Bone Regeneration Using Porous Titanium Particles versus Bovine Hydroxyapatite: A Sinus Lift Study in Rabbits

France Lambert, DDS, MS, PhD;* Geoffrey Lecloux, DDS, MS;† Angelique Léonard, Ir, PhD;‡ Sophie Sourice, MSc;§ Pierre Layrolle, PhD;¶ Eric Rompen, DDS, MS, PhD**

ABSTRACT

Aim: The first objective of this study was to qualitatively and quantitatively assess the bone formation process, particularly the long-term behavior and three-dimensional volume stability of subsinusal bone regeneration, using titanium (Ti) or bovine hydroxyapatite (BHA) granules, in a rabbit model. The second objective was to evaluate the effect of the hydration of the BHA particles with a therapeutic concentration of doxycycline solution on the osteogenesis and biomaterial resorption.

Materials and Methods: Rabbits underwent a double sinus lift procedure using one of three materials: grade 1 porous Ti particles, BHA, or BHA hydrated with doxycycline solution (0.1 mg/ml) (BHATTC). Animals were sacrificed after 1 week, 5 weeks, or 6 months. Samples were analyzed using μCT and nondecalcified histology.

Results: The materials used in each of the three groups allowed an optimal bone formation; bone quantities and densities were not statistically different between the three groups. At 6 months, more stable three-dimensional volume stability was found with Ti and BHATTC ($p = .0033$). At 5 weeks and 6 months, bone to material contact corroborating osteoconduction was significantly higher with BHA and BHATTC than with Ti ($p < .0001$).

Conclusions and Clinical Implications: Even though the studied biomaterials displayed different architectures, they are relevant candidates for sinus lift bone augmentation prior to dental implants because they allow adequate three-dimensional stability and osteogenesis. However, to recommend the clinical use of Ti, both an observation on the drilling effects of Ti particles and clinical trials are needed.

KEY WORDS: bone regeneration, histomorphometry, hydroxyapatite, osteoconduction, titanium

INTRODUCTION

Trauma, infection, or even simple teeth extractions often lead to alveolar bone defects that impair dental implant placement. Preservative or reconstructive surgical techniques have been developed to preserve or regenerate an adequate bone volume for dental implants.[1,2] Autogenous bone grafting was considered the gold standard for such procedures because it contains the patient's own bone cells and growth factors, leading to osteoinductive properties. However, autologous bone grafting requires a second surgical site and has variable and unpredictable resorption rates.[3] The use of biomaterials in extraction socket preservation, implant site development (guided bone regeneration) and sinus lift

*Assistant professor, Department of Periodontology and Oral Surgery, Faculty of Medicine, University of Liège, 4000 Liège Belgium; †assistant professor, Department of Periodontology and Oral Surgery, Faculty of Medicine, University of Liège, Belgium; ‡professor, Department of Applied Chemistry, University of Liège, 4000 Liège, Belgium; §research technician, INSERM, U 791, Laboratory for Osteoarticular and Dental Tissue Engineering, University of Nantes, France; ¶professor, Inserm U957, Laboratory of Physiopathology of Bone Resorption, Faculty of Medicine, University of Nantes, France; **professor, head of the Department of Periodontology and Oral Surgery, Faculty of Medicine, University of Liège, 4000 Liège, Belgium

Correspondence to: Dr. France Lambert, Service de Médecine Dentaire, Domaine du Sart Tilman Bat B35, B-4000 Liège, Belgium; e-mail: france.lambert@chu.ulg.ac.be

DOI 10.1111/j.1708-8208.2011.00374.x

procedures are currently a well-documented and reliable approach.[1,4–6] For instance, systematic reviews have clearly shown that sinus lift procedures were successful when they used biomaterials exclusively.[1] Nonresorbable biomaterials are of great interest in the dental field because they are subjected to external loads such as tongue and lip forces or intrasinusal positive pressure that might lead to bone resorption.[3,7] The long-term stability of three-dimensional bone augmentation is a determinant factor for dental implant and aesthetic success.

The biological concept of subsinusal bone augmentations is well described in animal models.[8–12] If bone regeneration can be obtained with a simple blood clot, then regenerated bone is progressively resorbed concomitantly with a reexpansion of the sinusal cavity because of positive air pressure. When using autologous bone particles in sinus lifts, particles are completely remodeled into woven bone and subsequently into rarefied trabecular bone, a process that is associated with a significant decrease in the regenerated volume. In contrast, nonresorbable biomaterials seem to withstand the reexpansion of the sinus cavity and provide a long-term three-dimensional stability.[11]

Titanium (Ti) is known to be highly compatible in vivo and poorly corrosive in bodily fluids. For these reasons, this material is often used in dental implants and orthopedic prostheses. Both the mechanical stability and nonresorbable properties of titanium (Ti) are potential advantages for bone augmentation prior to dental implantation.[3,7] Ti particles were recently used to support bone regeneration in sinus lifts and in the treatment of peri-implantitis.[13,14] Nevertheless, tissue integration and three-dimensional bone regeneration with Ti particles were poorly explored in these previous reports.

The association of a therapeutic agent such as tetracycline that can prevent bacterial infection and influence regenerative cell behavior to a space filler such as bovine hydroxyapatite (BHA) could contribute to more predictable clinical outcomes.[15,16] Indeed, the nonantibiotic effects of doxycycline on fibroblastic cells, mesenchymal stem cells and osteogenic cells were clearly demonstrated in vitro.[15,17–19] Doxycycline seems to promote cell adhesion and consequently to induce the proliferation and osteogenic differentiation of mesenchymal stem cells as well as to inhibit osteoclast formation, thus decreasing bone or biomaterial resorption. Hydrating biomaterial with a tetracycline solution might be of interest to promote osteogenesis and decrease the material resorption. However, such effects of the local delivery of tetracycline in vivo have not been extensively investigated.

The first objective of the present study was to observe the behavior of grade 1 Ti porous particles in a rabbit model of sinus lifts and to compare the results with the outcomes of BHA, a commonly used biomaterial for alveolar bone regeneration. Qualitative and quantitative assessments of both early osteogenesis and the long-term behavior of the regenerated bone were performed. The three-dimensional volume changes, cell colonization, bone density, osteoconductivity, and resorption rates were explored in this study. The second objective was to evaluate the effect of the hydration of the BHA particles with a therapeutic concentration of doxycycline solution on the osteogenesis and biomaterial resorption.

MATERIALS AND METHODS

Animals

New Zealand White rabbits (adult, males, average body weight of 3.0 kg) were used in the study. All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Ethics Committee of the University of Liège, Belgium. The "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, was followed carefully.

Study Design

This study is part of an overall project where 96 sinus-lift procedures performed on 48 rabbits using 10 different types of space fillers were assessed at three distinct time points: 1 week, 5 weeks, and 6 months, respectively. Specifically, the space fillers were randomly allocated to the sinuses and 16 rabbits were sacrificed at each time point, so that at least three sinuses were available for each space filler at each time point, yielding a two-factor experimental design (space filler and time) with repeated measurements. In the present study, three space fillers were compared: BHA (Geistlich Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland, 0.25–1 mm particle size), grade 1 porous Ti particles (Natix®, Tigran, Malmö, Sweden, 0.7–1 mm particle size), or BHA hydrated with a doxycycline solution (0.1 mg/mL) (BHATTC;

Bio-Oss®, Geistlich Pharma AG). A total of 28 sinus-lift procedures were analyzed from 28 different rabbits.

Surgical Procedure

Anesthesia of the rabbits was induced by administration of a ketamine/xylazine bolus (respectively 65/4 mg/kg, IM) 20 min after a fentanyl/dehydrobenzperidol premedication (0.22 mL/kg of a bolus 25 µg/1.25 mg/mL IM). Two hours before surgery, animals also received buprenorphin at a dose of 0.05 mg/kg. This was administered twice a day for 2 days. Surgical interventions were performed under strict sterile conditions. The surgical area was shaved and disinfected with iodine, and a straight incision was made to expose the nasal bone and the naso-incisal suture lines. The soft tissues were reflected with the periosteum in order to access the upper bone wall of the sinus. Two ovoid windows (approximately 6 × 4 mm) were created bilaterally using a round diamond bur. The membrane was carefully raised from the floor and lateral walls and the space-filling material was inserted into the created compartment. The volume of filling material was standardized to 0.4 mL per sinus using insulin syringes. The bony windows were covered with a resorbable membrane (Biogide, Geistlich, Wolhusen, Switzerland) and the wounds were sutured with 4/0 polyester thread (Permasharp, Hu Friedy, Rotterdam, the Netherlands) (Figure 1). Animals were sacrificed by injection of pentobarbital (200 mg/kg, IV, after the same premedication as for surgeries). Samples were dissected (Figure 2) and soaked in fixative (6% formol).

X-ray Microtomography Analysis

All collected samples were first submitted to x-ray microtomography. Before scanning, the samples were transferred to an Eppendorf® tube containing fixative. The tube was affixed to the brass stub and examined with a Skyscan 1,172 high-resolution desktop micro-CT system (Skyscan®, Kontich, Belgium). The cone beam source operated at 100 kV and 100 µA. The detector was a two-dimensional, 1,048 × 2,000 pixel, 16-bit x-ray camera. The sample was rotated through 180° with a rotation step of 0.49°, giving an acquisition time of 30 min per sample. Taking into account the camera definition and the source-object-camera distance, two-dimensional images with a pixel size of 17.28 µm were obtained, using a cone-beam reconstruction algorithm. The corresponding three-dimensional images were produced by stacking all the two-dimensional cross-sections.

