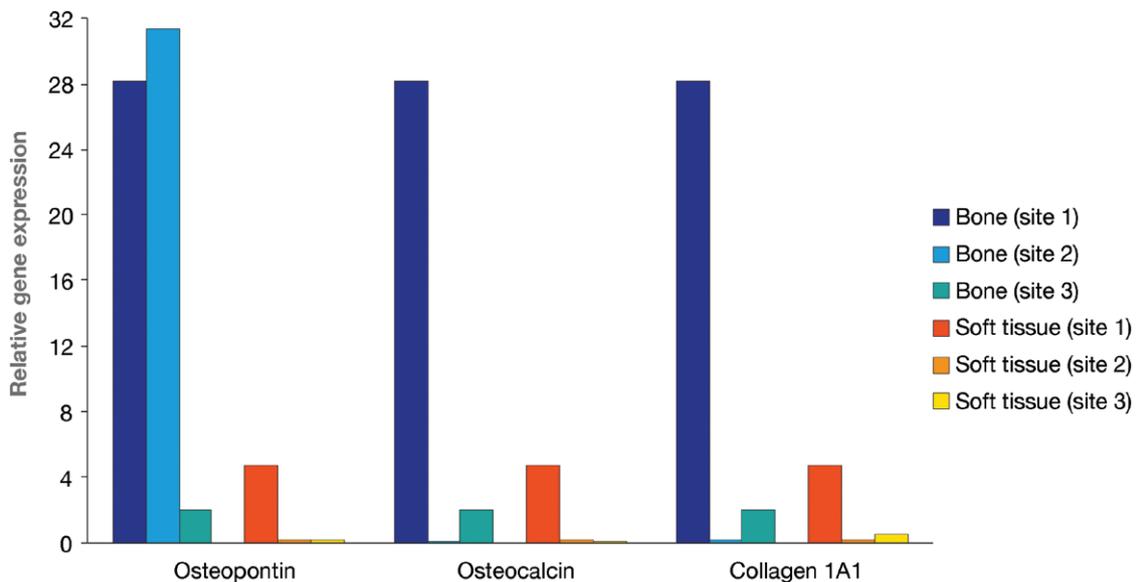
Fig. 2. The high expression of genes associated with bone formation: osteopontin, osteocalcin, and collagen 1 imply intense osteoblastic activity at the implant site 9 months after surgery (patient 1). Site 1 = center of defect; site 2 = border between defect and parietal bone; site 3 = parietal bone distant to defect. Bone = tissue at the level of the implant; soft tissue = tissue between the level of the implant and the skin.

not shown). The expression of transcription factor runx2 was low in all samples tested.

Patient 2 (50 Months Postoperatively)

