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Bone regeneration with glass ceramic implants and calcium phosphate cements in a rabbit cranial defect model

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Abstract Hydroxyapatite cement (BoneSource®) and brushite calcium phosphate cement (chronOS™ Inject) were tested for fixation of glass ceramic implants (Bioverit®) in experimentally created cranial defects in 24 adult New Zealand White rabbits. Aim of the in vivo study was to assess and compare the biocompatibility and osseointegration of the implanted materials. Macroscopic and histological evaluations were performed 1 month, 3 months, and 6 months postoperatively. All implanted materials were well tolerated by the surrounding tissue. Both bone cements exhibited osteoconductive properties. Differences could be detected regarding to the rates of cement resorption and new bone formation. The brushite cement was resorbed faster than the hydroxyapatite cement. The chronOS™ Inject samples exhibited a higher rate of connective tissue formation and an insufficient osseointegration. BoneSource® was replaced by bone with minimal invasion of connective tissue. New bone formation occurred faster compared to the chronOS™ Inject group. Bioverit® implants fixed with BoneSource® were successfully osseointegrated.

E. Beleites—Deceased.

The authors dedicate this article to the memory of Prof. Dr. Eggert Beleites (1939–2006).

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1 Introduction

Deformity of the craniofacial skeleton may arise from various causes like tumor resection, traumatic injuries, infection, or congenital anomalies. Autologous bone grafts are still regarded as the gold standard for many indications in craniomaxillofacial reconstruction. However, the use of autografts is associated with significant disadvantages including increased operation time, donor site morbidity, and limited graft size. There has been an ongoing search to find alternatives for reconstruction with allograft bone substitutes. Last years various nonresorbable alloplastic implant materials have been utilized for functional and structural restoration of craniofacial bone deficiencies, such as metals, silicone elastomers, acrylics, and ceramics. The glass ceramic Bioverit® has been introduced for otosurgical purposes by Beleites and coworkers in 1985 [1]. Bioverit® is not resorbable, biocompatible, corrosion-resistant, and very easily shaped during surgery [2]. This material has been successfully applied in middle ear surgery as in neurocranial and craniofacial surgery [3]. Such glass bioceramic implants individually prefabricated via computer-aided manufacturing have found good acceptance in the reconstructive procedures in skull surgery [4, 5] and in orbital floor reconstruction [6]. Fixation of implants in craniofacial bones sometimes is very difficult because placement of drill holes is limited or not possible in some regions. An alternative possibility for fixation of nonresorbable implants is the usage of free mouldable, resorbable bony substitutes, for example calcium phosphate cement. Calcium phosphate cements have the ability to be replaced by native bone when placed directly adjacent to a bony surface. There are two main types of calcium phosphate cements: hydroxyapatite cements and brushite cements. Most studies have been performed with

hydroxyapatite cements. This material is available for clinical use in cement paste form since 1992 [7]. Several clinical applications in craniofacial surgery are reported with excellent results using hydroxyapatite cement, especially BoneSource® [8–13]. Biocompatibility and bone remodeling of brushite calcium phosphate cements were examined in various applications in vivo [14–17]. Kuemmerle and coworkers [18] evaluated the suitability of the brushite cement chronOS™ Inject for use in craniofacial reconstruction.

In many cases the reconstruction of bone deformities is not possible with bone cements alone but in combination with a permanent implant. Bone cements have limited resistance and should not be used in stress-bearing defects. Free modeling of great and especially of curved defect areas with cement material is connected with major difficulties and provides often poor aesthetic results.

It may be assumed that calcium phosphate cements can be used for fixation of implants in craniofacial bone defects. At the present time, little is known about combinations of such materials and the resulting implant stability depending on equilibrium between bone deposition and cement resorption. The goal of the current study was to evaluate the suitability, biocompatibility, and osseointegration of Bioverit®-implants in combinations with commercially available bone cements (hydroxyapatite cement or brushite cement, respectively) in an animal model studying the effects at 1 month, 3 months, and 6 months after implantation.

2 Materials and methods

2.1 Cement materials

Two bone cements were tested. The first was the apatite cement BoneSource® (Stryker Leibinger Freiburg, Germany). BoneSource® is a mixture of tetracalcium phosphate and dicalcium phosphate. After mixing with water, the components react isothermically and transform into a paste-like substance. The final product is hydroxyapatite.

