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Bone Augmentation by Replica-Based Bone Formation

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Abstract

Objectives:
The sources of iliac crest bone grafts are limited. Alternatives are evaluated due to the progress in biomaterial sciences. Synthetical hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) or biphasic compounds, or even a mélange of HA and β-TCP will replace bovine ceramics. The goal is maintenance of replica-based-bone formation (RBBF) for bone augmentation.

Methods:
2 female and 2 male patients between 41 and 73 years with 5 sinus elevations were evaluated. Sinus elevations with lateral fenestration, trapezoidal-muco-periosteal flaps and filling with micro-chambered beads (1.5 mm) was performed. A porcine-collagenous membrane and the refixated flap covered the defect. A biopsy program over 20 months was confirm confirm the maintenance of the newly formed bone.

Results:
A fast bone formation was pronounced. The biopsies revealed mature lamellar bone and full osseointegration of the β-TCP implant. The biopsy after 20 months showed compact bone with osseointegration of minor rests of the ceramic implant. The defect revealed a mature bone stock already after 5 weeks.

Significance:
The introduction of the replica-based-bone formation (RBBF) around micro-chambered beads will change the paradigm of bone augmentation. The next step of the ongoing study has to redefine the interval for implant insertion.
The clinical approach confirms the breakthrough to primary mature lamellar bone formation and will permit reduction of placement time for a dental implant.
Introduction

Autologous iliac crest bone-graft (ICBG) is still considered the gold standard for bone grafting. The amount of autologous bone which can be gathered from the iliac crest is defined and only accessible by surgeons. The arguments in favor of searching for alternatives are first, not to prolong the operation by a second intervention, and second, to avoid donor bed morbidity [1]. The science of bone substitutes has made enormous progress, the results of which are approaching the success of autologous cancellous bone or are even surpassing it, if combined with growth factors or micro-chambered beads (MSCs) [2].

The main objective of this study was to evaluate a bone graft substitute (BGS) which provides fast bone formation, full biocompatibility, no immune response, osteogenicity and osteoinduction as well as osteoconduction to replace ICBG, thus changing the paradigm. Another objective concerns the indication of hydroxyapatite (HA) or β-tricalcium-phosphate (β-TCP)? Do we need both or even a mélange due to their different biomechanical properties? Finally, which morphology and what's about the combination with autologous morselized bone, growth factors or even MSCs?

The terminology is mainly influenced by the market. Starting with market research, we find the NIH definition of biomaterials: "Any substance (other than a drug) or combination of substances, synthetic or natural in origin, which can be used for a long time, as a whole or as a part of a system that treats, augments, or replaces any tissue, organ, or function of a body. The market can be segmented based on the material used for implants into metallic, ceramic, polymer and natural biomaterials", including all arthroplasties and dental implants. The definition of orthobiologics is more restricted: "Orthobiologics mainly refers to products that combine both biology and biochemistry for replacement or regeneration of musculoskeletal structures. These products include allografts, bone and soft-tissue substitutes, tissue-engineered substances, and cell-based matrices" [Allied Market Research: Portland, OR 2018]. Demineralized bone matrix (DBM), as well as allografts are predominant products in US, whereas in Europe and especially in Asia, synthetic ceramics are common. Collagenous membranes and mixtures of collagen with calcium phosphates or sulfates, as well as bone-morphogenetic protein (BMP) and MSC for local tissue engineering, play a more dominant role in those disciplines [3].

The results with DBM are often reported as comparable to ICBG [4]. In a study on sheep, comparing DBM with β-TCP + BMP-7, delayed healing with clear signs of immune response were reported, whereas bone-healing processes, bone remodeling and even resorption
of β-TCP were enhanced by BMP-7 [2]. rhBMP-2 and rhBMP-7 represent the most frequently studied growth factors for orthopedic and maxillo-facial indications [5].

Side effects of BMP are not only heterotopic-bone formation, but also local edema and infection. Even osteolysis and antibody reactions were published [6]. The risk of malignancy was considered more theoretically [7]. In the Draenert et al. sheep study [2], no heterotopic bone formation was observed due to the slow release from the carrier.

Mineralized cancellous-bone allografts are common in enhancing augmentations with autologous-cancellous bone; demonstrating good results and complete healing after a 14-year follow up [8,9]. Despite any histocompatibility matching [10], allografts remain controversial with respect to delayed healing, transmission of tumors and infections [11]. In summary, allografts are in fact unlimited with respect to the amount needed. There are, however, several arguments in favor of searching for bone substitutes which fulfill all of the criteria for replacing autologous and, particularly, homologous grafts.