Analysis of the three-dimensional images allowed the calculation of the total volume of the regenerated space at baseline, at 5 weeks and at 6 months. The three-dimensional measurements were carried out with the CTscan software (release 2.5, Skyscan®).

Histological Analysis

The samples were processed for nondecalcified histology using polymethacrylate (PMMA) resin. After fixation for about 1 week, the samples were dehydrated in ascending graded ethanol series (24 h each grade) and then placed in pure acetone for 24 h. Finally, samples were impregnated with methylmethacrylate for 4 days with one refreshment before embedding in PMMA at 4°C for 4 days. Each resulting nondecalcified block was cut sagittally with a circular diamond saw (Leica SP1600, Leica Microsystems, Wetzlar, Germany) at two different levels in the central region. The first cut was in the area of the window and the second, 1.5 mm outwards. The two slices were then polished with a grinding machine

Figure 1 Surgical procedure. (A) The maxillary sinus was opened and the membrane was pushed inwards. (B) Space fillers were calibrated and inserted into the created cavity using a customized syringe. (C) The windows were covered with collagen membranes before suturing.

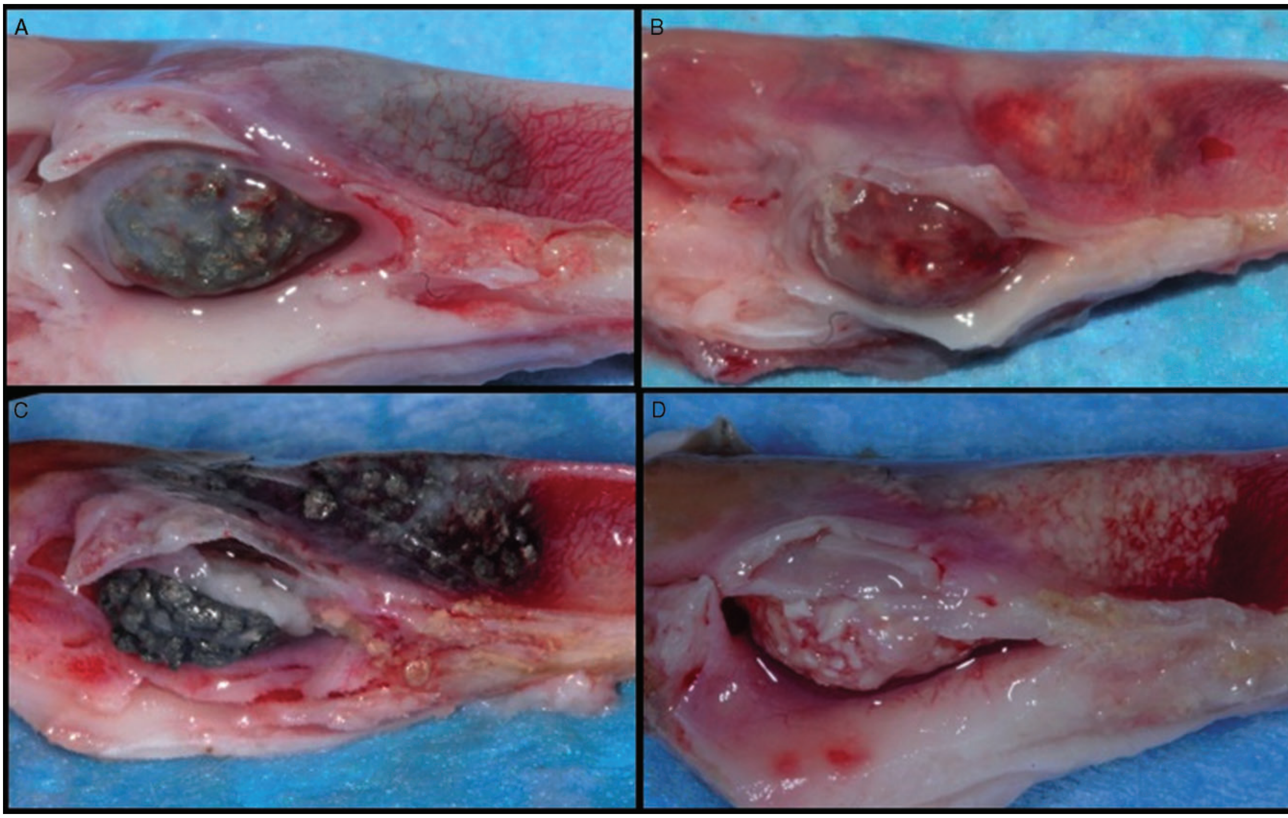

Figure 2 Samples of rabbit augmented maxillary sinuses at 1 week (A, C) and 5 weeks (B, D) with Ti and BHA. Membrane thickness seems to be thinner at 5 weeks than at 1 week postsurgery. BHA = bovine hydroxyapatite; Ti = titanium.

(Metaserv®2000, Buehler, Düsseldorf, Germany) and sputter coated with a thin layer of gold/palladium on both sides. Samples were observed under scanning electron microscopy (SEM; Leo 1450 VP, Zeiss, Berlin, Germany). SEM observations were made using backscattered electron mode (BSE). Moreover, 30-µm sections were cut and polished using the same material from the rest of each block in the close vicinity of the central area and were stained with HTX-eosin and counterstained with toluidine blue. To allow a better observation of cells, thin 7-µm sections were also created using a hard tissue microtome (Leica Polycut SM 2500) and stained with Goldner trichrome. However, samples from the Ti group could not be treated this way because Ti particles were too hard to cut using the microtome.

Histomorphometry

Scanning electron micrographs (Leo 1450 VP) were taken using the BSE mode at 30× magnification and assembled to visualize the entire sinus. These contiguous BSE pictures allowed a quantitative evaluation of the mineralized bone, the remaining biomaterial, and the soft tissue areas based on their respective gray levels using a semiautomatic image analyzer (Leica Qwin). The regions of interest were manually defined, and the different areas were automatically calculated. The following measurements were made:

- augmented area, defined as the raw surface of the secluded space underneath the sinus membrane;
- bone formation, space-filler area and noncalcified tissues, all expressed as percentages of the augmented area;
- regenerated area, defined as the raw surface invaded by new bone, calculated in mm^2 (Figure 3);
- bone density: defined as the percentage of bone inside the regenerated area
- Bone to material (BMC) contact: defined as the percentage of particle perimeter in contact newly formed bone.

Statistical Analyses

For each variable studied, one value was obtained for each sinus. Results were expressed as mean, standard deviation (SD), minimum and maximum. The

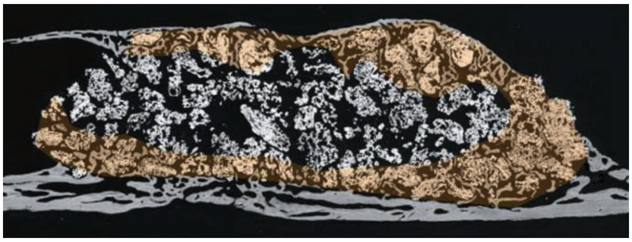

Figure 3 Scattered electron microscopy micrographs of subsinusal spaces filled with Ti at 5 weeks. Representation of the regenerated area in orange: surface invaded by newly formed bone inside the augmented area. Ti = titanium.

experimental data were analyzed by two-way analysis of variance (ANOVA) with repeated measurements, allowing a test for interaction between the two factors. When the interaction term was significant, space fillers were subsequently compared at each time point by one-way ANOVA. Otherwise, the overall time and space-filler effects were tested. Results were considered to be significant at the 5% level ($p < .05$). A Bonferroni correction was applied to account for multiple comparisons. Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

A total of 28 sinuses were available from 28 different rabbits in this study. The sinuses were considered as statistically independent experimental units. There were at least three sinuses in each experimental cell.

Macroscopically, at the time of dissection, the sinusal membranes appeared to be thicker 1 week postoperative compared with 5 weeks (see Figure 2).

Descriptive Histological Analysis

The normal sinus cavity was surrounded by respiratory mucosa and a thin layer of cortical bone. The mucosa was composed of ciliated stratified cuboidal epithelium, lamina propria, and submucosal tissues containing serous glands (Figure 4). The histological analysis did not display any difference between the BHA and the BHATTC groups.

One Week after Implantation. No evidence of inflammation was found in any of the three groups. A penetration of connective and vascularized buds into the subsinusal space was observed along the bone walls and under the lifted sinusal membrane. Early steps of centripetal new bone formation were observed progressing from the anterior part of the sinus and the bone walls (Figure 5). In the Ti group, cell proliferation was only visible at the periphery of the created space, while the blood clot was still noninvaded by cells in the center of the cavity on the 30-µm sections (see Figure 5, C and D). With BHA and BHATTC, on the 7-µm sections, cell colonization was found throughout in the cavity, and remnants of the clot were sometimes visible. Osteoclasts were sometimes present along the BHA particles (Figures 4 and 6).