The second tested calcium phosphate cement was chronOS™ Inject (Mathys Medical Ltd., Switzerland). ChronOS™ Inject consists of β-tricalcium phosphate (β-TCP) powder, monocalcium phosphate monohydrate, magnesium hydrogen phosphate trihydrate, β-TCP granules, and some additives for better setting time. The dry components were mixed with a 5% solution of sodium hyaluronate. The end product of the setting reaction is biphasic consisting of β-TCP granules embedded in a dicalcium phosphate dihydrate matrix (brushite). The setting process of chronOS™ Inject is not adversely affected by fluid contacts. In contrast, BoneSource® must be kept dry to set.

2.2 Study design and surgical procedure

Our established rabbit skull model for calvarial defects [2] was used for this study. With permission for the animal study (Registration number 02-19/01, Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz), a total of 24 female New Zealand White rabbits were used as the experimental model. Anesthesia was achieved with ketamine (15 mg/kg) and xylazine (6 mg/kg). The dorsal area of each rabbit's cranium was shaved. Every rabbit underwent a procedure involving a creation of three skull defects, two 7 mm and one 5 mm in diameter (Fig. 1). The underlying dura was removed. A cylindrical glass ceramic implant (Bioverit II®, 3di GmbH Jena, Germany) was placed in the 5 mm defect (using as controls). A Bioverit®-implant was fixed with bone cement in one 7 mm defect (Fig. 2). Two groups of 12 rabbits were used: in one group the implants were fixed with BoneSource® and in the other group chronOS™ Inject was used for fixation of the implants. The third bony defect was closed with the respective bone cement alone. After a setting time of 5–10 min for BoneSource® and 12 min for chronOS™ Inject the skin was closed with resorbable suture (Polyglactin). After 1, 3, and 6 months, four animals at each time period and from each group, respectively, were sacrificed.

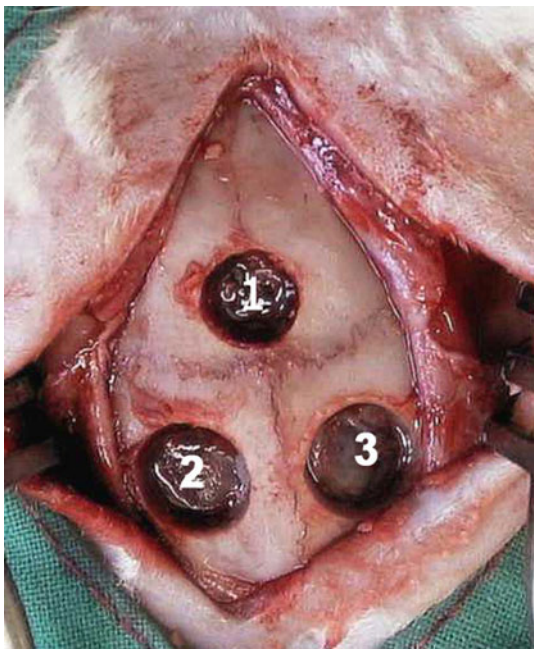

Fig. 1 Intraoperative view of the rabbit skull after drilling the defect holes to fill with Bioverit®-implant without BoneSource® (control) (1), BoneSource® (2), and Bioverit®-implant with BoneSource® (3)

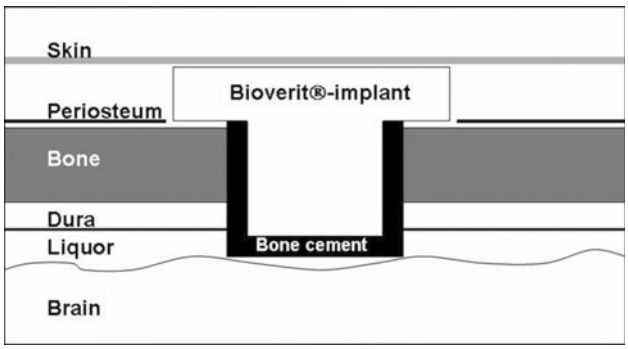

Fig. 2 Schematic drawing of the skull model with a prepared calvarial defect and implanted materials. In the animal experiments the diameter of the defect hole was 7 mm, of the implant 5 mm, and of the implant head 9 mm

2.3 Macroscopic and microscopic evaluation

Macroscopic appearance of the defects was assessed considering the stability of the implants, the cement incorporation, obvious cement resorption and tissue reaction adjacent to the implanted materials. The area carrying the reconstructed skull defects was removed en bloc for histological analysis. Brain biopsies were taken selectively from areas under the skull defects and from control areas.