**Alloplasts and Heteroplasts:**

The following materials are available on the market: HA and β-TCP or biphasic materials combining both in a defined composition [12]. FDA approved ceramic BGSs for dental, oral and maxillo-facial application are the synthetic Cerasorb®, a β-TCP and Osbone® a synthetic hydroxyapatite, as well as the bovine HA Endobon® and BioOss®, mainly for augmentation or reconstruction of the alveolar ridge and filling of infra-bony periodontal defects and after root resection [13,14].

All ceramic BGSs on the market morphologically present scaffolds similar to cancellous bone, which implies that ingrown bone presents a replica of all bone marrow spaces, forming so-called bony balls, and needing remodeling during the following 4 to 6 months [15].

The stiffness of HA-implants results in slow osseointegration due to stress protection, whereas bone ingrowth into the more soluble β-TCP is faster due to its decrease in stiffness as water is absorbed [2]. As a result of several animal experiments, it was concluded that the bone-forming element takes the shape of a bead. A conglomerate of beads presents an osteoconductive replica of all bone-marrow spaces, thus providing primary formation of physiological-cancellous bone, or *replica-based bone formation* (RBBF) [16].

**Purpose**

The purpose of this study is to evaluate RBBF with micro-chambered-beads®(MCB®) for bone augmentation specifying early mature bone formation in monthly steps and its maintenance for
implant anchorage as a scientific basis for an ongoing clinical study to evaluate the earliest possibility for implant insertion. In order to achieve this, the strength of the newly formed bone at different postoperative intervals must be examined.

Material and Methods

4 patients, requiring a total of 5 sinus elevations, were operated on: 2 female patients, aged 59, on both sides, and 73 underwent operations, as well as two male patients, aged 66 and 41, respectively (Fig. 1 d). MCB® (Fig.1 a), consisting of pure β-TCP measuring 1.5 mm in diameter and providing high capillary forces, were implanted. In all cases, a porcine-collagen membrane was applied. Precise diamond-coated instrumentation was used for gathering biopsies. The inclusion criteria for this first follow up were sinus-floor augmentation for implant anchorage in otherwise healthy patients. The indication was based on the panoramic radiograph measuring the bony-sinus floor less than 5 mm. Anesthesia was achieved by means of local vestibular anesthesia by infiltration in combination with block anesthesia of the major palatine nerve. The incision was started along the maxillary ridge with the small base of a trapezoidal muco-periosteal flap preserving the vascularization of the tissue. The bone fenestration comprised a longitudinal rectangle or ellipsoid of 15 – 20 mm x 10 – 15 mm and was performed with a round, water-cooled bur. The preparation of the Schneider membrane was performed until a satisfying cavity was formed for the beads. The still adherent bone cover was carefully placed craniomedially to form the apical roof of the cavity. The ceramic beads had been prepared in a bowl or in a syringe with blood and Ringer solution and then precisely inserted into the cavity without damaging the bead shape. A tight package was achieved by covering the defect with a wet sponge and gently injecting Ringer or physiological NaCl solutions to distribute the beads within the defect.

In all cases, the filled cavity was covered by a porcine collagenous membrane and the flap was repositioned and sutured with single-stitch sutures. Wound healing was checked on a daily basis and the sutures were removed on the 7th day. The interval between sinus elevation and augmentation was between 4.5, 11 and 20 months, respectively, depending upon the patience of the recipients, thus fulfilling the study design. An examination of the bone quality at various intervals was conducted to confirm the strength of bone under unloaded conditions. The preservation of strong bone is referred to as its maintenance.
Results