Five Weeks after Implantation. In the BHA groups, newly formed bone bridged the particles of hydroxyapatite. Most of the particle surfaces were in tight contact with a layer of newly formed bone. Only the center of the regenerated area was not filled with new bone (Figure 7). Some osteoclastic cells could be found along the particles (Figure 8), and osteoblastic cells were observed only in the central region.

In the Ti group, the newly formed bone was still mostly immature with few areas of bone marrow. The newly formed bone was sometimes in contact with the particles, and the ingrowth seemed to be independent of the Ti space filler (Figure 9). Under the sinusal membrane, the mucosal epithelium was in close contact and matched the shape of the granules (see Figure 9C).

Six Months after Implantation. In the BHA groups, bone marrow and adipocytes were much more frequently observed than at 5 weeks, whereas multinucleated cells

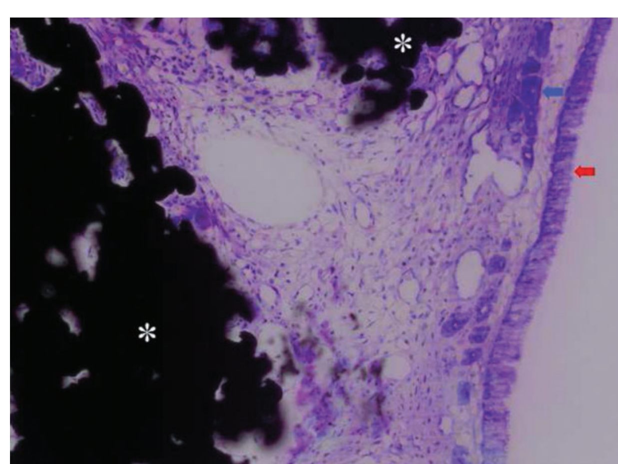

Figure 4 Ti group at 1 week: nondecalcified section of an augmented sinus floor in the region under the sinusal membrane (10×; staining: HTX-eosin/toluidine blue). Note the ciliated stratified cuboidal epithelium (red arrow), submucosal tissues containing serous glands (blue arrow) and the Ti porous particle (white asterisk). Ti = titanium.

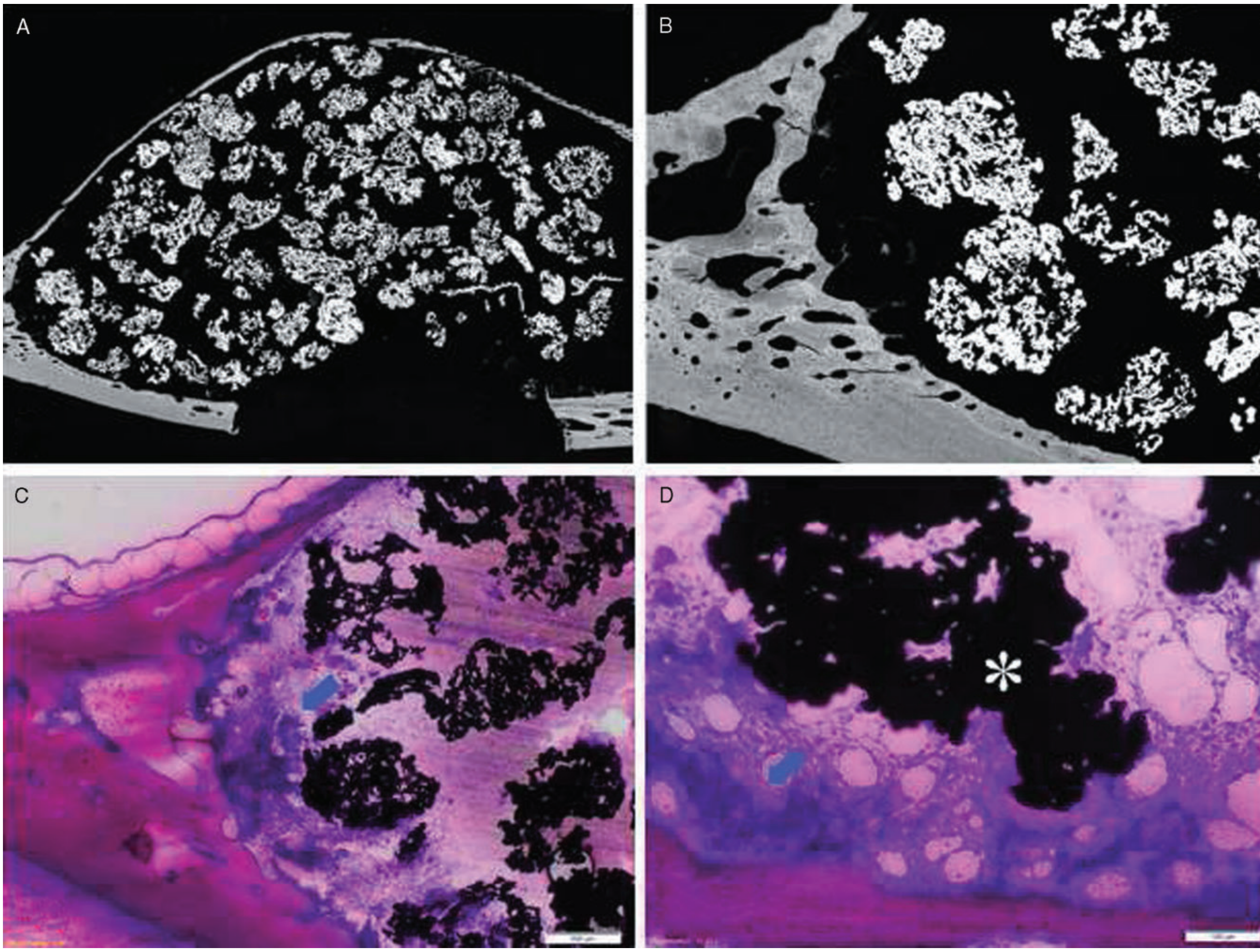

Figure 5 Ti group, 1 week. (A) Scattered electron microscopy micrographs: reconstruction of the full sagittal section. (B) Scattered electron microscopy (magnification 30×). (C) Nondecalcified histology (2×, staining: HTX-eosin/toluidine blue). (D) Nondecalcified histology (10×, staining: HTX-eosin/toluidine blue). Note newly formed bone in the anterior part of the sinus (blue arrow) and porous Ti particle (white asterisk). Ti = titanium.

were no longer visible. Lamellar bone was only found in intimate contact with the particles and bridged them together (Figure 10). The BHA granules located just under the sinusal membrane sometimes protruded into the sinusal cavity. Bone trabeculae were covered with a unicellular flat layer of cells; neither osteoid tissue nor osteoblasts were noted. At 6 months, in the Ti group, bone density was very low, without visible interconnections between trabeculae; large bone marrow spaces with adipocytes were observed (Figure 11). Still, few

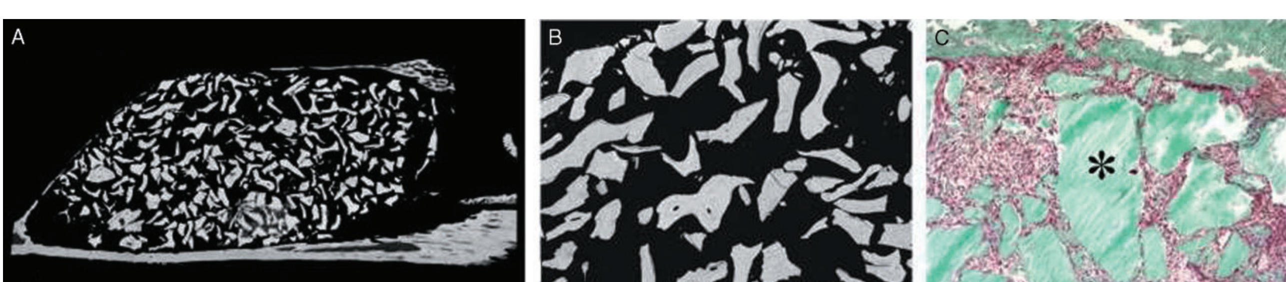

Figure 6 BHA, 1 week. (A) Scattered electron microscopy micrographs: reconstruction of the full sagittal section. (B) Scanning electron microscopy (magnification 30×). (C) Nondecalcified histology (10×, staining: Goldner trichrome). BHA particles are stained in light green (asterisk). BHA = bovine hydroxyapatite.