Gross inspection showed bleeding bone that appeared to cover all ceramic tiles (Fig. 3A). Tiles located heterotopically at the border of the implant seemed integrated with host bone, whereas solid ectopic bone growth appeared on ceramic tiles located in the middle of the implant without bone bridging between tiles. Histological examination from this area revealed compact bone in direct contact with remnants of inert ceramic materials (Fig. 3B). Blood vessels were present within the compact bone.
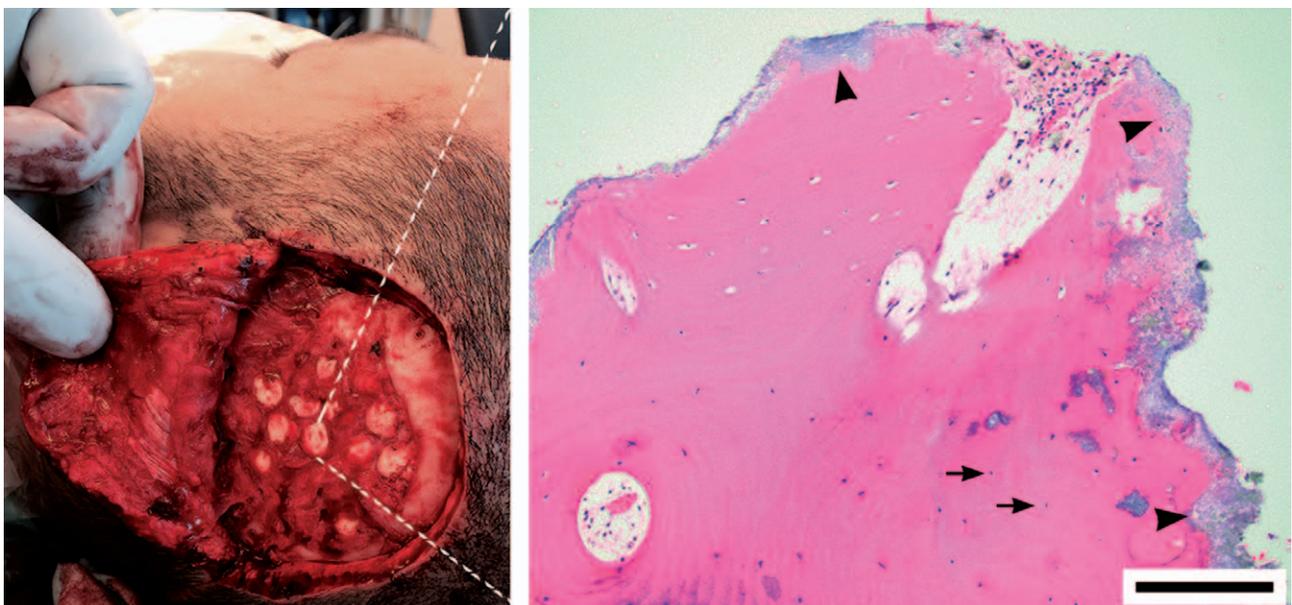
Fig. 3. In patient 2, the reconstructed area was inspected 50 months after implantation. Ceramic tiles were integrated with adjacent soft tissues and osseointegrated with parietal bone in the periphery of the implant. Transformation of ceramic tiles into bleeding bone was macroscopically apparent also in the middle of the reconstructed area. Histological examination from a centrally located tile shows compact bone with numerous osteocytes (some of which are denoted by arrows) and blood vessels. The bone appears in direct contact with remnants ceramic materials (arrowheads). Bar = 200 µm.

DISCUSSION

Although caution is a prime requirement when concluding biological processes based on analyses in few patients, the present case studies provide important information with respect to the feasibility of regenerating bone in major cranial defects. Implants with osteoconductive and possibly osteoinductive properties were shown to induce bone healing of cranial defects in patients as demonstrated by gene expression analyses and histology. The ceramic compound of the implant, comprising monetite, β-calcium pyrophosphate (PPi), β-tricalcium phosphate, and brushite, is intended to chemically resemble native bone and to be part of the process of coupled bone formation. Gene expression analysis 9 months postoperatively indicated osteoclastic activity in parallel with new bone formation. This suggests that cell-mediated resorption of calcium phosphates occurs, which may be a prerequisite for deposition of new bone.[17,18] However, the process implicates a narrow balance between resorption rate and new bone deposition. PPi is essential because removal of this phase provokes enhanced resorption and complete resolution of ceramics within months without new bone formation as shown in large animals (not published). Thus, PPi plays an essential role in controlling resorption rate of calcium phosphates and differentiation of osteoprogenitors and, as a consequence, new bone formation.[19,20] A chart review including more than 100 patients is currently underway to assess clinical outcome and complication rates in patients reconstructed with the use of the bone-stimulatory cranial implant.

CONCLUSION

A bioactive calcium phosphate-based implant was developed as an alternative to conventional inert alloplastic materials used for cranial repair. The implant was shown to stimulate bony healing of cranial defects as demonstrated by gene expression analysis and histology in two patients. We hypothesize that the bone regenerative effects may be advantageous by reducing complication rates in cranioplasty procedures as compared to the use of plastics and titanium.

