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A Human Clinical, Histological, Histomorphometrical, and Radiographical Study on Biphasic HA-Beta-TCP 30/70 in Maxillary Sinus Augmentation

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ABSTRACT Background: By mixing hydroxyapatite (HA) and tricalcium phosphate (TCP), biphasic calcium phosphate ceramics can be obtained, and by varying their ratio it is possible to tailor the characteristics of the biomaterial. Purpose: The aim of the present human study was to evaluate the histological and radiographical aspects of bone formation in maxillary sinus augmentation using a 30/70 HA-beta-TCP with a reticular structure. Materials and Methods: A total of 12 patients, undergoing two-stage sinus augmentation procedure using HA-beta-TCP at a ratio of 30/70, were included in the present study. After a 6-month healing period, during implant insertion, radiographical analysis was performed, and then the bone core biopsies were harvested and processed for histology. Results: At radiographic evaluation, the bone gain was on average 6.85 1 0.60 mm. HA-beta-TCP 30/70 appeared to be lined by newly formed bone, with no gaps at the interface. The histomorphometric analysis revealed 26 1 2% of residual grafted biomaterial, 29 1 3% of newly formed bone, and 45 1 2% of marrow spaces. Conclusions: The present results indicate histologically the high biocompatibility and osteoconductivity of HA-beta-TCP 30/70, and clinically its successful use for sinus augmentation procedures.

KEY WORDS: biphasic calcium phosphate, bone regeneration, histology, hydroxyapatite, sinus augmentation

INTRODUCTION

Implant placement could be difficult when an insufficient bone volume is present. This evidence may frequently occur due to the expansion of the maxillary sinus following tooth extraction and the atrophic resorption of the alveolar process of the posterior maxilla with aging. Since 1986 when Tatum1 first described the maxillary sinus augmentation procedure, different bone graft materials, such as autografts, allografts, xenografts, and synthetic grafts, have been developed to increase the residual maxillary bone height. Autografts are considered the golden standard for sinus augmentation procedures as they show osteoinductive, osteoconductive, and osteogenic properties, due to the presence of matrix proteins, bone matrix, and viable cells.2 However, they presented several disadvantages, such as limited availability from the intraoral donor site, tendency to undergo partial

resorption, morbidity at bone graft donor site, and necessity of an additional surgery in case of extraoral donor site.3,4 Allografts and xenografts have been developed to overcome these weaknesses.5–8 Among the bone grafts currently available, calcium phosphate (CaP) materials are probably the most ancient9,10 and widely used biomaterials for sinus augmentation procedures.11,12 They have been tested for the following applications: periodontal and bone defects, bone atrophies, endodontic lesions, maxillary sinus augmentation, and as membrane barriers.13–15 Few studies showed the biocompatibility and osteoconductivity of CaP ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP), which are considered the most suitable ceramic materials for bone reconstructions.16–18 Animal studies reported ectopic bone formation following extraskeletal implantation of CaP, indicating that osteoinduction might be its intrinsic property.19–22 HA Ca5(PO4)3OH (HA) and betatricalcium phosphate Ca3(PO4)2 (beta-TCP), differ in their Ca/P ratio and, therefore in their resorption potential. It has been reported that HA is slowly resorbed,18,23 whereas materials consisting of TCP are quickly resorbed.12,24,25 The fast resorption of the latter leaves a calcium sulfate lattice, which is thought to promote osteogenic activity. By mixing HA and beta-TCP, biphasic calcium phosphate (BCP) ceramics of various phase compositions (HA/beta-TCP ratios) can be obtained. They are the most important CaP ceramics for dental and medical applications, as they are both biocompatible and osteoconductive.18 Moreover, biological and mechanical properties of the ceramics can be changed and improved by varying HA/TCP ratio.12,18,26 Indeed, by modifying some parameters (such as Ca/P ratio, structure, surface, porosity, and chemistry) specific materials with peculiar customized activity as resorption, mechanical resistance, porosity, and granulometry, can be obtained. One of the most important criteria for an ideal scaffold is the presence of a highly interconnected porous network with both pore sizes, and interconnections, large enough for cell migration, fluid exchange, and eventually tissue ingrowth and vascularization (penetration of blood vessels).27 Scaffolds porosity is of paramount importance; the optimal pore size is still a debate issue and considered to be optimal within a range between 50 and 400 microns.28 Nowadays, CaP ceramics of various phase compositions, different formulations (bone cements, granulates, or blocks), and several sizes and shapes are used.17,18 Most of the studies available report on 60% HA and 40% TCP.19,22,29 They state that the dissolution of TCP might lead to more interparticular space for new bone formation. Furthermore, the levels of released calcium and phosphorous ions could stimulate new bone formation.30,31 In general, one of the main limitations of CaP ceramics is their low degradation rate, especially in the case of dense ceramic blocks. In this direction, the use of materials whose degradation can be tailored by varying their chemical composition, together with the incorporation of pores seems to be a good strategy to overcome the already mentioned drawbacks. Consistent with these observations, the aim of this prospective study was to evaluate the histological and radiographical behavior of a new bioceramic, a 30/70 HA-beta-TCP with a reticular structure in maxillary sinus augmentation in humans. The HA component will serve as space maintainer and will not be resorbed, while the beta-TCP will dissolve, and hopefully, enhance new bone formation by leading to more interparticular space and releasing ions able to influence the progress of resorption and healing processes. The reticular structure characterized by well-defined pores might have significant effects both in material resorption and bone formation.

