Healing of Extraction Sockets Filled with BoneCeramic® Prior to Implant Placement: Preliminary Histological Findings

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ABSTRACT

Background: Various grafting materials have been designed to minimize edentulous ridge volume loss following tooth extraction by encouraging new bone formation in healing sockets. BoneCeramic® is a composite of hydroxyapatite and bèta-tricalcium phosphate with pores of 100–500 microns.

Purposes: The aim of this study was to evaluate bone regeneration in healing sockets substituted with BoneCeramic® prior to implant procedures.

Materials and Methods: Fifteen extraction sockets were substituted with BoneCeramic® and 14 sockets were left to heal naturally in 10 patients (mean age 59.6 years). Biopsies were collected only from the implant recipient sites during surgery after healing periods ranging from 6–74 weeks (mean 22). In total, 24 biopsies were available; 10 from substituted and 14 from naturally healed sites. In one site, the implant was not placed intentionally and, in four substituted sites, implant placement had to be postponed due to inappropriate healing, hence from five sites biopsies were not available. Histological sections were examined by transmitted light microscope.

Results: At the time of implant surgery, bone at substituted sites was softer than in controls, compromising initial implant stability. New bone formation at substituted sites was consistently poorer than in controls, presenting predominantly loose connective tissue and less woven bone.

Conclusion: The use of BoneCeramic® as a grafting material in fresh extraction sockets appears to interfere with normal healing processes of the alveolar bone. On the basis of the present preliminary findings, its indication as a material for bone augmentation when implant placement is considered within 6–38 weeks after extraction should be revised.

KEY WORDS: BoneCeramic®, bone grafting material, extraction sockets, human histology, hydroxyapatite, tricalcium phosphate

Healing of an extraction socket implies a series of events including the formation and maturation of a coagulum that subsequently will become replaced by a provisional matrix and woven bone. Undisturbed sockets heal uneventfully with new bone formation 1–2 months following extraction.[1,2] This healing process usually occurs with reduction of the height and width of the alveolar bone,[3] which in some cases may aesthetically compromise prosthodontic treatment following implant surgery. With increasing awareness of dental implants, there is an increasing demand for the treatment of more complex cases where preliminary grafting is indicated. The ideal extraction regimen for preserving or minimizing the edentulous ridge volume loss has been described[2] and various ridge preservation techniques following tooth extraction have been proposed, such as the placement of different graft materials and the use of occlusive membranes to cover the extraction

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socket entrance.[4] Although grafting materials are known to encourage new bone formation by a variety of processes,[5,6] the use of these materials in fresh extraction sockets has been questioned because they seem to interfere with the normal healing process.[7–9] Studies in humans using autogenous bone,[7] demineralized freeze-dried bone allograft,[10,11] deproteinized bovine bone mineral,[8,12–18] bioactive glass,[19–21] hydroxyapatite,[4] calcium sulphate,[22–24] tricalcium phosphate[25–27] or polylactide/polyglycolide polymers[28–31] show the presence of particles of the grafting material in the alveolar sockets 6–9 months following insertion. Autogenous bone induces bone formation through osteogenesis, whereas allogenic bone is thought to be osteoinductive due to the presence of growth factors. Xenografts, such as bovine bone material and alloplastic substitutes, encourage the apposition of new bone by osteoconduction.[18] Eventually, anorganic bone allograft, bioglass and hydroxyapatite become incorporated, whereas demineralized bone allograft, calcium sulphate or tricalcium phosphate are completely restituted during bone regeneration.

BoneCeramic® (Straumann AG, Basel, Switzerland) is a synthetic bone-graft substitute designed for augmenting bone to support dental implant procedures (launched May 2005). It consists of biphasic calcium phosphate, a composite of 60% hydroxyapatite (100% cristalline) and 40% β-tricalcium phosphate sintered at temperatures of 1,100 to 1,500°C. BoneCeramic® is 90% porous with interconnected pores of 100–500 microns in diameter.[32] The aim of the present experiment was to evaluate bone regeneration in healing sockets substituted with BoneCeramic® both clinically and histologically in a first-wave patient sample.

MATERIALS AND METHODS

Patient Selection and Extraction Procedure

Patients presenting for multiple extractions and selected for later implant treatment, were consecutively treated with BoneCeramic® as a bone filler after tooth extraction and scheduled for delayed implant placement. Subjects were referred for periodontal and/or implant treatment. Teeth were extracted for multiple reasons such as periodontal disease, root or crown fractures, or root caries. In the waiting period between extraction and implant surgery, the periodontal condition was improved by nonsurgical therapy and oral hygiene instructions to avoid or minimize infection risks during implant surgery. All patients were systemically healthy and could be treated under local anesthesia. Mucoperiosteal flaps were raised and, after extraction followed by thorough debridement, sockets with intact buccal and palatal bone plate were filled with BoneCeramic®, which was mixed with some of the patient's own blood aspirated from the extraction area. The assumption was that a bone filler would support the soft tissues and enhance the aesthetical outcome. A bleeding bone bed was considered an essential prerequisite for placement of the biomaterial because the natural supplement of platelet-derived growth factors and transforming growth factors can reduce the healing time and enhance bone formation environment.