The bony specimens were fixed in 5% buffered formalin and dehydrated in serial concentrations of ethanol. According to the method of Donath and Breuner [19] for the investigation of undecalcified bone, the specimens were embedded in hydroxyethylmethacrylate (Technovit 7200 VLC, Heraeus Kulzer Germany). After embedding the samples were cut into slices of approximately 200–300 μm using a micro-grinding system (Exakt Apparatebau Norderstedt, Germany). The samples were ground and polished to a thickness of 5–10 μm and were stained with hematoxylin-eosin.

The brain biopsies were fixed in formalin, embedded in paraffin, sectioned at 8 μm, and stained with hematoxylin-eosin (HE stain) or modified Masson's trichrome stain (MT stain), respectively. All slices were analyzed with a light microscope.

3 Results

3.1 Surgeries

All surgeries of the rabbits went well. All animals in the experimental study survived the duration of the experiments. Postoperative healing was uneventful. There were no wound infections or wound-related complications. During the observation time none of the animals exhibited any behavioral changes.

3.2 Macroscopic evaluation

All Bioverit®-implants and cements seemed to be well incorporated and did not elicit an obvious inflammatory reaction in the adjacent soft tissue. In all animals a thin layer of connective tissue was apparent overlying both implants and the bone cement. The implanted materials were incorporated within the surrounding bony structures. In the drill holes with cement alone the material appeared to be partially resorbed after 3 and 6 months but it was still detectable macroscopically. In the chronOS^{TM} Inject group, displacement of cement particles from drill holes in surrounding tissues was noticed in three of the specimens after 1 month and in one specimen after 3 and 6 months, respectively.

At all time periods, most of the Bioverit®-implants with BoneSource® or chronOS^{TM} Inject were better fixed than the implants without any bone cement. No difference of the fixation effectiveness verified by palpation could be found between the BoneSource® group and the chronOS^{TM} Inject group.

3.3 Histological evaluation

3.3.1 BoneSource® group

Over the subsequent months, the histological examinations demonstrated a progressive cement resorption and new bone formation around the Bioverit®-implants. BoneSource® was replaced by bone with minimal invasion of fibrous tissue. At all time periods the nonresorbable implants fixed with BoneSource® were surrounded by a thin connective tissue layer. At some points new bone formation was visible in direct contact to the Bioverit®-implant without fibroconnective tissue (Fig. 3a). Bone regeneration could also be seen to extend under the cement layer that was filled in the drill hole. The resorption and replacement of the hydroxyapatite cement by native bone occurred not only at the periphery. New bone and blood vessel formation was also detected within the cement matrix. Six months postoperatively, intensive bone formation was ongoing although a thin core of connective tissue remained. BoneSource® was present in many fragments incorporated within the new surrounding bony structures. But maximum half of the original cement matrix was replaced by bone (Fig. 5c, d).

Bioverit®-implants without bone cement distinctly have more fibrous tissue than implants with bone cement at all time points. Some animals exhibited a gross layer of connective tissue (Fig. 4a) and in sections from other animals a conspicuous gap between implant and tissue was detected. Formation of new bone did not occur in close proximity of the implants.