3 beads of MCB\textsuperscript{®} suctioned in 1/20 cc of blood and bone marrow (Fig.1 b,c). All interventions were performed uneventfully. There was a single one-tooth alveolar gap (OTAG), one two-tooth alveolar gap (TTAG) and three free-end maxillary ridge (FEMR) segments. The step-by-step operation was identical for all patients: ridge incision, trapezoidal-flap preparation, fenestration of the bone and preparation of the Schneider membrane, filling with MCB\textsuperscript{®}, and packaging the sponge-covered beads with a jet. In all patients, bleeding was stopped by the MCB\textsuperscript{®}. On the OTAG digital volume tomography (DVT), the augmented floor bottom was presented (Fig.2 a-d). Wound healing was uneventful in all cases, the suture material was removed after 7 days. After 5 weeks, the X-ray of the OTAG revealed an already complete osseointegration of the implant: new mature bone had formed extending from the adjacent alveoli (Fig.2 e, arrows), newly-formed bone had grown over the beads like a shell and had extended into the pores of the MCB\textsuperscript{®}. Most of the ß-TCP ceramic had already been reabsorbed. Eleven weeks after the operation, newly formed bone had been reinforced (Fig.2 f) and the mineralization process had advanced. Three and a half months after the operation, the implantation was preplanned with a DVT. Minor residuals of the implant had not yet been reabsorbed. The implant could be inserted into a compact bone stock with a perfect seat (Fig.2 g,h).

The TTAG was filled with 2.5cc of MCB\textsuperscript{®} Syntricer\textsuperscript{®} (Fig.3 a) which was covered by a porcine collagenous membrane and the mucosa flap. The patients and an ethics committee could follow the augmentation over 20 months under unloaded conditions without implant. The implants were inserted after preoperative planning. One implant bed was prepared using a diamond-coated tool to collect a biopsy (Fig.3b,c). The newly formed bone was very hard, and the biopsy gathered with the wet-grinding diamond technology comprised a cylinder of nearly compact bone: The 3D µ-CT (Fig.3 d) and the single 2D-slices of the µ-CT revealed mature compact bone with lamellar trabeculae and marrow honeycombs. Residuals of the ceramic were still osseointegrated (Fig.3 e). The non-de mineralized histology of alkaline-fuchsin-stained sections revealed fully mineralized trabeculae with red osteocytes and red-osteoid seams with osteoblast layers; the bone marrow spaces were comprised of fibrous tissue revealing the path of the tricalcium-phosphate material under degradation and final reabsorption, as well as cell-rich bone marrow; microradiographs showed fully mineralized mature lamellar bone, which was still integrating ceramic residual (Fig. 4a-c). The implants were firmly screwed into hard and compact bone and the follow up after 4 years and 5 months confirmed stable implantation (Fig.4 d). Even the free-end situation had been successfully
augmented with 2.5 cc MCB® β-TCP. The X-ray after 7 weeks showed neat, newly formed bone stock. After 7 months, the DVT demonstrated complete reabsorption of the ceramic and a physiological and strong bone stock, into which the implant could be safely implanted (Fig. 5 a-d, arrow). In all patients, a mature and lamellar bone stock allowed the insertion of tooth implants, which could be followed for up to 4 years and 5 months. All implants were firmly osseointegrated without radiolucencies.

**Discussion**
There are some limitations to the study. The number of patients is small, the bone quality before intervention was not classified, and there was no direct comparison in the study with granulated material offered on the market. The first step of the follow up, however, had aimed to show just how physiologically and rapidly the new bone had formed like a shell around the bead. This key finding should be made accessible for further studies. A key finding does not require many cases.

Kaufmann [17] accurately summarized the task of sinus elevation or sinus graft as a development which increases the height of bone available for implant placement. He identified four categories for the placement of graft material between the sinus membrane and the mucosa: autografts, allografts, synthetical alloplasts and xenografts.

The autologous grafts are still considered the gold standard [18] in comparison to bovine hydroxyapatite. The Benlidayi et al. [19] retrospective study with 34 patients, however, observed no difference between the two materials. Szabo et al. [20] published a retrospective study of a 6-month follow up of 42 bilateral sinus elevations with the implantation of β-TCP implant Cerasorb® compared with autologous grafts and found no differences between both materials. Autologous bone, together with Cerasorb® gathered either from the mandibula or symphysial bone, were compared by Weijs et al. [21]. The authors found no difference with respect to osseointegration. Stevanovic et al. [22] compared a biphasic calcium phosphate as compound to a polylactide formulation with the β-TCP Cerasorb® and found here a slightly greater reduction of the bony pocket depth than with the stiffer biphasic calcium phosphate.