Since multiple extractions were often performed during the same treatment session, extraction sockets that were not in need for additional filling were left untreated. This was partly done to minimize the treatment cost. As a consequence, it was possible to select in each patient at least one socket for natural healing without bone substitute (control sockets). The flaps were closed after mucoperiosteal release and meticulously sutured over the alveolar crest. In multiple extraction cases, this adequate coronal repositioning of the mucoperiosteal flap for perfect wound closure is easy to achieve. This technique may enhance wound stability and an undisturbed healing process.[2] Hence, the use of occlusive membranes was not advocated in none of the cases.

Because the first cases showed disappointing healing at the time of implant surgery, however, it was decided to perform a histological analysis of the bone healing. The principle of biopsy taking for histological analysis was approved by the Ethics Committee of the University Hospital of Ghent since it could be included as a procedure of implant bed preparation during surgery without jeopardizing treatment outcome.

Sampling and Histological Processing

Biopsies were collected only from the implant recipient sites during surgery. In total, 23 implants were placed in the previous extraction sockets. In one additional site the implant was not placed but the biopsy was taken. In four substituted sites, implant placement was impossible due to inappropriate healing whereby taking of biopsies was impossible. Hence, biopsies were available from 10 substituted and 14 naturally healed sites. Cylindric biopsies from these 24 sockets were sampled for histological

analysis with a hollow trephine bur (2.7 mm × 8 mm core) and placed in a fixative (10% buffered formalin). The biopsies were randomly assigned to two groups to be processed either as decalcified or undecalcified sections; the same treatment protocol was used for biopsies retrieved from the same patient. About 10–12 sections were examined from each biopsy. These were compared as per the location in the biopsy cylinder. Specimens selected for undecalcified sections were dehydrated in an ascending series of alcohol rinses and embedded in glycolmetacrylate resin (Technovit® 9100 VLC, Kulzer, Friedrichshof, Germany). After polymerization, the specimens were cut at 5 µm in the vertical plane using an automatic osteomicrotome with a carbide blade (SM2500, Leica Microsystems, Wetzlar, Germany). The sections were stained with hematoxylin and eosin (routine combination staining method), by Masson's trichrome (three-color method for distinguishing cells from surrounding connective tissue) and by Von Kossa method (silver-nitrate stain for demonstrating deposits of calcium or calcium salt). At baseline, it was also planned to visualize the activity status of osteoblasts (OB) in the retrieved samples by using markers for cbfa1 (stains immature OB; cbfa1 anti-goat 1:50, Acris) on paraffine sections and osteocalcine (stains mature OB; polyclonal osteocalcin 10 µg/mL, Acris) on nondecalcified sections. Specimens selected for paraffin sections were decalcified in 10% EDTA for 5–14 days at room temperature (depending on sample hardness) and processed to paraffin wax according to routine methods. Specimens were sectioned at 5 µm and stained with hematoxylin and eosin, and by Masson's trichrome method. The sections were examined by transmitted light microscope. In addition, undecalcified and decalcified BoneCeramic® particles were treated and prepared with the same protocols as samples for paraffin and resin sectioning to serve as a baseline reference.

Implant Treatment

Osteotomies were completed according to routine protocol followed by placement of the appropriate size of titanium dental implants. BoneCeramic® particles that were not removed during implant bed preparation remained in contact with the implant (see Figure 1A). Three different implant systems were used according to the guidelines of the manufacturers (Astra Tech, Mölndal, Sweden; Nobel Biocare, Gothenburg, Sweden; Biomet3i, Palm Beach, FL, USA). Care was taken to obtain primary stability and whenever necessary underpreparation of the socket or wider implants were used. All implants were placed with a one-stage surgical approach in that the healing abutments were installed simultaneous with implant placement. In some cross-arch rehabilitations the implants were provisionalized and immediately loaded.

RESULTS

Ten patients (eight men and two women) were included in the histological study. They had a mean age of 59.6 years (range 41–81). Three patients were heavy smokers of at least 20 cigarettes per day and seven were nonsmokers. In total, 29 teeth had been extracted prior to surgery of which 15 were treated with BoneCeramic®. After two weeks, the clinical appearance of the soft tissues around the extraction sockets was similar in both groups and registered as good in 26 of the cases. At the time of implant surgery, there was less resorption of the alveolar crest at the substituted sites as compared with the naturally healed sites (Figure 2). Bone at substituted sites, however, was softer than bone in naturally healed sites which was irrespective of the individual healing time. In the majority of cases, large amounts of loose biomaterial were found at the time of surgery. These sites were thoroughly debrided prior to implant installation but sometimes the recipient beds were too large to get normal diameter implants initially stable. Hence, wider implants were necessary. Due to differences in healing, radiographic evidence of graft consolidation, and patient availability, the time between augmentation and implantation ranged from 6–74 weeks, with a mean of 22 weeks. Of the 29 extractions sites, one was not prepared for implantation and hence not available for biopsy taking. In two patients (#9 and #10) with four BoneCeramic® grafts (after 8 and 21 weeks of healing respectively) sampling of a bone core was impossible due to a complete lack of mineralization. Implants, although planned, could not be installed at these sites and treatment was postponed. In those two patients, as a consequence, no biopsy was taken from the nongrafted sites. Hence, in total, 14 control and 10 substituted sites were harvested. Of the 15 originally substituted sites, five could not be implanted because of impaired healing and two of the 10 inserted implants failed within 3 months after insertion. Thirteen of the 14 naturally healed sites were implanted; however, three failed of which two in an immediate loading case. The clinical results and the