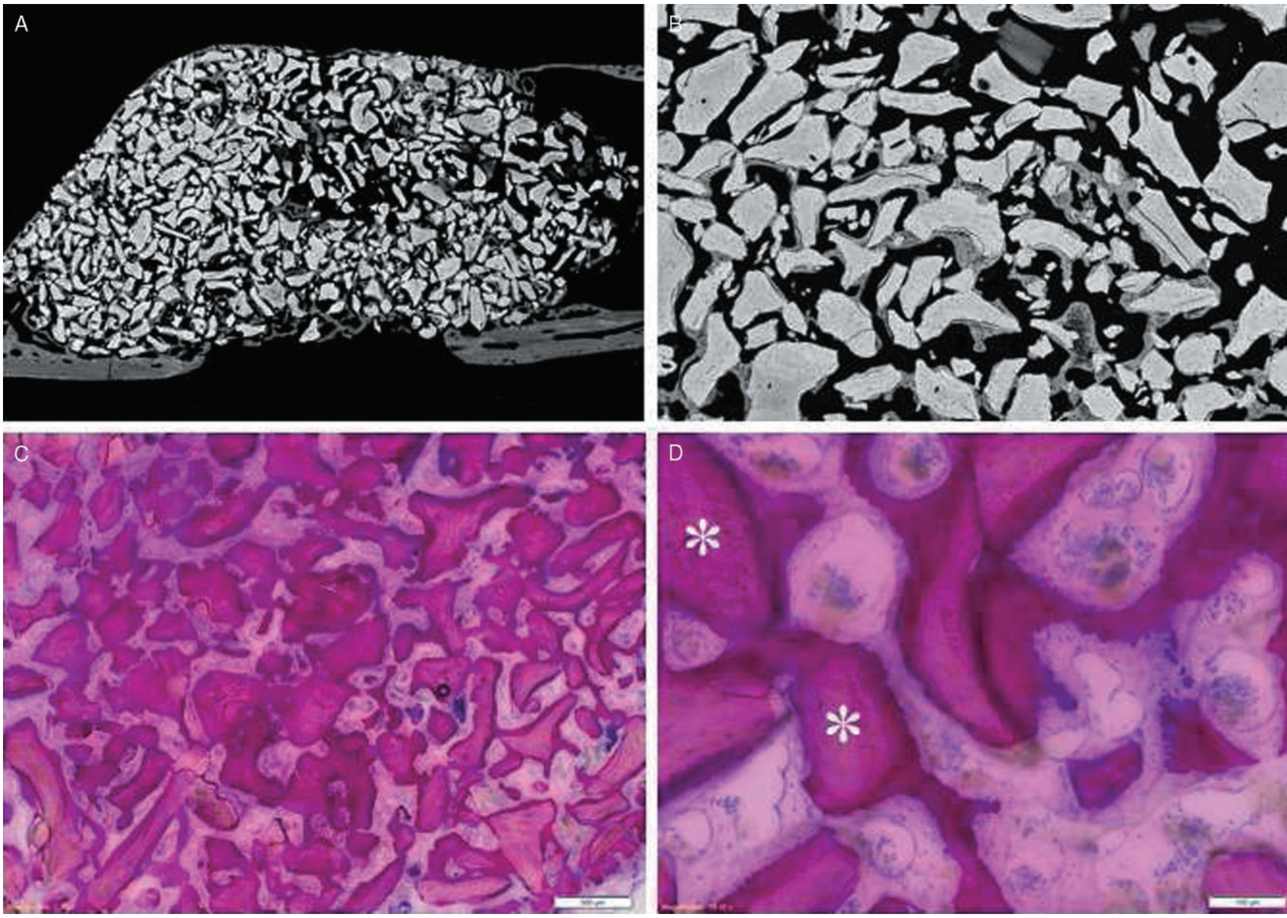

Figure 7 BHA group, 5 weeks. (A) Scattered electron microscopy micrographs: reconstruction of the full sagittal section. (B) Scattered electron microscopy (magnification 30×); newly formed bone (grey) bridges the BHA particle (white) together. (C) Nondecalcified histology (2×, staining: HTX-eosin/toluidine blue). (D) Nondecalcified histology (10×, staining: Goldner trichrome). Note newly formed bone in dark red around the BHA particle (white asterisk). BHA = bovine hydroxyapatite.

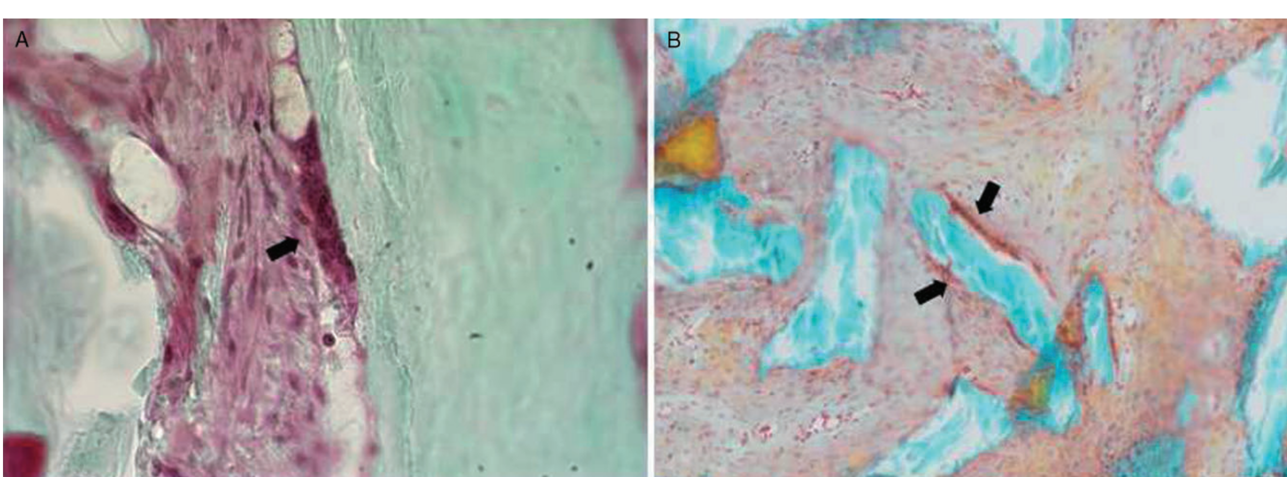

Figure 8 At 5 weeks. (A) BHA. (B) BHATTC. Note the presence of osteoclasts along the biomaterial particles (black arrows). BHA = bovine hydroxyapatite; BHATTC = chemically modified bovine hydroxyapatite.

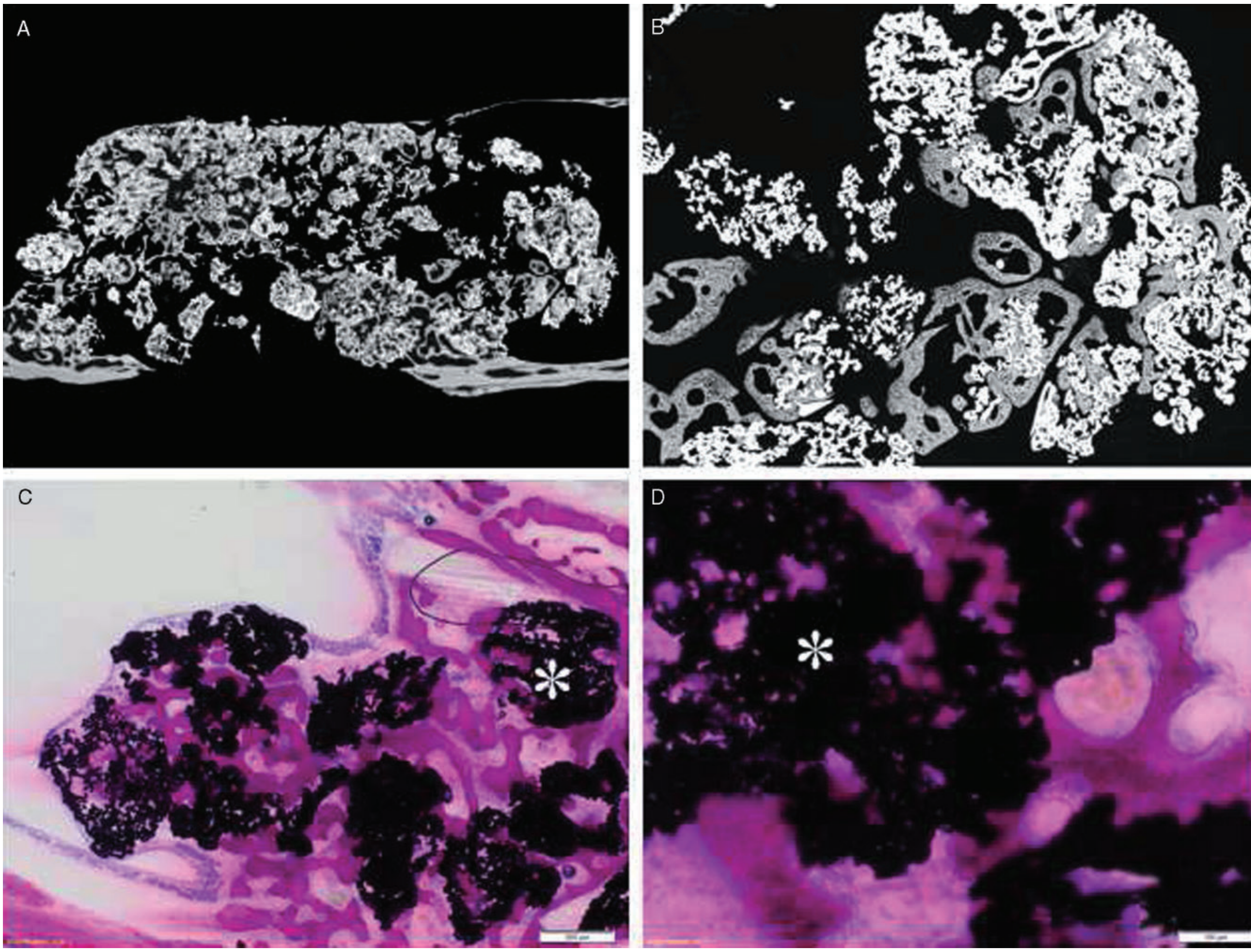

Figure 9 Ti group, 5 weeks. (A) Scattered electron microscopy micrographs: reconstruction of the full sagittal section. (B) Scattered electron microscopy (magnification 30×). (C) Nondecalcified histology (2×, staining: HTX-eosin/toluidine blue). (D) Nondecalcified histology (10×, staining: HTX-eosin/toluidine blue). Ti = titanium.

contacts were observed between the bone and the Ti biomaterial.