BioOss® was always combined with autologous grafts [23,24]. De Angelis et al. [25] observed increased marginal bone along the interface of bovine HA implants, which was interpreted by Draenert et al. [15] biomechanically by the stiffness of the material. A study was published which made a direct comparison between both bovine HA materials Endobon® and BioOss® in a prospective and randomized control trial. It revealed no differences between these two materials and all 38 patients showed complete osseointegration.
Despite a lack of observable differences in all these studies, their limitations still have to be considered, since no morphometry was performed and the study designs did not allow for differentiated conclusions. The search for an alternative graft material is still significant because autologous bone is limited, especially if a second surgical approach is requested. This is a situation in which donor bed morbidity represents a dreaded complication [26].

The allograft bone was applied as DBM or mainly deep-frozen, fully mineralized fresh cadaver bone, or sometimes freeze-dried or gamma-irradiated, and often applied as a mélange of autogenous and allogeneic bone. The results in clinical studies were often successful [27,28]. In an animal study on rabbits, however, the delay in osseointegration of 2 - 3 weeks was analyzed using polychromatic sequential labeling, even if the implants had been press fitted into contact with their recipient bone [29]. The direct comparison of β-TCP with DBM versus β-TCP combined with BMP-7 showed a difference in osseointegration of up to 4 - 6 weeks [2] even in vascularized periosteal flaps, immune reactions, infections and tumor transmissions with allografts [11].

The alloplasts and xenoplasts comprise synthetical calcium phosphate compounds like HA or β-TCP and bovine HA, respectively. Bone substitutes consisting of either HA or β-TCP are considered to be biocompatible materials. The FDA regards these materials to be medical devices, which implies that there is no sign of local or systemic toxicity, genotoxicity, foreign body reaction, immune response or cancerogeneity.

Osteogenicity was postulated for all autologous grafts, since MSCs are transferred with the bone [30]. Because the MCB® suction in blood and bone marrow, there is clear osteogenic activity after revascularization, which explains the resulting rapid and complete osseointegration.

With respect to osteoinduction, the β-TCP ceramic does not induce bone formation; disregarding the bioactive surface for the colonization with osteoblasts of all calcium phosphate ceramics. However, mechnano-induction of the stiffer HA-implants is present, which is obvious and has been published [16]. The observation of De Angelis et al. [25] also indicates mechnano-induction. The combination with rhBMP-7 resulted in very impressive induction of mesenchymal processes, and not only bone formation, but also bone remodeling and resorption [15].

In terms of osteoconduction, all marketed calcium phosphates display physiological structures of cancellous bone or try to imitate that morphology representing marrow spaces and interconnections. The bone, ingrown into a single pore of a spongy structured substitute, however, represents a bony ball which must be remodeled [16]. The authors defined the bead
as the bone-forming element and requested a replica of all bone marrow spaces as osteoconductive ladder. Another phenomenon occurs with cancellous-bone-structured stiff ceramics: stress shielding is seen in the pores resulting in a weak stimulus for new bone formation. The newly formed bone represents mainly woven bone which is unable to carry any load, and is therefore followed by time-consuming remodeling. In contrast is the osteoconduction of a conglomerate of ceramic beads: The newly formed bone grown over a ball-like shape represents mature lamellar bone which has been under load from the beginning and requires no remodeling. With this key finding, the authors could explain fast healing in even large defects [16,31].

β-TCP granules with their high water-uptake and resulting fast degradation might not be strong enough to maintain osteoconductive properties during the bone healing phase [32; 33]. This is one of the reasons for the introduction of biphasic calcium phosphate materials which combine the stable phase of HA with the more soluble phase of β-TCP [33]. The behavior in the body of biphasic implants, however, is no longer predictable. β-TCP is completely reabsorbable, whereas the HA portion is not [15].

In our approach, pure β-TCP was implanted, thus representing replicas of the marrow spaces and acting as bone-forming elements which were quickly overgrown by newly formed bone before their structures weakened due to the water uptake. Other than the stress-protected ingrown bone in the pores of a spongeous-like structure, the replica-based newly formed bone is directly in the load-line, thus forming mature and fast-mineralized bone. The newly formed scaffold is able to carry the load because it was formed under a load acting upon it.