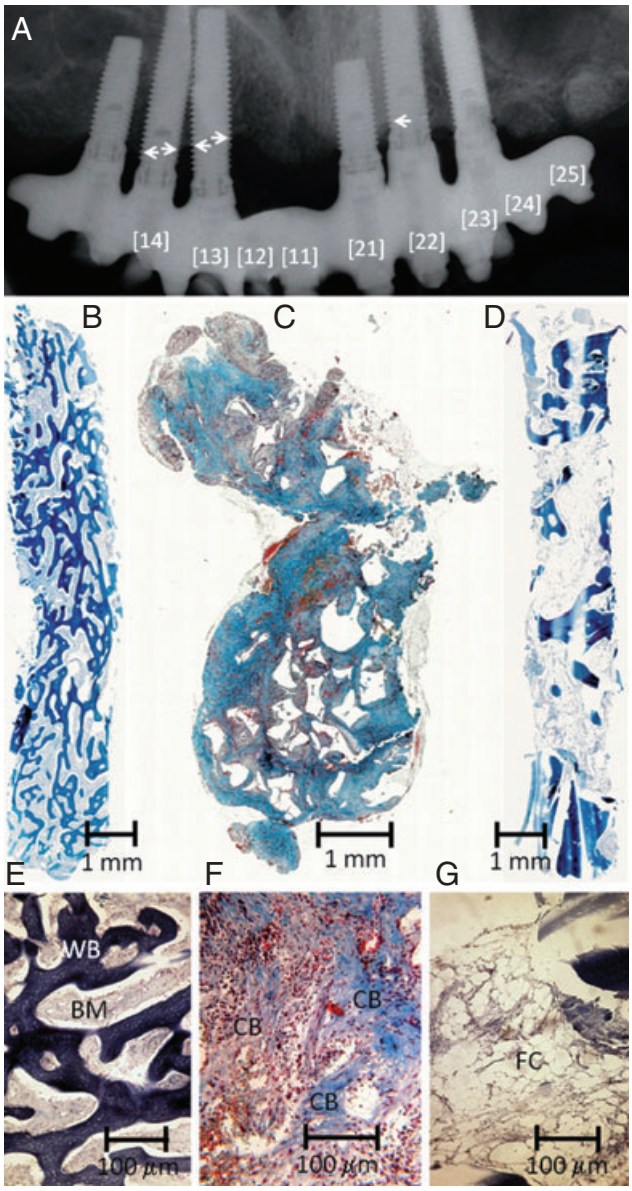

Figure 1 *A*, Clinical radiographs (patient 1) taken after 15 months of implant loading. In total, seven teeth were extracted for periodontal reasons; three extraction sockets remained ungrafted (22, 13, 14) and four were filled with BoneCeramic® (12, 23, 24, 25). After a healing period of 10 weeks, implants could be placed with excellent initial stability (13, 14, 15, 21, 22, 23) followed by immediate loading with a provisional resin bridge. Implants did not obtain initial stability on 24 and 12, both BoneCeramic®-grafted sites, due to insufficient healing. After 9 months, the final prosthesis was put into loading. Bone-to-implant contact is indicated with arrows and bone loss below the first implant thread is eminent on nongrafted sites (14, 13, 22). Furthermore and additionally, a radio-opacity is visible around the implant in the grafted site (23) and granulae are present at the grafted but not implanted nor harvested bone site on position (25), indicating that integration of BoneCeramic® in the newly-formed bone was incomplete. *B*, Photomicrograph of a control site biopsy (13) after 10 weeks of healing, showing excellent bone formation with woven bone (WB) and bone marrow (BM). Mineralized bone areas are stained dark blue, while bone marrow stains grey to light blue. Masson's trichrome stain on paraffine section, ×10. *C*, Photomicrograph of a substituted site biopsy (12) after same 10 weeks of healing, showing predominantly dense connective tissue (CT) without evidence of bone formation. Most of BoneCeramic® particles that were still abundantly present as a granular mass at the time of biopsy taking were lost during histological processing. Because of ineffective healing, bone at this site was too soft and no implant could be placed. Masson's trichrome stain on paraffine section, ×10. *D*, Photomicrograph of a substituted site biopsy (24) displaying a combination of loosely arranged connective tissue (CT) and few islands of immature woven bone (WB). Note residual lamellar bone flakes (LB) at the apical portion of the biopsy. At this position, implant placement also was impossible due to bone softness. Immature woven bone stains blue to purple with central red spots indicating areas of advancing mineralization. Masson's trichrome stain on paraffine section, ×10. *E*, Detail of (B) displaying woven bone (WB) with bone marrow (BM); ×250. *F*, Detail of (C) presenting dense, blue to greyish stained collagen bundles (CB) and proliferation of vessels, fibroblasts, and lymphocytes. The bright red cells are erythrocytes issuing from local hemorrhage during biopsy taking; ×250 (G). Detail of (D) showing loose connective tissue abundant with fat cells (FC) and some peripheral islands of mineralized bone (B); ×250.

characteristics of patients, extraction sites, and implants are summarized in Table 1.