Histomorphometric Analysis

By applying two-way ANOVA to histomorphometrical data (augmented area, bone formation, space-filler area, and noncalcified tissues), no significant interaction was found between space filler and time for any of the variables. Thus, the time evolution of histomorphometrical parameters was similar in each space-filler group. As seen in Table 1, for the augmented area, BHA differed significantly from Ti and BHATTC with lower values ($p = .0078$). The time effect was not significant ($p = .62$). There was a significant increase of bone formation along time ($p < .0001$) but no difference was seen between the three biomaterials ($p = .28$). The space-filler area did not change with time ($p = .22$) but differed significantly ($p = .0028$) between space-fillers BHA and Ti. Finally, the percentage of noncalcified (soft) tissues clearly decreased over time ($p < .0001$) and a significant statistical difference appeared between Ti and BHA/BHATTC ($p = .001$).

Regenerated Area. The interaction term was not significant. No difference was observed between the three biomaterials ($p = .094$) but the augmented area significantly increased over time ($p < .0001$) (Table 2).

Bone Density. The interaction term was not significant. The percentage of bone inside the regenerated area was comparable in each biomaterial groups ($p = .17$), but it decreased between 5 weeks and 6 months ($p = .0007$) (see Table 2, Figure 12).

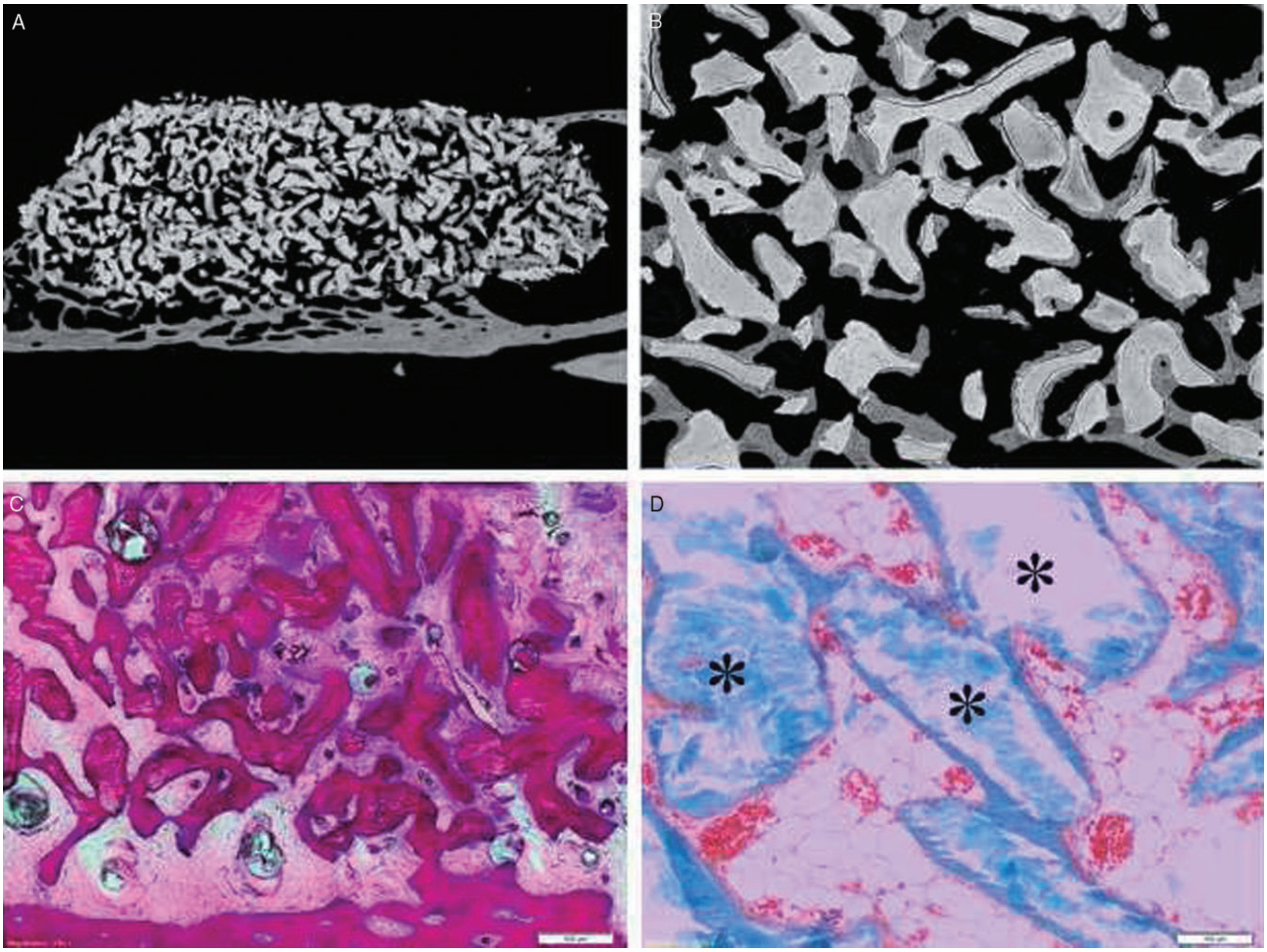

Figure 10 BHA, 6 months. (A) Scattered electron microscopy micrographs: reconstruction of the full sagittal section. (B) Scattered electron microscopy (magnification 30×). (C) Nondecalcified histology (2×, staining: HTX-eosin/toluidine blue). (D) Nondecalcified histology (7-µm sections) (10×, staining: Goldner trichrome). BHA particules (asterisk) appear lost or disrupted because of the thin section processing. BHA = bovine hydroxyapatite.

Bone to Material Contact. The interaction term was not significant. The percentage of material perimeter in contact with regenerated bone was significantly lower ($p < .0001$) in the Ti group (see Table 2, Figure 13).

MicroCT Analysis: Three-Dimensional Volume Variation. Two-way ANOVA applied to three-dimensional volumes did not reveal any significant interaction ($p = .12$) between space filler and time. An overall negative time trend of three-dimensional volume variation was observed ($p = .0081$) while a significant statistical difference of three-dimensional volume was found between Ti and BHA ($p = .0033$) (Table 3). Figure 14 gives the correlation between volume variation and the percentages of bone, space filler, and soft tissues within augmented tissues for each time point.

DISCUSSION

The first objective of this study was to observe the short and continuing behavior at a tissue level of grade 1 Ti porous particles used as a space filler in a rabbit model of sinus lift and to compare the outcomes to BHA. The second objective was to evaluate the potential effect of treating BHA with tetracycline.

Porous Ti particles were recently used in clinical human models.[13,14] Nevertheless, tissue integration and three-dimensional bone regeneration with this material were poorly explored, and only a few investigations have been carried out for alveolar bone regeneration. Wolfang and colleagues,[20] in a tibial defects model in rabbits, primarily studied the in vivo biological performance of metallic or oxidized porous Ti granules. They hypothesized that Ti has thrombogenic properties