MATERIALS AND METHODS

Material Fabrication

Biphasic HA ceramic scaffolds were developed by the direct rapid prototyping technique dispense-plotting.32 A virtual scaffold model was designed with a cylindrical outer geometry by using three-dimensional computer aided design software. The size of the model was adapted to the shrinkage of the ceramic material in the subsequent sintering process. The inner geometry, that is, the pathway of the material rods, was defined by a custommade software, which generates the control commands of the rapid prototyping machine. To build up the green bodies, material rods, consisting of a paste-like aqueous ceramic slurry, were extruded out of a cartridge through a nozzle and deposited using an industrial robot (GLT, Pforzheim, Germany). In the present study HA and TCP powders (Merck,

Darmstadt, Germany) were blended to get a powder mixture with an HA/TCP weight ratio of 30/70. The characteristic rheological behavior of the aqueous ceramic slurry was achieved by thermal treatment of the raw HA powder at 900°C for 1 hour and by adding a compatible binder/dispersant system of organic additives of 10.5 weight % relative to the mass of ceramic powder. The rod deposition was controlled in x, y, and z direction to assemble three-dimensional scaffolds layer by layer on a building platform. By rotating the direction of the rod deposition by 60° from layer to layer, a three-dimensional network with an interconnecting pore structure was generated. The assemblies made of ceramic slurry were dried at room temperature and subsequently sintered at 1250°C for 1 hour. Finally, the sintered scaffolds were manually reduced to smaller blocks with a volume of about 1.4 cm3 in order to remove the solid rim that resulted from the turning points at the edge of the printed pathways (Figure 1A). HA/TCP appears to have a regular and reticular surface structure by scanning electron microscopy (Figure 1B). Specifically, the macrostructure of the blocks is characterized by the extruded rods, the distance between two parallel rods inside one layer and the changed orientation of the rods from layer to layer (60°). The rods of the scaffolds had a diameter of 342 1 30 µm. Total porosity of the scaffolds was measured to be 64 vol-%, mainly determined by the pore-channels between rods. The lay-down-pattern 0/60° resulted in pore-channels in z-direction (section: equilateral triangle with side length about 340 µm). The pore-channels in x and y direction have nearly rectangular section (about 300 µm side length). The uniaxial compression strength was measured to be about 140 N. Patient Selection Twelve patients (five females and seven males, mean age 56 years; range 35–72 years), who required a sinus floor elevation for implant–prosthetic rehabilitation, were selected for this study. The protocol of the study was approved by the Ethical Committee of the University of Guarulhos, São Paulo, Brazil, and all the patients signed a written informed consent form. Inclusion criteria were: maxillary partial edentulism involving the premolar/molar areas, and the presence of a residual bone height, between the sinus floor and alveolar ridge, as measured on the serial sections of the computerized axial tomography (CAT) scan in each case, ranging from 1 to 4 mm. Exclusion criteria were: smoking, patients with systemic diseases, and maxillary sinus pathology. At the initial visit, all patients underwent a clinical and occlusal examination and periapical and panoramic radiographs; CAT was also performed (Figure 2, A and B). A unilateral two-stage approach was performed in all patients for a total of 12 sinus lift procedures using biphasic HA and beta-TCP (in a 30/70 ratio). After a 6-month healing period, at the reentry surgery, biopsies were taken and 25 implants (Leone Implant System®, Florence, Italy) were inserted. The implants had been placed exactly in the sites where biopsies were harvested, under the guide of CAT template for guided bone surgery. Finally, 6 months after implant placement, a definitive prosthetic rehabilitation with ceramic-metal fixed prostheses was delivered. Surgical Procedure Prior to surgery, the patients' mouths were rinsed with a 0.2% chlorhexidine digluconate solution for 2 minutes. Local anesthesia (Xilestesin, Espe Dental, Seefeld, Germany) with 2% adrenaline was administered. The maxillary sinus augmentation was performed as