Histological Findings

In decalcified sections of biopsies harvested from untreated control sites, mature bone was present, mainly comprising lamellar bone outlining the original socket, and newly formed woven bone at the center of the previous socket (Figure 3, A and B). Formation of new bone at substituted sites was poorer than controls, mainly exhibiting loosely arranged connective tissue with sparse evidence of mineralized bone in the grafted portion of the cylinders (Figure 4, A–C). In most of the substituted samples, the coronal portion of the biopsies was predominantly comprised of irregularly arranged bundles of collagen fibers, proliferation of blood vessels and fibroblasts, fat cells and moderate numbers of lymphocytes. At the apical portions of some of the samples, impaction of small clusters of BoneCeramic® particles could be observed in the fibrotic tissue with weak to no evidence of new bone formation. The bone tissue apically from the previous socket area was of a lamellar type. These findings of poor bone formation were most pronounced as compared with control sites in substituted sites of patients 2, 4, 5, and 7, and less explicit but still clearly present in patients 1, 3, and 8. Bone in the substituted socket of patient 6 harvested after 74 weeks

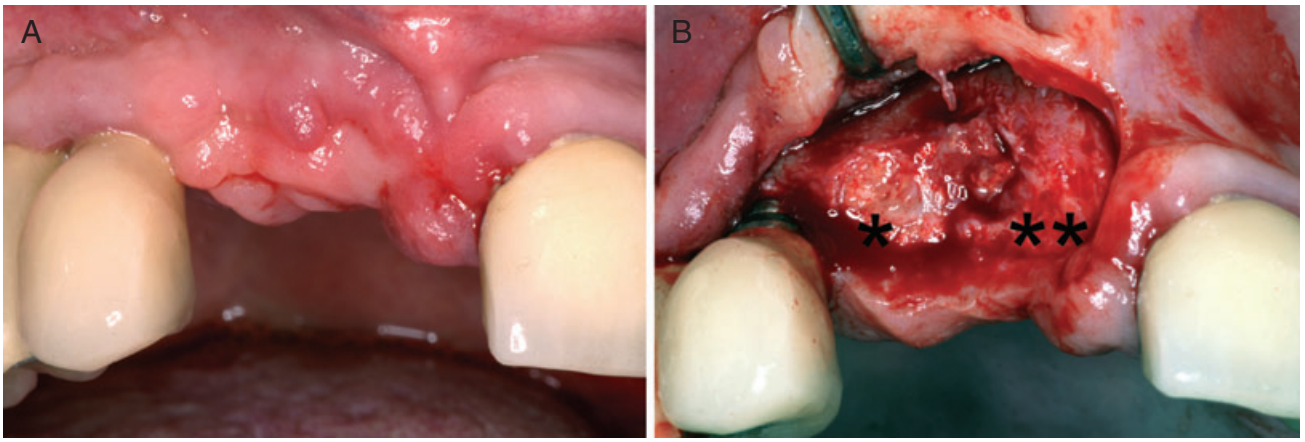

Figure 2 *A*, Clinical aspect during implant surgery after 16 weeks of healing (patient 2), showing (B) elevated and granulomateous/fibrous tissue at substituted site (*) versus slightly depressed and compact aspect at naturally healed site (**).

of healing was histologically similar to that of naturally healed bone. In the majority of both undecalcified and decalcified substituted specimens, large amounts of nonresorbed or integrated BoneCeramic® particles found at the time of surgery were, for the greater part, almost completely lost during histological preparation. Comparative baseline treatment and preparation of BoneCeramic® particles, however, had shown no essential disintegration of the material suggesting that the particles were lost due to poor tissue integration.

Processing of the undecalified sections went well in the first samples but, further on, technical problems came up hampering comparative immunostaining of the complete set of samples. In a considerable part of the substituted sections, the material broke up from the carrier during antigen retrieval procedures, despite trying out several adhesives. It was felt that this was related to the poor tissue quality of the retrieved bone core. Hence, data on immunostaining could not be presented in the manuscript. In addition, for the same reason histomorphometry was not performed.

No relation was detected between bone quality and age, pre-extraction status, healing period, wound closure, and/or smoking. In only one available biopsy from a substituted site (patient 4), poor initial wound healing was associated with poor bone formation and a large inflammatory cell infiltrate (lymphocytes and macrophages) throughout the biopsy. One should keep in mind, however, that from 4 substituted sites, a biopsy could not be analyzed because of clearly ineffective healing. Overall, five of the 15 substituted sites showed jeopardized healing.