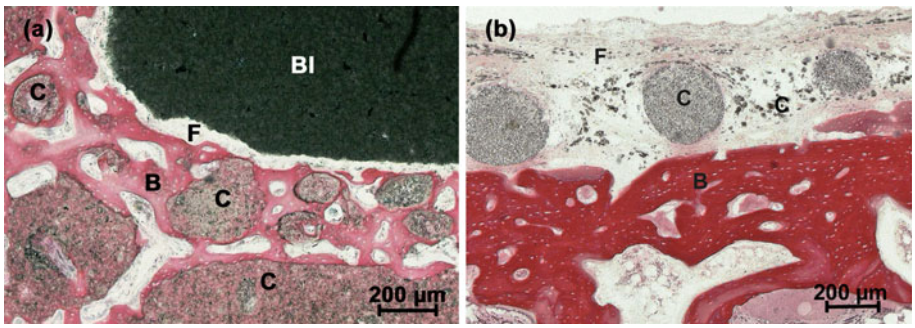

Fig. 3 One month postoperatively; HE stained slices. **a** Defect filled with Bioverit®-implant (BI) and BoneSource® hydroxyapatite cement. (C) Bone (B) growth along the sides of the implant is seen with a thin fibroconnective interface (F). Note the cement fragmentation and the direct bone bonding to the material. **b** Displaced chronOS™ Inject cement material (C) in the fibroconnective tissue (F) above the cranial bone (B) The brushite matrix and the TCP-granules are remote to the original implantation site. [HE stain]

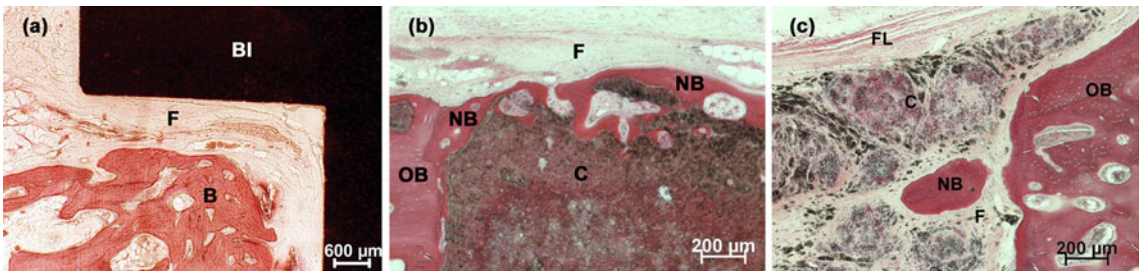

Fig. 4 Three months postoperatively; HE stained slices. **a** Control defect with Bioverit®-implant (BI) without cement. A gross layer of connective tissue (F) developed between the cranial bone (B) and the implant. **b** Defect treated with BoneSource® cement (C) alone demonstrating the border between old bone (OB) and new bone (NB). A good bone contact against the hydroxyapatite cement material is found. A fibrous layer (F) with adipocytes overlays the defect hole. **c** At the edge of the original drill hole treated with chronOS™ Inject (C) alone. The area of the defect is filled with partially degraded cement material surrounded by connective tissue (F) and sparse islets of new bone (NB). A fibrous layer (FL) overlays the defect; old bone (OB) on the right

Sections of bone defects filled with BoneSource® alone showed that the area of residual cement decreased over the observation period. But at 6 months, nearly two-thirds of BoneSource® was still present. In the 1 month specimens, the hydroxyapatite cement appeared as a consolidated bulk. Three and 6 months postoperatively, a layer of new bone over and under the cement matrix was observed. In addition, there was new bone as insular pattern in the cement area. Most bony islands were in direct contact with BoneSource® without an intervening layer of connective tissue. At the edges of the defect holes old and new bone adjoined (Fig. 4b).

In all samples no acute or chronic inflammatory cells were seen at any time point. Only a mild inflammatory reaction with granulocytes was seen in three specimens. In the brain sections no inflammatory changes and no cellular infiltrates occurred.

3.3.2 *ChronOS™ Inject group*

The histological examination of the specimens treated with chronOS™ Inject demonstrated progressive cement resorption, which did not occur parallel to new bone formation. Much connective tissue and adipocytes were noted around the Bioverit®-implants. Non-resorbed chronOS™ Inject material was distributed in that tissue layer. The remaining cement was a combination of brushite matrix and β-TCP granules (granule diameter 0.2–0.25 mm) (Fig. 5a). The brushite matrix was resorbed faster than the TCP granules. In some cases the connective tissue partly showed a mild inflammatory reaction. One month postoperatively, no osseointegration of the implants was found. At 3 months, partially newly formed bone was noticed as bony islets in the connective tissue around the Bioverit®-implants. New bone could also be found in direct contact with resorbing β-TCP granules. Three months after surgery, foreign body giant cells were noticed in two animals. In the 6 months specimens more bone formation occurred and the amount of remaining cement decreased (Fig. 5b).

Defects in calvarial vault treated with chronOS™ Inject alone were characterized by a great amount of fibroconnective tissue. No bone formation was visible 1 month postoperatively. After 3 and 6 months, the defect holes exhibited decreased amount of cement material and several new bony islets embedded in connective tissue (Fig. 4c).