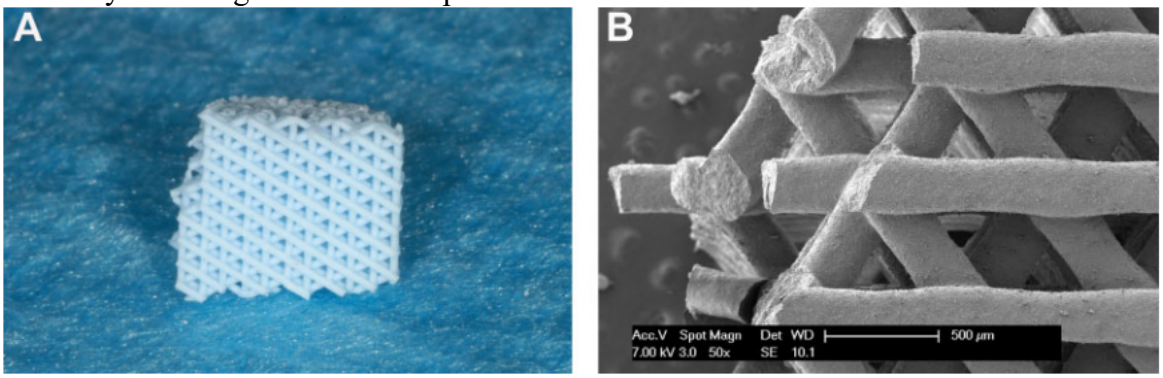

Figure 1 (A) Macroscopic appearance of hydroxyapatite (HA) and tricalcium phosphate (TCP) 30:70 scaffold sintered in a small block. (B) Scanning electron microscopy appearance of regular and reticular HA/TCP surface structure. 50×.