DISCUSSION

The present study was based on preliminary and disappointing findings of extraction socket substitution with BoneCeramic® prior to implant procedures. As can be seen in Figure 2B, loosely packed granular tissue was found at the coronal part of the substituted sockets at the time of implant surgery. The latter contained dispersed amounts of BoneCeramic® granules, jeopardizing the placement of dental implants with a good initial stability. As a consequence and based on ethical considerations, the present study was not designed on forehand and hence the patient's inclusion, selection of sites, filling of the defects in the substituted sites, flap handling and suturing was merely depending on the surgeon's clinical choice during the treatment. As a consequence, the treatment protocol may be somewhat inconsistent. On the other hand, many new biomaterials are CE marked but not always supported with adequate studies backing them up. At the time of the launch and the clinical application in the cases presented in this study, no clear clinical guidelines were included by the manufacturer; the provided description of the product composition was vague and suggestions regarding healing time were lacking. Hence, it was the feeling of the authors that the poor outcome achieved with the performed treatment protocol had to be reported.

In the present study, implant installation at extraction sites that had previously been filled with BoneCeramic® was generally compromised by poor formation of new bone although the mucosal wound healing seemed overall clinically acceptable with a proper socket closing. From five out of the 15 grafted sockets –

TABLE 1 Case Description of All Patients, Treatment Modality of the Extraction Sockets, Implant Location, and Implant Characteristics. Sites with Ineffective Healing of Bone and Absence of Biopsy are Indicated with #

Case	Age (Years)	Reason for Extraction	Smoker	Tooth Site	BoneCeramic in Socket	Healing in Weeks	Implant/Biopsy in Extraction Site	Failed Implant	Implants per Patient	Failed/Installed Implants per Patient	Implant Length (mm)	Implant Width (mm)	Implant Stability
1	55	Perio	No	22	No	11	Yes / Yes	N	4 extraction sites + 2 healed sites	0/6 astra	11.5	4	Good
1	55	Perio	No	23	Yes	11	Yes / Yes	N			15	4	Good
1	55	Perio	No	24	Yes	11	No / Yes	not placed			—	—	—
1	55	Perio	No	12	Yes	11	No / No	not placed			—	—	—
1	55	Perio	No	13	No	11	Yes / Yes	N			15	4	Good
1	55	Perio	No	14	No	11	Yes / Yes	N			10	4	Good
2	58	Caries	Yes	11	No	16	Yes / Yes	N	2 extraction sites	0/2 nobel	13	4	Good
2	58	Caries	Yes	12	Yes	16	Yes / Yes	N			13	4	Good
3	57	Fracture	No	11	Yes	23	Yes / Yes	N	3 extraction sites + 2 healed sites	1 /5 astra	15	4	Good
3	57	Fracture	No	23	No	23	Yes / Yes	N			15	4	Good
3	58	Caries	Yes	22	No	23	Yes / Yes	N			15	4	Good
4	66	Caries	Yes	44	No	21	Yes / Yes	Y	2 extraction sites	2/2 nobel	11.5	4	Good
4	66	Caries	Yes	45	Yes	21	Yes / Yes	Y			10	4	Good
5	81	Fracture	No	12	No	6	Yes / Yes	Y	5 extraction sites	3/5 Biomet 3i	10	5	Good
5	81	Caries	No	22	Yes	6	Yes / Yes	Y			15	4	Good
5	81	Caries	No	23	No	6	Yes / Yes	Y			15	4	Good
5	81	Caries	No	21	No	6	Yes / Yes	N			15	4	Good
5	81	Caries	No	11	Yes	6	Yes / Yes	N			15	4	Good
6	41	Perio	No	41	No	74	Yes / Yes	N	2 extraction sites + 2 healed sites	0/4 nobel	15	4	Good
6	41	Perio	No	43	Yes	74	Yes / Yes	N			15	4	Good
7	62	Fracture	No	12	No	21	Yes / Yes	N	2 extraction sites + 2 healed sites	0 /4Biomet 3i	15	4	Good
7	62	Fracture	No	22	Yes	21	Yes / Yes	N			15	4.8	Poor
8	58	Caries	Yes	44	Yes	38	Yes / Yes	N	3 extraction sites	0 /3Nobel	10	3.7	Good
8	58	Caries	Yes	24	No	38	Yes / Yes	N			15	4	Good
8	58	Fracture	Yes	25	No	38	Yes / Yes	N			11.5	4	Good
9	58	Caries	No	11	Yes	8	No / No #	not placed	0 inserted	*	*	*	*
9	58	Caries	No	37	Yes	8	No / No #	not placed	*	*	*	*	*
10	60	Perio	No	26	Yes	21	No / No #	not placed	0 inserted	*	*	*	*
10	60	Perio	No	37	Yes	21	No / No #	not placed	*	*	*	*	*
$n = 10$	**Mean age 59.6**	**Perio: $n = 9$ Caries: $n = 9$ Fracture: $n = 6$**	**Yes: $n = 7$ no: $n = 16$**	**$n = 24$**	**Yes: $n = 15$ No: $n = 14$**	**Mean time 22.7 weeks**	**Yes/Yes: $n = 23$ No/Yes: $n = 1$ No/No: $n = 5$**	**Failed: $n = 5$ Not placed: $n = 6$**		**Failed: 6/31**			**Good: $n = 22$ Poor: $n = 1$**