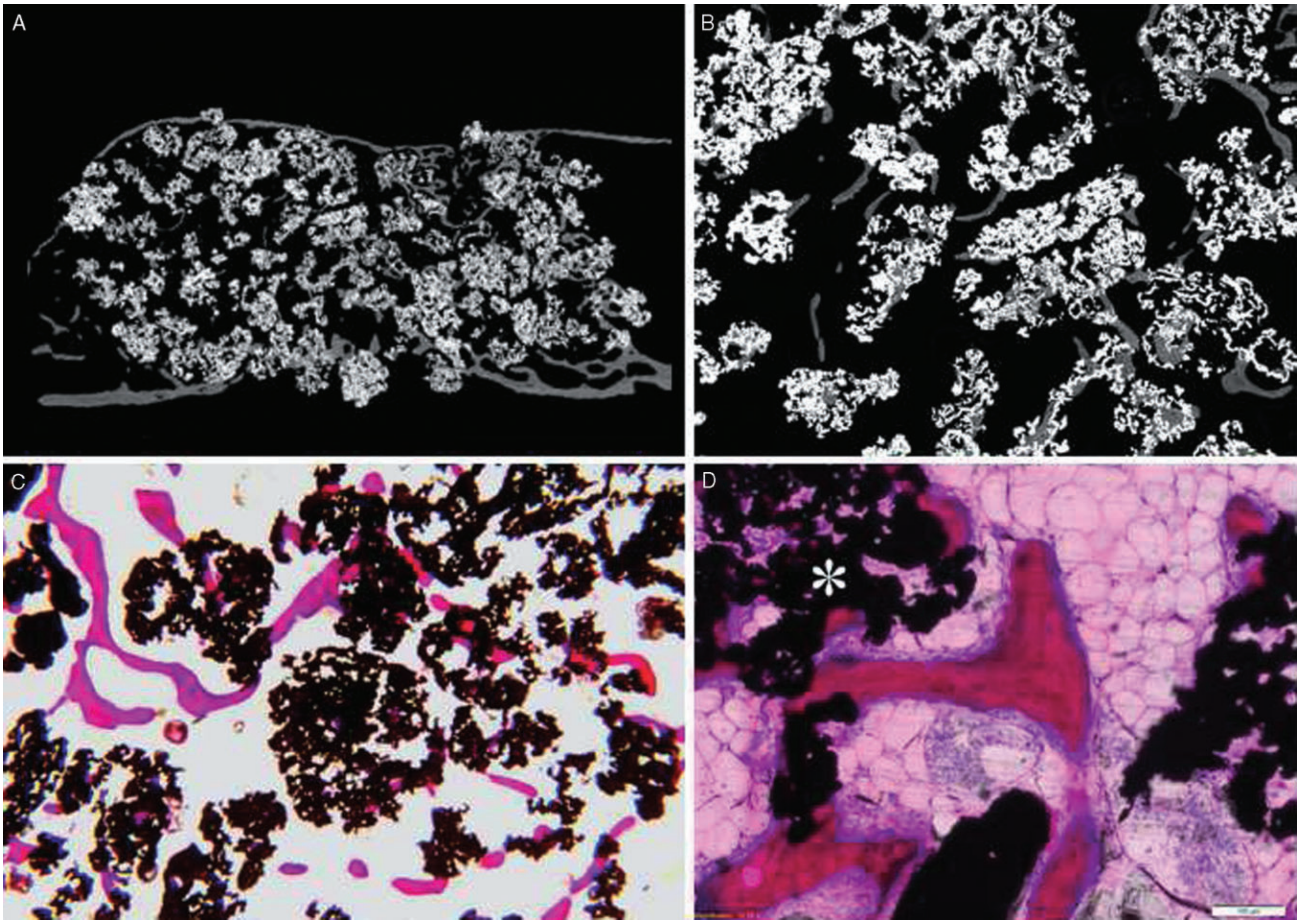

Figure 11 Ti group, 6 months. (A) Scattered electron microscopy micrographs: reconstruction of the full sagittal section. (B) Scattered electron microscopy (magnification 30×). (C) Nondecalcified histology (2×, staining: HTX-eosin/toluidine blue). (D) Nondecalcified histology (10×, staining: HTX-eosin/toluidine blue). Note that the rarefied bone trabeculae (red staining) are not in close contact with Ti particles (white asterisk). Ti = titanium.

which might result in the release of higher levels of growth factors into the blood clot and enhanced osteogenesis.

In the present study, bone formation quality and quantity, osteoconductivity (BMC), three-dimensional volume changes, and material resorption rates were explored. The model used was based on a previously described model for studying the performance of biomaterials.[8–12,21]

Both Ti and BHA showed here an excellent biocompatibility with no signs of inflammation. Moreover, both biomaterials proved to be as effective in terms of newly formed bone quantity and regenerated area (surface invaded by bone) at each time point (see Table 2).

In terms of bone density, an effect of time was observed with reduction of the density from 5 weeks to 6 months, most likely related to the bone maturation. At 6 months, the bone quantity and density (see Figure 12) were lower in the Ti group than in the BHA and BHATTC groups, but this difference did not reach the level of statistical significance.

Another parameter evaluated in this study was the osteoconduction potential of the various materials as measured by BMCs. Osteoconduction is a physical effect by which the biomaterial's matrix forms a scaffold that allows outside cells to penetrate the graft and form new bone.[22] Our results clearly indicate that BHA has a high osteoconductive potential, superior to that of Ti (see Figure 13): BHA could potentially exhibit calcium and phosphate ions, leading to the precipitation of biological carbonated apatites on their surface. This layer of biological apatite, which incorporates various proteins, favors the adhesion, proliferation, and differentiation of the osteoprogenitor cells that produce the bone extracellular matrix, resulting in direct bone apposition on calcium phosphate biomaterials.[23] In the case of Ti, unless bioactive surface treatments such as alkaline or calcium phosphate coatings are used, the metal is in

TABLE 1 Augmented Area and Histomorphometric Data – Mean (SD) (Min–Max)

	BHA	Ti	BHATTC	*p* Value* Time Effect	*p* Value* Space-Filler Effect
Augmented area mm^2					
1 week	**46.8** (6.4)	**54.4** (2.8)	**49.1** (12.0)	0.62	0.0078
	(38.0–52.3)	(51.8–57.3)	(37.7–61.6)		
5 weeks	**47.5** (2.1)	**52.0** (7.1)	**59.6** (3.9)		
	(45.1–49.1)	(45.9–59.8)	(55.1–62.3)		
6 months	**43.8** (1.9)	**55.9** (4.8)	**55.9** (3.5)		
	(42.1–45.8)	(50.9–60.5)	(51.9–58.0)		
% Bone					
1 week	**0.1** (0.1)	**0.1** (0.1)	**0.3** (0.2)	<0.0001	0.28
	(0.0–0.3)	(0.0–0.2)	(0.0–0.4)		
5 weeks	**14.8** (2.1)	**13.7** (4.5)	**12.6** (2.4)		
	(12.8–16.9)	(8.7–17.3)	(10.1–14.9)		
6 months	**16.0** (3.7)	**11.0** (2.1)	**16.5** (3.0)		
	(13.1–20.2)	(8.9–13.2)	(13.6–19.6)		
% Filler					
1 week	**42.7** (3.9)	**32.8** (1.4)	**36.6** (9.8)	0.22	0.0028
	(38.5–47.7)	(31.2–33.8)	(26.1–45.5)		
5 weeks	**40.3** (3.1)	**29.6** (1.9)	**37.6** (5.9)		
	(37.5–43.6)	(27.9–31.6)	(31.9–43.7)		
6 months	**34.9** (5.1)	**31.3** (3.6)	**35.0** (1.9)		
	(30.8–40.6)	(28.6–35.4)	(33.2–36.9)		
% Soft tissues					
1 week	**57.2** (3.9)	**67.1** (1.3)	**63.2** (9.9)	<0.0001	0.001
	(52.3–61.4)	(66.2–68.6)	(54.2–73.7)		
5 weeks	**45.7** (2.1)	**56.7** (5.5)	**49.9** (7.4)		
	(43.7–47.8)	(51.1–62.2)	(41.4–55.4)		
6 months	**49.2** (4.5)	**57.7** (5.4)	**48.7** (2.8)		
	(44.8–53.7)	(51.4–61.2)	(45.5–50.9)		

*The interaction term was not significant.

contact with a fibrous tissue layer and is in partial contact with the bone.[24–26]

Biomaterials having non- or slowly resorbable properties are of great interest in alveolar ridge augmentation because these sites are often subjected to external pressure that might induce the resorption of the regenerated bone and therefore impact implant success. In the present study, the three-dimensional volume stability of the bone regenerations was also measured, by using µCT. The main advantage of this three-dimensional-imaging technique lies in its nondestructive approach, as opposed to SEM, which requires cutting of the sample and two-dimensional determination. With its large field of view, x-ray microtomography allows scanning of the entire region of interest within the samples. This technique has been primarily used to characterize highly porous materials.[27–33]

Ti proved to efficiently prevent the remodeling of the augmented volume: our results displayed stable three-dimensional volumes and two-dimensional surfaces (augmented area) at each time point, corroborating the interlocking and unpackable properties described by the manufacturer of this material. Moreover, the invariable surface percentages of the remaining space filler confirmed the nonresorbable properties of Ti, which is probably also a factor in the volume stability.

The volume of the samples treated with BHA appeared to be slightly less stable, with a decrease limited to about 15% compared with the baseline volume; this

TABLE 2 Regenerated Areas and Tissue Densities – Mean (SD) (Min–Max)

Time	BHA	Ti	BHATTC	*p* Value* Time Effect	*p* Value* Space Filler Effect
Regenerated area mm^2					
1 week	**0.3** (0.2)	**0.5** (0.4)	**0.6** (0.6)	<0.0001	0.094
	(0.1–0.5)	(0.0–0.9)	(0.1–1.2)		
5 weeks	**32.4** (4.3)	**34.6** (10.0)	**38.1** (11.4)		
	(28.7–37.1)	(25.3–45.3)	(28.4–50.6)		
6 months	**42.4** (3.3)	**54.0** (4.4)	**55.0** (5.6)		
	(40.5–46.1)	(49.1–57.3)	(48.7–59.3)		
Bone density %					
1 week	NA	NA	NA	0.0007	0.17
5 weeks	**21.8** (1.9)	**20.5** (1.2)	**20.2** (3.9)		
	(19.7–23.3)	(19.1–21.3)	(17.8–24.6)		
6 months	**16.5** (3.9)	**11.4** (2.5)	**16.7** (2.4)		
	(14.1–21.0)	(8.7–13.7)	(14.5–19.2)		
Bone-material contact %					
1 week	NA	NA	NA	0.22	<0.0001
5 weeks	**60.5** (5.4)	**13.5** (5.2)	**51.3** (7.0)		
	(56.2–65.6)	(7.6–17.4)	(44.1–58.1)		
6 months	**50.8** (2.3)	**14.5** (5.2)	**50.5** (0.9)		
	(49.1–53.4)	(8.8–19.0)	(49.8–51.5)		

*The interaction term was not significant.
NA = not applicable.

observation might be related to the slight resorption of the filling material.