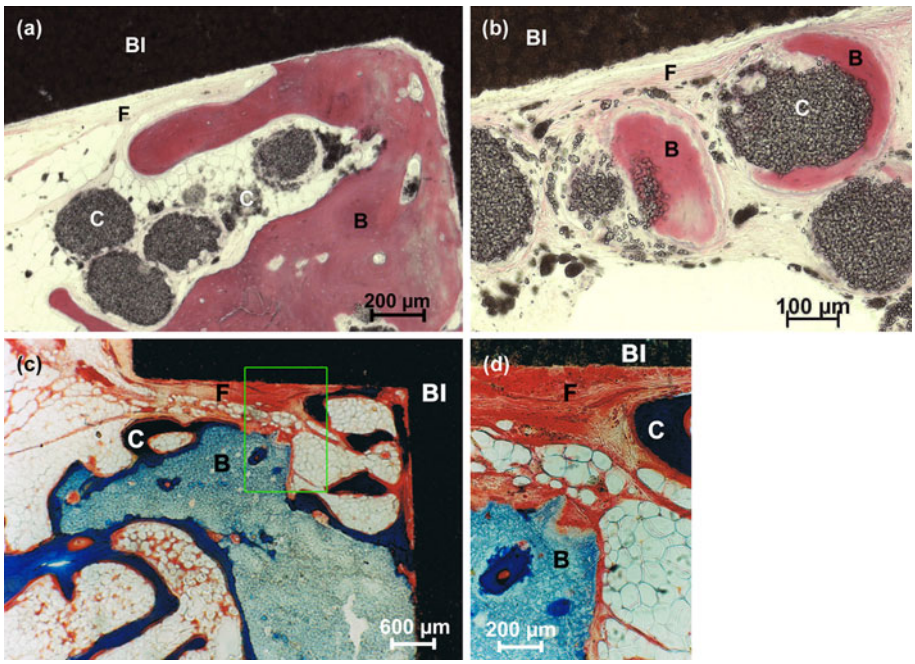

Fig. 5 Six months postoperatively; **a**–**b**: HE stained slices; **c**–**d**: MT stained slice. **a** Defect treated with Bioverit®-implant (BI) and chronOS^{TM} Inject brushite cement (C) Fibroconnective tissue (F) with residual brushite matrix and β-TCP granules is filling large areas. The section shows good bone (B) contact to the implant at some sites. **b** ChronOS^{TM} Inject (C) and fibrous tissue (F) beneath the Bioverit®-implant (BI). Note newly formed bone (B) adjacent to the dissolving TCP-granules. **c** Defect filled with Bioverit®-implant (BI) and BoneSource® cement (C). A fibrous layer (F) with adipocytes enclosing bone cement particles (C) overlays large parts of defect area. The Bioverit®-implant has partially direct contact to bone (B). **d** Enlarged clipping of (**c**), indicated by a green frame in (**c**)

In half of the specimens loss of implant volume was observed. Impressions at the upper and the lower side of the calvarium were visible.

In all animals of this group cement displacement concerning the brushite matrix and the TCP granules was noted in the histological examination. Migrated cement particles were found in the connective tissue layer over the Bioverit®-implants, around the implants without chronOS^{TM} Inject and in the tissue overlying the cranial bone at a great distance from the initial implanted cement material. Displaced brushite matrix and granules were always surrounded by connective tissue (Fig. 3b).

The same results as in the BoneSource® group could be found in the sections of the control drill holes (Bioverit®-implants without cement) except for displaced cement matrix. On histological examination there was no evidence of adverse reaction in brain underlying the reconstructed area.

4 Discussion

In the present study, the biocompatibility of Bioverit®-implants in combinations with calcium phosphate cements was investigated in calvarial defects in a rabbit animal model over a time span of 6 months. Important properties of biomaterials for bone reconstruction are osteoconductivity, biocompatibility, and mechanical strength. Bone cement could be an excellent bone replacement material in combination with internal fixation devices that guarantee the mechanical stability within the bone defect. Such resorbable cement should be replaced by normal bone tissue ideally with close contact between cement and bone and no fibrous tissue may be interposed. The ingrowth of fibrous tissue inhibits bone regeneration.