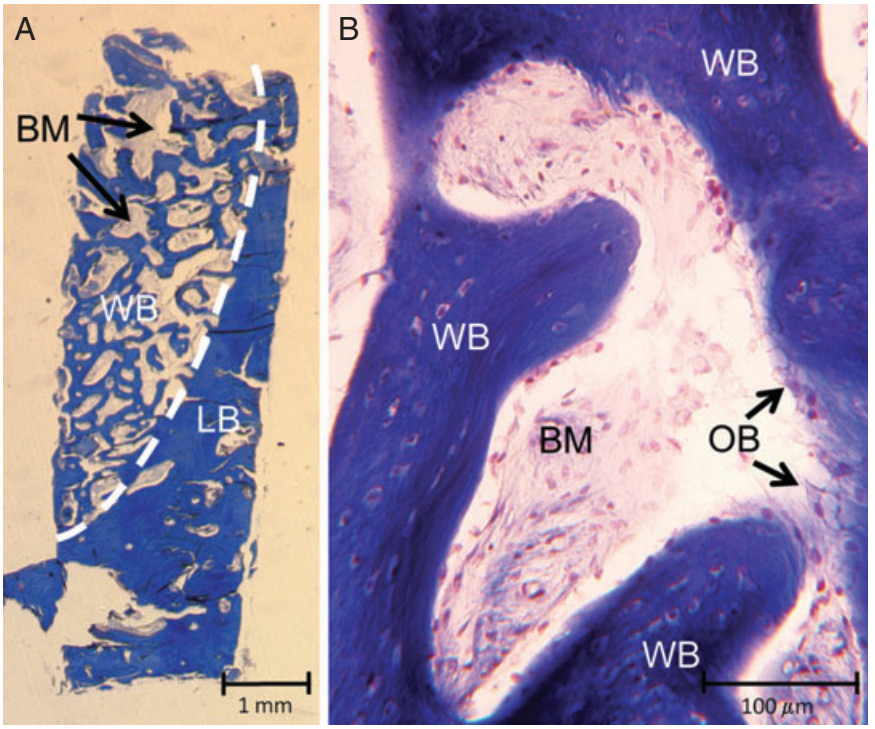

Figure 3 *A*, Photomicrograph illustrating a control site specimen (23 weeks of healing – patient 3), comprised of woven bone (WB) and bone marrow (BM) in the coronal part and lamellar bone (LB) in the apical part. Dotted line indicates outline of the original socket. Masson's trichrome stain on paraffine section, ×10. *B*, Higher magnification of (A) showing woven bone (WB) and bone marrow (BM) with osteoblasts (OB). Mineralized bone areas are stained dark blue. Masson's trichrome stain on paraffine section, ×250.

representing three of the 10 patients – there were no biopsies analyzed. One because the site was not prepared for an implant recipient site and four because there was no bone core to harvest. It was merely infectious soft tissue with graft particles that could be removed easily. In those patients, after proper socket debridement and removal of the granulation tissue mixed with the graft material, the dimensions of the socket did not allow proper implant placement. As a consequence, in two patients, the placement of four implants had to be postponed until proper healing had occurred. Hence, biopsies of the control bone and the grafted bone were lacking and the clinical protocol was unforeseeably changed. This, of course, is of clinical importance and affects patient-centered outcome with the treatment.

The histological examination of biopsies collected at the time of implant surgery showed that sites filled with BoneCeramic® had less mineralized bone tissue in the grafted portion as compared to their naturally healed homologues. The better results in patient 6 (74 weeks of healing) and, to a lesser degree, patient 8 (38 weeks) suggest that the biomaterial in focus may be responsible for a considerable delay of new bone formation. Because it is well established that implant surgery can already take place in naturally healed sockets 8 weeks after tooth extraction,[33] the present findings compel to revise the indication of BoneCeramic® as a grafting material for bone augmentation of extraction sockets when insertion of a dental implant is considered within a period of 6–38 weeks after extraction.

Because the present number of patients is too limited and three different implant types were used with different loading protocols, it is not scientifically appropriate to make a statistical analysis of implant survival of the grafted versus nongrafted sockets. Nevertheless, the total number of four out of 19 implant failures (21%) is unacceptably high. The early loss of one implant supporting an immediately loaded rehabilitation may, of course, jeopardize other implants because of overloading. Consequently, it is difficult to link implant loss to the nature of the socket. It is nevertheless tempting to suggest that the impaired healing of the recipient sites may affect implant survival. Further investigation needs to focus on possible relationships between implant survival and site characteristics, such as healing period of the augmented sockets but also, for example, wound