The secondary objective of this study was to evaluate the effect of hydration of the BHA particles with therapeutic concentrations of a doxycycline solution on osteogenesis and biomaterial resorption. The use of doxycycline in guided bone regeneration might be relevant to induce bone formation while preventing

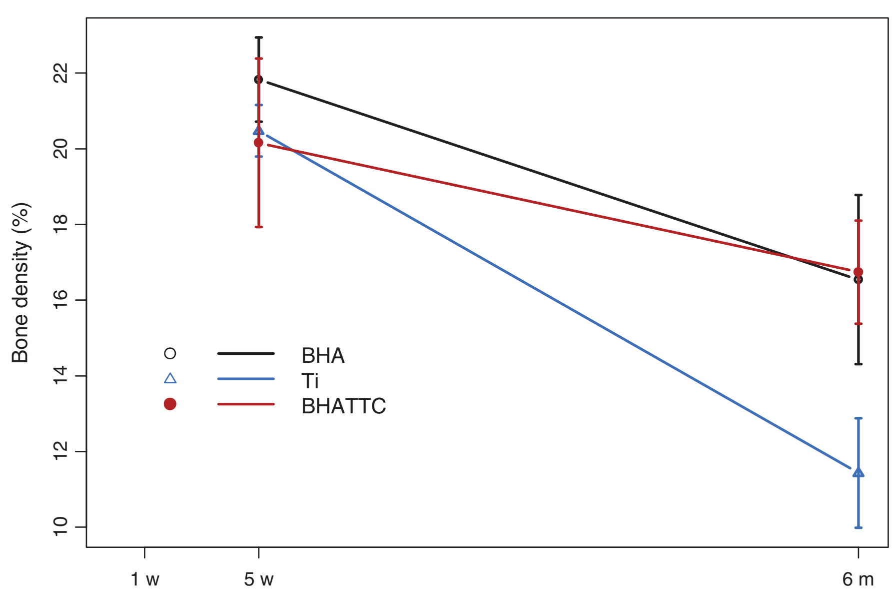

Figure 12 Evolution of the bone density from 5 weeks to 6 months.

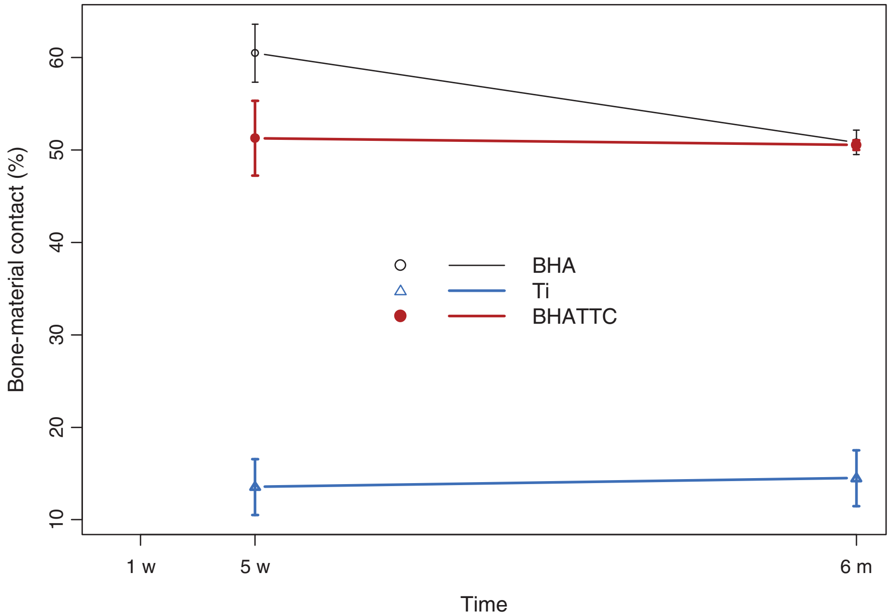

Figure 13 Level of bone to material contact (BMC) at 5 weeks and 6 months.

bacterial infection: Gomes and colleagues[15] demonstrated in vitro that ceramic-based biomaterials loaded with tetracyclines combine the antimicrobial activity in implant-related bone infections with an induction of osteoblastic proliferation and maintenance of the characteristic biological activity of the cultured cells. Further properties of doxycycline, such as inhibition of matrix metalloproteases as well as osteoclast and osteoprogenitor cell adhesion were emphasized in vitro and in vivo.[16–19,34–37]

In the present study, BHA treated with doxycycline displayed similar levels of osteogenesis and of osteoconductivity (BMC) as compared with native BHA. The percentage of residual filling material at 6 months was not influenced by the presence of doxycycline; however, the volume appeared to be more stable with BHATTC. The effect of doxycycline on biomaterials with higher resorption rates should be investigated preferably using immunohistology and gene expression analyses.

It must be emphasized that a limitation of the present study is linked to the limited number of samples for each condition. A higher number of samples might have emphasized the intergroup differences.

CONCLUSION

Titanium porous particles and BHA allowed optimal bone formation in sub-sinusal bone regeneration in a rabbit model. Nevertheless, the BMC proved to be significantly higher on BHA/BHATTC than on Ti. The studied biomaterials are relevant candidates for sinus lift

TABLE 3 Volume Variations for the Different Space Fillers at Each Time Point – Mean (SD) (Min–Max)

Time	BHA	Ti	BHATTC	*p*-Value* Time Effect	*p*-Value* Space Filler Effect
1 week	**344** (16) (323–363)	**374** (21) (350–387)	**386** (41) (352–431)	0.0081	0.0033
5 weeks	**321** (12) (314–335)	**340** (7) (332–346)	**330** (39) (286–360)		
6 months	**287** (23) (262–308)*	**375** (6) (369–381)*	**338** (30) (309–368)		

*The interaction term was not significant.

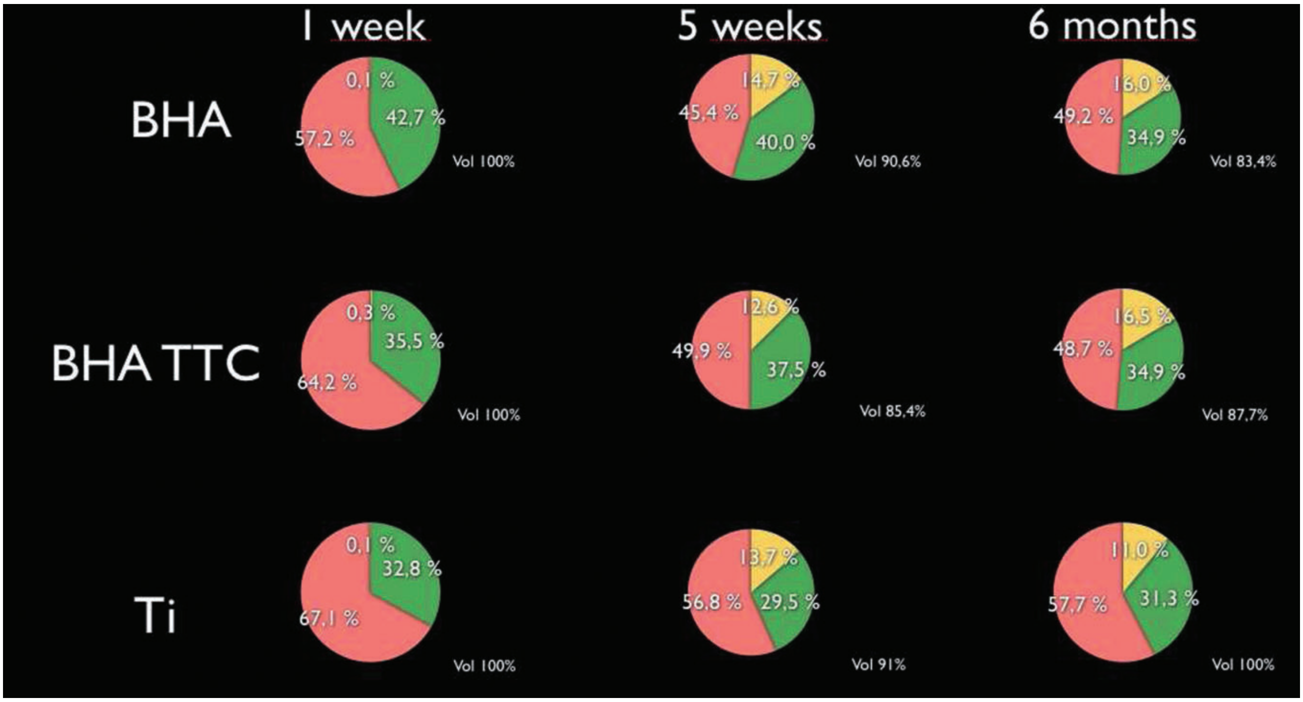

Figure 14 Pie charts of the mean proportions of new bone, space filler, and noncalcified soft tissues, taking into account the volume variation of the bone augmentations.

bone augmentation prior to dental implants because they allow three-dimensional stability. However, observations on the drilling effects of Ti particles as well as on the effect of Ti incorporation on mechanical properties of peri-implant bone should be further investigated to clinically recommend the used of porous Ti particles in bone alveolar bone regeneration.