Due to the capillary forces of the MCB®, blood is suctioned in and coagulates, yielding a very stable implantation in which all beads preserve a certain distance to each other, as visible in the post-operation X-rays. Over time, the scaffold develops an even more regular arrangement due to the bony shells colonizing the shape of the beads. The overgrown bone represents mature lamellar bone. Another important feature tremendously simplifies the intervention. Since all beads preserve a certain distance and are fixated in the coagulated blood, no ceramic disease [34] can occur. Meiss [34] found that the sharp-edged hydroxyapatite granules have to be combined with morselized bone in order to avoid fibrous encapsulation, which is induced by micromovements of the granules. It is recommended to combine hydroxyapatite granules at least with 20% of morselized bone [23]. Meiss [34] suggested a mixture of 50:50% morselized bone and HA granules. There is no need to gather morselized bone in combination with MCB®. The introduction of the RBBF can be further endorsed
because granulated material as well as any putty of HA or calcium phosphate or sulfate does not yield a physiological scaffold. The fast healing, namely in weeks instead of months, can thus be explained since there is no remodeling of the newly formed bone. Therefore, ongoing studies have already been defined: newly formed bone has to be measured at different intervals after the sinus elevation with respect to its strength, thus identifying the point in time for inserting the implant. One question remains for continuing studies with regard to the indication for HA-MCB® or even a mélange of both dependent upon the quality of the maxillary bone: it might be reasonable to use the mechano-induction of HA in osteoporosis or osteomalacia.

Conclusion

The introduction of RBBF with MCB® initiated a new principle for osteoconduction combined with local tissue engineering: suctioning-in blood and bone marrow. All five sinus elevations confirm the key finding of rapid osseointegration of the whole implant by primarily formed mature lamellar bone. This concept will change the current paradigm of bone augmentation and bone healing.

Compliance with Ethical Standards

Funding:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval:

This study was approved by Ethics committee LMU Munich No.760-16UE. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.


References


Figure 1

(a) Micro-Chambered Bead® (MCB®); (b) and (c) MCB® suction in blood and bone marrow 1/20 cc; after coagulation, the MCB® implant represents a stable implantation.

(d) Cohort of patients (w = weeks, m = month, Y = year, FEMR = free-end maxillary ridge, TTAG = two tooth alveolar gap, OTAG = one tooth alveolar gap)
Figure 2
Sinus elevation No. 5
(a, b, c) sagittal, frontal; horizontal projection.
(a-c) The sinus floor measured less than 5 mm and the indication for augmentation was given: The height could be augmented. The newly forming scaffold of MCB* was loaded by the close teeth on both sides;
(d) The end-result was a compact filling with MCB* (β-TCP);
(e-f) From both natural teeth and their alveolar bone thick mature lamellar bone is growing deep into the scaffold of the osseointegrated implant (arrows); the ceramic beads are completely overgrown by mature and fully mineralized newly formed bone, surrounding the beads’ shape like shells. The trabeculae supporting the implant onto the alveolar bone have been further reinforced after 11 weeks (arrows).
(g) Sagittal projection. Preoperative planning of the implantation 5 months after sinus-floor elevation; the bone stock was very strong at the time of implant insertion, only single ceramic beads were still visible in the X-ray (white dots);
(h) 6 months after implantation, the bone structure reveals cleared scaffolds of physiological mature cancellous bone.
Figure 3
Sinus elevation No. 2
(a) Operating situs: Complete package of MCB, bleeding has been stopped.
(b) To confirm the strength of the newly formed bone, without implants, the insertion of the two implants was performed late after 20 months;
(c) before placement, a biopsy (arrow) was gathered for non-demineralized histology, using a diamond with an inner-cooling system;
(d) 3-D µ-CT of the biopsy;
(e) 2-D µ-CT slices revealing strong mature lamellar bone, still presenting minor rests of the osseointegrated β-TCP ceramic.
Figure 4
Sinus elevation No. 2
(a) Osseointegration of minor rests of β-TCP (cer) in mature lamellar bone (non-demineralized cross section, Alkaline fuchsin staining);
(b) The vital bone is comprising bone marrow spaces that show the path of the degraded β-TCP (arrow), the so-called remodeling-resorption (non-demineralized cross section, Alkaline fuchsin staining).
(c) which is fully mineralized lamellar bone (high-resolution microradiograph).
(d) Follow up: 4 years and 5 months after implantation, both implants reveal a firm and osseous anchorage.
Figure 5
Sinus elevation No. 4
(a, b) Preoperative planning of the implant insertion, 7 months after augmentation.
The bone stock was arranged in a physiological scaffold of cancellous bone. Minor rest of the β-TCP implant are still osseointegrated (DVT: a sagittal, b horizontal).
(c, d) X-ray: Very stable primary fixation (0 d) and stable situation after 11 months. Fully mineralized bone in a physiological structure.