previously described.32 After a horizontal crestal incision and two vertical incisions extending beyond the mucogingival junction, a full-thickness flap was reflected, in order to expose the maxillary sinus lateral bone wall. Under constant irrigation with copious saline solution, an osseous window of approximately 1 × 1 cm was demarked and isolated, using a piezotome equipment (Satelec, Acteon Group, Merignac, France). The isolated osseous window was subsequently removed and conserved in saline solution. The Schneiderian membrane was exposed and carefully isolated, using specially designed elevators, to avoid undesired perforations. The cavity produced was filled with HA/TCP 30/70 small cubes (4 × 4 mm). After the sinus augmentation procedure was completed, the previously isolated bone window was repositioned to close the sinus lateral wall. Sutures were performed (Supramid®, Novaxa Spa., Milan, Italy) to ensure complete flap closure. Antibiotic prophylaxis (1 g Zimox, Pharmacia & Upjohn, Milan, Italy) was administered 1 hour before surgery and for 3 days afterward; the patients were given inflammatory and analgesic medication as well (Synflex 550 mg, Recordati, Milan, Italy). Clinical and radiological follow-up examinations were performed immediately after the surgery and 6 months later. Histological Processing Six months after sinus floor elevation, at the time of dental implants placement, biopsies were taken under local anesthesia with a 2.5 mm diameter trephine bur (ACE Surgical Supply Company, Brockton, MA, USA) under copious irrigation with sterile saline. Five bone cores for HA/beta-TCP-regenerated sites were harvested. The specimens were immediately fixed in 10% buffered formalin and processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy).33 The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned along their longitudinal axis with a high-precision diamond disc at about 150 μm and ground down to about 30 μm with a specially designed grinding machine. The slides were stained with acid fuchsin and toluidine blue. The slides were observed in normal transmitted light under a Leitz Laborlux microscope (Laborlux S, Leitz, Wetzlar, Germany). The histomorphometry of residual grafted biomaterial, newly formed bone and marrow spaces was performed using a light microscope (Laborlux S, Leitz) connected to a high-resolution video camera (3CCD JVC KYF55B, Milan, Italy), and interfaced to a monitor and personal computer (Intel Pentium III 120 MXX, Intel, Milan, Italy). This optical system was associated with a digitizing pad (Matrix Vision GmbH, Milan, Italy) and a histometry software package with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc, Milan, Italy). Radiographic Measurements CT scans performed at the initial visit and 6 months after the augmentation were elaborated using a software (Mimics Materialise, Leouven, Belgium) in order to obtain a three-dimensional reconstruction of patient's jaws. Two measurements were done on anterior, medial, and posterior aspects of the bone crest: • residual bone crest height at the initial visit, defined as the distance between the intraoral bone margin and the highest point of the original floor of the maxillary sinus • bone crest height 6 months after augmentation, defined as the distance between the intraoral bone margin and highest point of the maxillary sinus graft after 6 months All the measurements were done along the longitudinal axes of the sections, in both frontal and lateral view. Histomorphometric and radiographic measurements are presented as means 1 standard deviations.