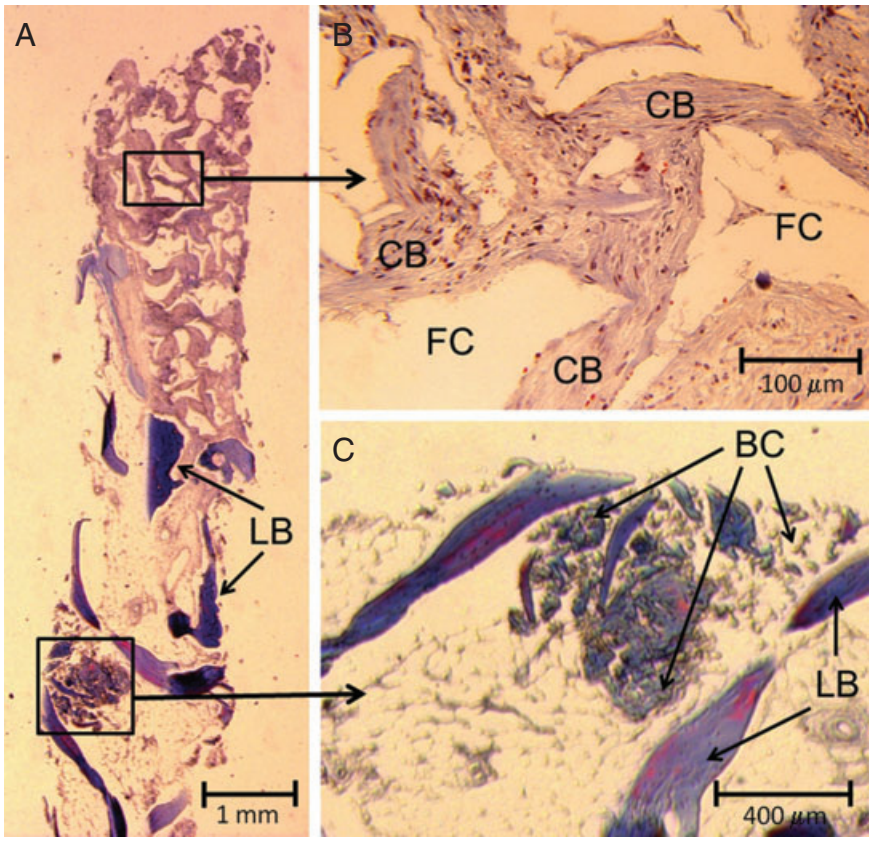

Figure 4 *A*, Photomicrograph of an experimental specimen from a substituted site (BoneCeramic®; 23 weeks of healing – patient 3), characterized by loosely arranged connective tissue at the coronal portion and proliferation of vessels, fat cells, and clusters of BoneCeramic® particles at the apical portion. Lamellar bone flakes (LB) are present at the apical portion, probably representing remnants from the original socket walls included during insertion of the graft material or during retrieval of the biopsy. Mineralized bone is stained dark blue to purple blue. Masson's trichrome stain on paraffine section, ×10. *B*, Higher magnification of the coronal portion of (A) showing collagen bundles (CB) and proliferation of fat cells (FC), fibroblasts, and lymphocytes. Collagen fibers stain light blue to greyish blue. Masson's trichrome stain on paraffine section, ×250. *C*, Higher magnification of the apical portion of (A). Note the presence of BoneCeramic® particles (BC) surrounded by remnants of fat cells and lamellar bone flakes (LB). BoneCeramic® particles do not stain and have a slightly phosphorescent aspect under light microscopy. Masson's trichrome stain on paraffine section, ×100.

closure procedures with or without the use of occlusive barriers.[34]

Many studies have reported on the efficacy of a variety of graft materials. Within the group of osteoconductive graft materials, synthetic hydroxyapatite (HA)[35] and α–tricalcium phosphate (TCP) ceramics[22,36–38] have been the subject of considerable investigation. Both HA and TCP – the two components of BoneCeramic® – are well-known biomaterials used for many decades as a bone substitute for small defects of the jaws. HA was used increasingly during the 1980s, not the least for its good biocompatibility. The ingrowth of bone tissue has been detected only at the direct contact interface with native bone, whereas more distant granulae displayed connective tissue encapsulation regardless of the type of HA applied.[39] Major disadvantages of HA are brittleness, little mechanical strength, poor resorption, and difficult control of pore size by conventional processing methods.[40] Because of these important drawbacks, it is recommended to limit application to areas that do not require mechanical strength.[41] Although previous studies of implant survival in extraction sockets augmented with HA have reported high success rates,[42,43] there are no sufficient data as to the long-term effects of HA incorporation on the metabolism and the biomechanical properties of the implant-supporting bone. In contrast, TCP has little biomechanical disadvantages and shows high biocompatibility and osteoconductivity.[25,44,45] TCP shows a more favorable resorption behavior but it was demonstrated that dissolution occurred before cell adhesion is possible, evoking a primary lymphocyte inflammatory.[39] When grafted into bone