REFERENCES

1. Pjetursson BE, Tan WC, Zwahlen M, Lang NP. A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation. J Clin Periodontol 2008; 35(8 Suppl):216–240.
2. Esposito M, Grusovin MG, Coulthard P, Worthington HV. The efficacy of various bone augmentation procedures for dental implants: a Cochrane systematic review of randomized controlled clinical trials. Int J Oral Maxillofac Implants 2006; 21(5):696–710.
3. Sbordone L, Toti P, Menchini-Fabris GB, Sbordone C, Piombino P, Guidetti F. Volume changes of autogenous bone grafts after alveolar ridge augmentation of atrophic maxillae and mandibles. Int J Oral Maxillofac Surg 2009; 38(10):1059–1065.
4. Chiapasco M, Zaniboni M. Clinical outcomes of GBR procedures to correct peri-implant dehiscences and fenestrations: a systematic review. Clin Oral Implants Res 2009; 20 (Suppl 4):113–123.
5. Barone A, Aldini NN, Fini M, Giardino R, Calvo Guirado JL, Covani U. Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study. J Periodontol 2008; 79(8):1370–1377.
6. Esposito M, Grusovin MG, Worthington HV, Coulthard P. Interventions for replacing missing teeth: bone augmentation techniques for dental implant treatment. Cochrane Database Syst Rev 2006(1):CD003607.
7. Hatano N, Shimizu Y, Ooya K. A clinical long-term radiographic evaluation of graft height changes after maxillary sinus floor augmentation with a 2:1 autogenous bone/xenograft mixture and simultaneous placement of dental implants. Clin Oral Implants Res 2004; 15(3):339–345.
8. Asai S, Shimizu Y, Ooya K. Maxillary sinus augmentation model in rabbits: effect of occluded nasal ostium on new bone formation. Clin Oral Implants Res 2002; 13(4): 405–409.
9. Sun XJ, Zhang ZY, Wang SY, Gittens SA, Jiang XQ, Chou LL. Maxillary sinus floor elevation using a tissue-engineered bone complex with OsteoBone and bMSCs in rabbits. Clin Oral Implants Res 2008; 19(8):804–813.
10. Xu H, Shimizu Y, Asai S, Ooya K. Experimental sinus grafting with the use of deproteinized bone particles of different sizes. Clin Oral Implants Res 2003; 14(5):548–555.
11. Xu H, Shimizu Y, Asai S, Ooya K. Grafting of deproteinized bone particles inhibits bone resorption after maxillary sinus floor elevation. Clin Oral Implants Res 2004; 15(1):126–133.

12. Xu H, Shimizu Y, Onodera K, Ooya K. Long-term outcome of augmentation of the maxillary sinus using deproteinised bone particles experimental study in rabbits. Br J Oral Maxillofac Surg 2005; 43(1):40–45.
13. Bystedt H, Rasmusson L. Porous titanium granules used as osteoconductive material for sinus floor augmentation: a clinical pilot study. Clin Implant Dent Relat Res 2009; 11(2):101–105.
14. Alffram PA, Bruce L, Bjursten LM, Urban RM, Andersson GB. Implantation of the femoral stem into a bed of titanium granules using vibration: a pilot study of a new method for prosthetic fixation in 5 patients followed for up to 15 years. Ups J Med Sci 2007; 112(2):183–189.
15. Gomes PS, Santos JD, Fernandes MH. Cell-induced response by tetracyclines on human bone marrow colonized hydroxyapatite and Bonelike. Acta Biomater 2008; 4(3): 630–637.
16. Luginbuehl V, Ruffieux K, Hess C, Reichardt D, von Rechenberg B, Nuss K. Controlled release of tetracycline from biodegradable beta-tricalcium phosphate composites. J Biomed Mater Res B Appl Biomater 2010; 92(2):341–352.
17. Rompen EH, Kohl J, Nusgens B, Lapiere CM. Kinetic aspects of gingival and periodontal ligament fibroblast attachment to surface-conditioned dentin. J Dent Res 1993; 72(3):607–612.
18. Vanheusden A, Nusgens B, Goffinet G, Zahedi S, Lapiere CM, Rompen E. In vitro modulation of human gingival epithelial cell attachment and migration by minocycline-HCL. J Periodontal Res 1998; 33(6):377–385.
19. Vanheusden AJ, Goffinet G, Zahedi S, Nusgens B, Lapiere CM, Rompen EH. In vitro stimulation of human gingival epithelial cell attachment to dentin by surface conditioning. J Periodontol 1999; 70(6):594–603.
20. Wohlfahrt JC, Monjo M, Ronold HJ, Aass AM, Ellingsen JE, Lyngstadaas SP. Porous titanium granules promote bone healing and growth in rabbit tibia peri-implant osseous defects. Clin Oral Implants Res 2010; 21(2):165–173.
21. Xu H, Shimizu Y, Ooya K. Histomorphometric study of the stability of newly formed bone after elevation of the floor of the maxillary sinus. Br J Oral Maxillofac Surg 2005; 43(6):493–499.
22. Newman TH, Klokkevold P, Carranza F. Carranza's Clinical Periodontology 2006; Saunders, Philadelphia, Pennsylvania, USA. 976, 1135.
23. LeGeros RZ. Calcium phosphate-based osteoinductive materials. Chem Rev 2008; 108(11):4742–4753.
24. Barrere F, van der Valk CM, Meijer G, Dalmeijer RA, de Groot K, Layrolle P. Osteointegration of biomimetic apatite coating applied onto dense and porous metal implants in femurs of goats. J Biomed Mater Res B Appl Biomater 2003; 67(1):655–665.
25. Le Guehennec L, Goyenvalle E, Lopez-Heredia MA, Weiss P, Amouriq Y, Layrolle P. Histomorphometric analysis of the osseointegration of four different implant surfaces in the femoral epiphyses of rabbits. Clin Oral Implants Res 2008; 19(11):1103–1110.
26. Le Guehennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent Mater 2007; 23(7):844–854.
27. Blacher S, Leonard A, Heinrichs B, Tcherkassova N, Ferauche F, Crine M, Marchot P, Loukine E, Pirard JP. Image analysis of X-ray microtomograms of Pd-Ag/SiO2 xerogel catalysts supported on Al2O3 foams. Colloids and Surfaces a-Physicochemical and Engineering Aspects 2004; 241 (1–3):201–206.
28. Blacher S, Maquet V, Leonard A, Chapelle G, Crine M, Jerome R, Pirard JP. Texture characterization of ultramacroporous materials using non-destructive methods. Characterization of Porous Solids Vi 2002; 144:331–338.
29. Leonard A, Blacher S, Marchot P, Pirard JP, Crine M. Image analysis of X-ray microtomograms of soft materials during convective drying: 3D measurements. Journal of Microscopy-Oxford 2005; 218:247–252.
30. Leonard A, Blacher S, Nimmol C, Devahastin S. Effect of far-infrared radiation assisted drying on microstructure of banana slices: An illustrative use of X-ray microtomography in inicrostructural evaluation of a food product. Journal of Food Engineering 2008; 85(1):154–162.
31. Leonard A, Calberg C, Kerckhofs G, Wevers M, Jerome R, Pirard JP, Germain A, Blacher S. Characterization of the porous structure of biodegradable scaffolds obtained with supercritical CO2 as foaming agent. Journal of Porous Materials 2008; 15(4):397–403.
32. Leonard A, Guiot LP, Pirard JP, Crine M, Balligand M, Blacher S. Non-destructive characterization of deer (Cervus Elaphus) antlers by X-ray microtomography coupled with image analysis. Journal of Microscopy-Oxford 2007; 225(3):258–263.
33. Tondi G, Blacher S, Léonard A, Pizzi A, Fierro V, Leban JM, Celzard A. X-Ray Microtomography Studies of Tannin-Derived Organic and Carbon Foams. Microscopy and Microanalysis 2009; 15(05):384–394.
34. Grevstad HJ, Boe OE. Effect of doxycycline on surgically induced osteoclast recruitment in the rat. Eur J Oral Sci 1995; 103(3):156–159.
35. Holmes SG, Still K, Buttle DJ, Bishop NJ, Grabowski PS. Chemically modified tetracyclines act through multiple mechanisms directly on osteoclast precursors. Bone 2004; 35(2):471–478.
36. Rifkin BR, Vernillo AT, Golub LM, Ramamurthy NS. Modulation of bone resorption by tetracyclines. Ann N Y Acad Sci 1994; 732:165–180.
37. Almazin SM, Dziak R, Andreana S, Ciancio SG. The effect of doxycycline hyclate, chlorhexidine gluconate, and minocycline hydrochloride on osteoblastic proliferation and differentiation in vitro. J Periodontol 2009; 80(6):999–1005.