The study showed that all implant devices were well tolerated by the tissue. All Bioverit®-implants with BoneSource® and chronOS^{TM} Inject exhibited good fixation on palpation and were better fixed than without any cement material. Over the time the amount of remaining cement decreased, but both cement materials were not resorbed completely. Differences between the two bone cements were found with regard to the rate of resorption and new bone formation. Published reports indicate that brushite phosphate cements seem to be resorbed much faster compared to apatite [14, 17, 20]. Our findings correspond to those reports. We observed the faster resorption of chronOS^{TM} Inject in comparison to BoneSource®. But the chronOS^{TM} Inject cement matrix was resorbed faster as new bone formation took place.

Good bone formation around the Bioverit®-implants occurred in the specimens treated with BoneSource®. The

newly formed bone was observed in close proximity to the nonresorbable implants partially with direct bone contact and no or less interposed fibroconnective tissue layer. Excellent bone attachment to the surface of the cement material was noted. It may be assumed that the cement provides a biological scaffold for bone formation. The growth of new bone in direct contact with the Bioverit®-implants and the resorbable bone cement is an indication of good biocompatibility. The replacement of BoneSource® by bone did not result in a loss of volume within the reconstructed area. Within our observation time of 6 months, a high proportion of incomplete resorbed cement remained. These findings are in agreement with those of other studies [21–23]. In contrast, in minipigs it was shown that BoneSource® was nearly completely resorbed and replaced by new bone 40 weeks after implantation [12, 24]. Long-term results from craniofacial reconstruction by patients did not show complete osseous replacement of hydroxyapatite cements [13].

There was a tendency for the chronOS^{TM} Inject samples to have a higher rate of fibroconnective tissue compared to the BoneSource® group. The calvarial defects treated with cement alone were filled partially with new bone but for the majority of the defect region with connective tissue. Insufficient osseous regeneration occurred also in the defect areas with Bioverit®-implants fixed with chronOS^{TM} Inject. This finding corresponds to the experimental study in the parietal bone of adult sheep [18]. The degradation of the cement, consisting of brushite matrix and β-TCP granules, is too quick in relation to the slow bony regeneration of the skull. As described in previous studies [14, 16, 25], the brushite matrix was resorbed faster compared to the TCP granules. These granules seemed to serve as nucleus for new bone formation. The TCP granules had either direct contact to newly formed bone or were incorporated in new bone. This is in accordance to other studies demonstrating this effect in a sheep model with bone defects in the limbs, but already after 4 weeks [14, 25].

Our results indicate that BoneSource® is a good bone replacement material for cranial defects. It exhibited a sufficient osseointegration in combination with Bioverit®-implants. On the other hand chronOS^{TM} Inject is not entirely suitable for reconstruction of craniotomy defects with or without nonresorbable implants.

5 Conclusion

In the present study all implanted materials were well tolerated by the surrounding tissue and showed good biocompatibility. Both cement materials exhibited osteoconductive properties, but the evaluation of osseointegration gave rise to diverse results. We conclude that BoneSource® produced better results than chronOS^{TM} Inject concerning the amount of bone formation as well as osseous fixation and anchorage of nonresorbable implants. Bioverit®-implants fixed with BoneSource® exhibited successful osseointegration. Due to the solid growth of fibroconnective tissue at the bone-implant interface and the insufficient bone anchorage of the implants in the chronOS^{TM} Inject group it can not be considered as successfully osseointegrated.