Thomas Engstrand, MD, PhD
Stockholm Craniofacial Centre
Department of Molecular Medicine and Surgery
Plastic Surgery Section
Karolinska University Hospital and Karolinska Institute
SE 171 76 Stockholm, Sweden
E-mail: thomas.engstrand@karolinska.se

REFERENCES

1. Hill CS, Luoma AM, Wilson SR, et al. Titanium cranioplasty and the prediction of complications. *Br J Neurosurg.* 2012;26:832–837.
2. Thien A, King NK, Ang BT, et al. Comparison of polyetheretherketone and titanium cranioplasty after decompressive craniectomy. *World Neurosurg.* 2015;83:176–180.
3. Wiggins A, Austerberry R, Morrison D, et al. Cranioplasty with custom-made titanium plates—14 years experience. *Neurosurgery* 2013;72:248–256; discussion 256.
4. Bowers CA, Riva-Cambrin J, Hertzler DA II, et al. Risk factors and rates of bone flap resorption in pediatric patients after decompressive craniectomy for traumatic brain injury. *J Neurosurg Pediatr.* 2013;11:526–532.
5. Cheng CH, Lee HC, Chen CC, et al. Cryopreservation versus subcutaneous preservation of autologous bone flaps for cranioplasty: comparison of the surgical site infection and bone resorption rates. *Clin Neurol Neurosurg.* 2014;124:85–89.
6. Dünisch P, Walter J, Sakr Y, et al. Risk factors of aseptic bone resorption: a study after autologous bone flap reinsertion due to decompressive craniotomy. *J Neurosurg.* 2013;118:1141–1147.
7. Ewald C. 122 Risk factors for aseptic bone necrosis following cranioplasty: a multivariate analysis after reinsertion of 500 bone flaps. *Neurosurgery* 2014;61(Suppl 1):199.
8. Martin KD, Franz B, Kirsch M, et al. Autologous bone flap cranioplasty following decompressive craniectomy is combined with a high complication rate in pediatric traumatic brain injury patients. *Acta Neurochir (Wien).* 2014;156:813–824.
9. Schoekler B, Trummer M. Prediction parameters of bone flap resorption following cranioplasty with autologous bone. *Clin Neurol Neurosurg.* 2014;120:64–67.
10. Yuan H, Fernandes H, Habibovic P, et al. Osteoinductive ceramics as a synthetic alternative to autologous bone grafting. *Proc Natl Acad Sci U S A.* 2010;107:13614–13619.
11. Afifi AM, Gordon CR, Pryor LS, et al. Calcium phosphate cements in skull reconstruction: a meta-analysis. *Plast Reconstr Surg.* 2010;126:1300–1309.
12. Arnander C, Westermark A, Veltheim R, et al. Three-dimensional technology and bone morphogenetic protein in frontal bone reconstruction. *J Craniofac Surg.* 2006;17:275–279.
13. Zins JE, Langevin CJ, Nasir S. Controversies in skull reconstruction. *J Craniofac Surg.* 2010;21:1755–1760.
14. de Monaco BA, Fonoff ET, Teixeira MJ. Early resorption of an artificial bone graft made of calcium phosphate for cranioplasty: case report. *Neuropsychiatr Dis Treat.* 2013;9:1801–1802.
15. Cushnie EK, Ulery BD, Nelson SJ, et al. Simple signaling molecules for inductive bone regenerative engineering. *PLoS One* 2014;9:e101627.
16. Engstrand T, Kihlström L, Neovius E, et al. Development of a bioactive implant for repair and potential healing of cranial defects. *J Neurosurg.* 2014;120:273–277.
17. Grossardt C, Ewald A, Grover LM, et al. Passive and active in vitro resorption of calcium and magnesium phosphate cements by osteoclastic cells. *Tissue Eng Part A.* 2010;16:3687–3695.
18. Elgali I, Igawa K, Palmquist A, et al. Molecular and structural patterns of bone regeneration in surgically created defects containing bone substitutes. *Biomaterials* 2014;35:3229–3242.
19. Grover LM, Wright AJ, Gbureck U, et al. The effect of amorphous pyrophosphate on calcium phosphate cement resorption and bone generation. *Biomaterials* 2013;34:6631–6637.
20. Kim HJ, Minashima T, McCarthy EF, et al. Progressive ankylosis protein (ANK) in osteoblasts and osteoclasts controls bone formation and bone remodeling. *J Bone Miner Res.* 2010;25:1771–1783.