defects, multinucleated giant cells incorporate TCP prior to osteoblastic activities.[46] Previous studies showed that TCP degrades either through osteoclastic resorption or by chemical dissolution by the interstitial fluid.[37] Although TCP is expected to degrade three times faster than HA, the predictability of ceramic degradation in vivo remains poor.[47,48] Furthermore, the extent of degradation depends on many factors, such as crystallinity, porosity, density, particle form and size,[49] the host, and implantation site.[50] In vivo experiments using rabbits[51] and rats[52] demonstrated that TCP may resorb and be replaced by newly formed bone within 1 month[52] to 3 months.[51] However, Nair and co-workers found that osseous healing was considerably delayed as compared with control sites due to the presence of TCP particles.[53] Several authors also reported on a predominant bone resorption at the early stage of bone healing associated with the use of α-TCP.[54–56] On the other hand, when dental implant placement and grafting with TCP are done concomitantly, new bone is formed resembling compact bone.[38]

At present, human studies on the clinical and histological outcome of socket supplementation with BoneCeramic® are scarce. Vlah and co-workers reported an excellent primary implant stability of at least 35 Ncm at sockets that had been augmented with BoneCeramic® 3 months before implant placement.[57] In the present study, a good primary wound closure was obtained without the use of occlusive barriers, such as membranes or gingival grafts. Although this approach was different from Vlah's (occlusion of grafting material with a gelatine tampone and a cross-suture of expanded polytetrafluorethylene), the difficulties experienced during implant insertion may not implicitly be attributed to differences in wound closure procedures. It was suggested by others[34,58] that the use of occlusive barriers in augmenting procedures does not warrant a more effective substitution of the grafting material by new bone. In the latter study, dense fibrous connective tissue was seen surrounding BoneCeramic® particles in the coronal part of a number of biopsies after 8 months of healing. At the cellular level, recent in vitro culture of mice L-929 fibroblasts in the presence of BoneCeramic® over a period of 28 days showed that the biomaterial does not affect cell viability. Nevertheless, the initial metabolic activity of the cells (proliferation rate, cell spreading) was reduced compared with that of control cells, suggesting an important role of material surface characteristics.[59] Future product research and development should focus on processing properties in order to enhance and increase initial cell adhesion, more in particular of young osteoblasts and differentiating endothelial cells, at the biomaterial surface.

Among both substituted and control sites, no differences were seen in bone formation between smokers and nonsmokers (Table 1). Although previous authors suggested that smoking may lead to a more significant dimensional reduction of the residual alveolar ridge and may postpone postextraction socket healing,[60] no valuable conclusions could be made on this subject due to the small sample size.

The present results indicate that bone formation at sites substituted with BoneCeramic® was poorer than controls after a mean healing period of 22 weeks. Although there was less resorption of the alveolar bone at substituted sites, it is worthy of note that the relative volume of new bone within grafts was smaller and was more comprised of loose connective tissue compared with sockets left to heal naturally. Although the present sample size was too small for statistical analysis, the implication in this series of cases was that the overall bone quality was inferior to that of naturally healed sockets, which compromised the insertion of normal-sized implants and may also have jeopardized implant survival. In a recent paper, BoneCeramic® was used in sinus floor augmentation.[61] After a waiting time of 6 to 8 months prior to implant placement human histology revealed that the bone to graft contact was 34.0%. Compared with anorganic bovine bone, the material showed less newly formed bone. This delayed healing would suggest a longer waiting time. Indeed, a prolonged healing time (case #6 and #8) showed an improved bone mineralization in the grafted portion. The feasibility to prolong the time between extraction and implant placement in clinical practice is, however, doubtful. Another possible reason for the treatment outcome with BoneCeramic® could be related to the fact that no occlusive membranes were used to cover the fresh extraction defects. There is a consensus that membranes are needed when immediate placement (extraction followed by implant insertion) is performed, certainly in conjunction with biomaterial use.[62] In normal extraction sockets without implant placement, wound closure with coronal flap displacement is also considered an alternative technique.[2] It is also known that membrane covered defects present with significantly less ridge atrophy than control

sites. This was, however, also observed in the present study in the substituted sites without the use of membranes. It is clear from the preliminary findings of our study that further studies should address the biological mechanisms as well as material, site and patient characteristics that influence implant integration/survival in extraction sockets supplemented with BoneCeramic®. This includes, among others, the long-term influence of the presence of incorporated HA bodies on both the metabolic characteristics and physical properties of the newly formed bone.

CONCLUSIONS

On the basis of the present preliminary findings, it can be concluded that socket supplementation with BoneCeramic® yields excellent results with respect to volume preservation of the alveolar crest but influences bone healing negatively. That is, new bone formation is retarded and inefficient compared with naturally healed sockets after equal healing periods (ranging 6–74 weeks). Further research, based on a proper designed study with adequate numbers for statistical analysis, is needed to scrutinize the biological mechanisms and the long-term clinical benefits of the procedure. The authors emphasize the need for clear instructions concerning the minimum healing period at the time of launching of new grafting materials, which should be based on multivariate studies implicating different patient and site characteristics. Unfortunately, until today, the manufacturer has not provided evidence based on histological studies to provide insight in the usage of the biomaterial discussed in the present study in extraction sockets.

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