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References

1. Beleites E, Neupert G, Augsten G, Vogel W, Schubert H. Scanning electron microscopy study of cell growth on mechanically produced biovitroceramic and carbon glass in vitro and in vivo. Laryngol Rhinol Otol (Stuttg). 1985;64:217–20.
2. Schneider G. Zur Osteoinduktion und Korrosion bei Implantation der Glaskeramik Bioverit - eine tierexperimentelle Studie. [Thesis]. Jena: Friedrich Schiller University; 1998
3. Beleites E, Rechenbach G. Implantologie in der Kopf-Hals-Chirurgie - gegenwärtiger Stand. In: Ganz H, Schätzle W, editors. HNO Praxis Heute. 12: Berlin, Heidelberg: Springer; 1992. p. 170–199.
4. Beleites E, Schneider G, Fried W, Schumann D, Linß W. 3-D-Artificial implants for bone defects of the skull. Dtsch Ärztebl. 2001;98:244–8.
5. Siebert H, Schleier P, Beinemann J, et al. Evaluation of individual ceramic implants made of Bioverit with CAD/CAM technology to reconstruct multidimensional craniofacial defects of the human skull. Mund Kiefer Gesichtschir. 2006;10:185–91.
6. Klein M, Glatzer C. Individual CAD/CAM fabricated glass-bioceramic implants in reconstructive surgery of the bony orbital floor. Plast Reconstr Surg. 2006;117:565–70.
7. Costantino PD, Friedman CD, Jones K, Chow LC, Sisson GA. Experimental hydroxyapatite cement cranioplasty. Plast Reconstr Surg. 1992;90:174–85.
8. Burstein FD, Williams JK, Hudgins R, et al. Hydroxyapatite cement in craniofacial reconstruction: experience in 150 patients. Plast Reconstr Surg. 2006;118:484–9.
9. Friedman CD, Costantino PD, Takagi S, Chow LC. BoneSource hydroxyapatite cement: a novel biomaterial for craniofacial skeletal tissue engineering and reconstruction. J Biomed Mater Res. 1998;43:428–32.
10. Friedman CD. Future directions in biomaterial implants and tissue engineering. Arch Facial Plast Surg. 2001;3:136–7.
11. Hollier LH, Stal S. The use of hydroxyapatite cements in craniofacial surgery. Clin Plast Surg. 2004;31:423–8.
12. Verheggen R, Merten HA. Correction of skull defects using hydroxyapatite cement (HAC)—evidence derived from animal experiments and clinical experience. Acta Neurochir (Wien). 2001;143:919–26.
13. Verret DJ, Ducic Y, Oxford L, Smith J. Hydroxyapatite cement in craniofacial reconstruction. Otolaryngol Head Neck Surg. 2005;133:897–9.

14. Apelt D, Theiss F, El-Warrak AO, et al. In vivo behavior of three different injectable hydraulic calcium phosphate cements. Biomaterials. 2004;25:1439–51.
15. Lu JX, About I, Stephan G, et al. Histological and biomechanical studies of two bone colonizable cements in rabbits. Bone. 1999;25:41S–5S.
16. Oberle A, Theiss F, Bohner M, et al. Investigation about the clinical use of brushite- and hydroxylapatite-cement in sheep. Schweizer Archiv für Tierheilkunde. 2005;147:482–90.
17. Ohura K, Bohner M, Hardouin P, et al. Resorption of, and bone formation from, new beta-tricalcium phosphate-monocalcium phosphate cements: an in vivo study. J Biomed Mater Res. 1996;30:193–200.
18. Kuemmerle JM, Oberle A, Oechslin C, et al. Assessment of the suitability of a new brushite calcium phosphate cement for cranioplasty - an experimental study in sheep. J Craniomaxillofac Surg. 2005;33:37–44.
19. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Säge-Schliff (sawing and grinding) technique. J Oral Pathol. 1982;11:318–26.
20. Munting E, Mirtchi AA, Lemaitre J. Bone repair of defects filled with phosphocalcic hydraulic cement: an in vivo study. J Mater Sci. 1993;4:337–44.
21. Indovina A Jr, Block MS. Comparison of 3 bone substitutes in canine extraction sites. J Oral Maxillofac Surg. 2002;60:53–8.
22. Gosain AK, Riordan PA, Song L, et al. A 1-year study of hydroxyapatite-derived biomaterials in an adult sheep model: III. Comparison with autogenous bone graft for facial augmentation. Plast Reconstr Surg. 2005;116:1044–52.
23. Friedman CD, Costantino PD, Jones K, et al. Hydroxyapatite cement. II. Obliteration and reconstruction of the cat frontal sinus. Arch Otolaryngol Head Neck Surg. 1991;117:385–9.
24. Rupprecht S, Merten HA, Kessler P, Wiltfang J. Hydroxyapatite cement (BoneSource) for repair of critical sized calvarian defects—an experimental study. J Craniomaxillofac Surg. 2003;31:149–53.
25. Theiss F, Apelt D, Brand B, et al. Biocompatibility and resorption of a brushite calcium phosphate cement. Biomaterials. 2005; 26:4383–94.