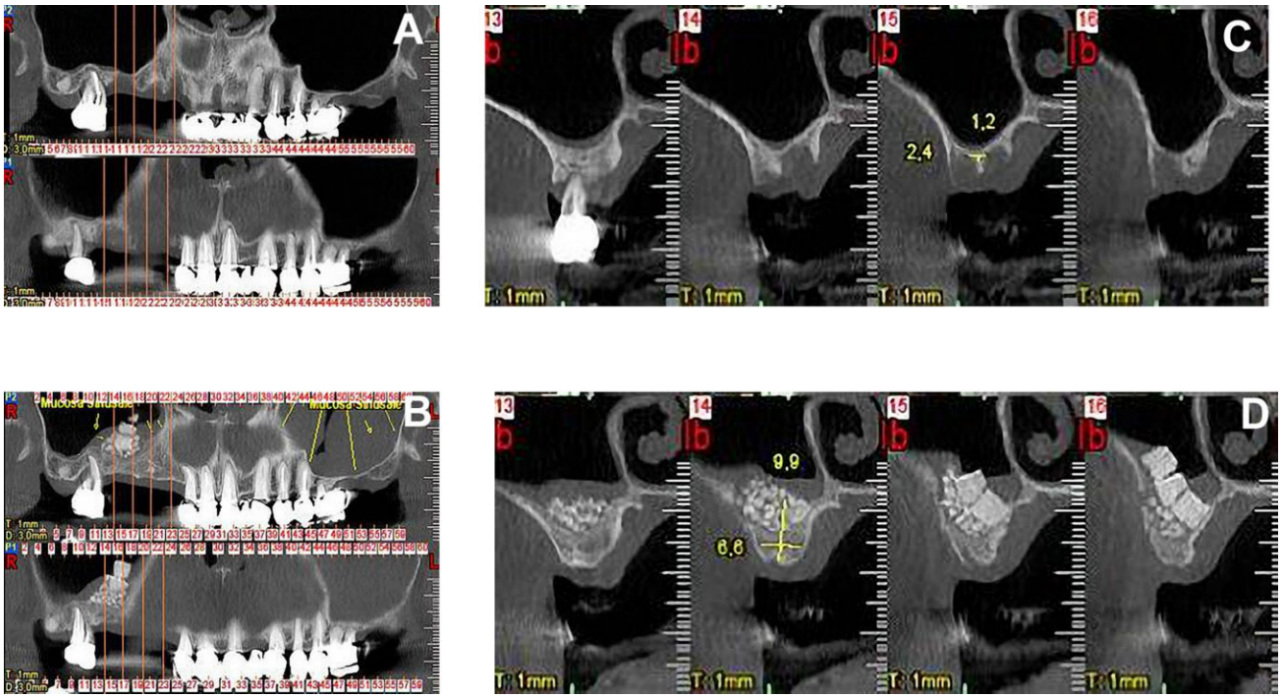

Figure 2 (A and B) Pre-operative cone beam computed tomography (CBCT) of the atrofied posterior maxilla; (C and D) CBCT 6 months postsurgery showing a significant average vertical bone gain with the biphasic calcium phosphate graft.

RESULT

Clinical Results

No perforations of the membrane and no signs of maxillary sinus disease were observed. The postoperative healing was uneventful and free of complications in all patients. Six months after augmentation, the CT examination showed, in all patients, the presence of dense bone in the maxillary sinuses where HA/betaTCP 30/70 was placed, and thus a significant average vertical bone gain (Figure 2, C and D). The implant stability quotient measured by resonance frequency analysis with the instrument Osstell (Integration Diagnostics AB, Gothenburg, Sweden) at the time of implant insertion (6–8 months after sinus lift) was 60 1 5. One year after placement, neither implant failures nor surgical or prosthetic complications occurred (Figure 3).

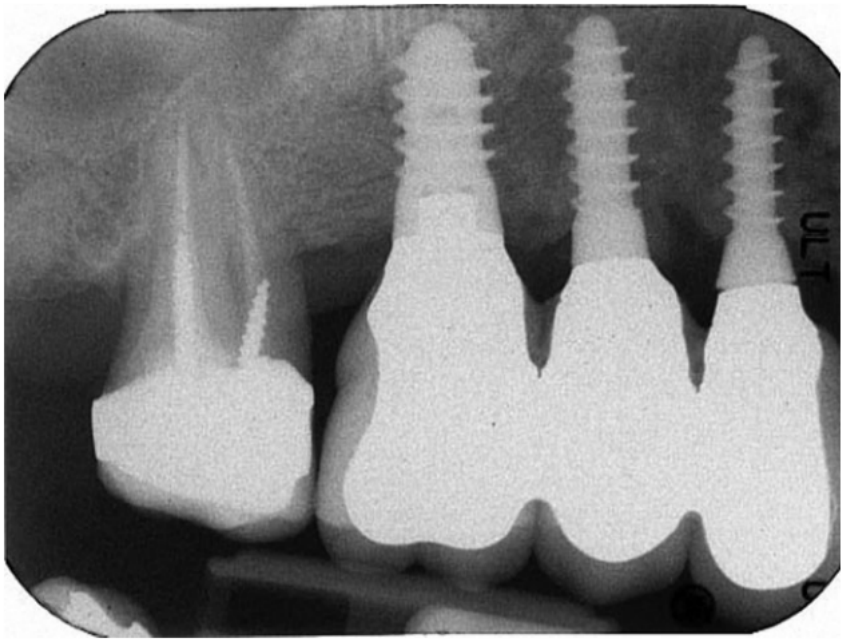

Figure 3 Periapical Rx 1 year after implant placement.

Histomorphometric Analysis

In the examined samples, trabecular bone with marrow spaces and residual biomaterial could be observed; they were mainly located in the apical portion of the samples (Figure 4).

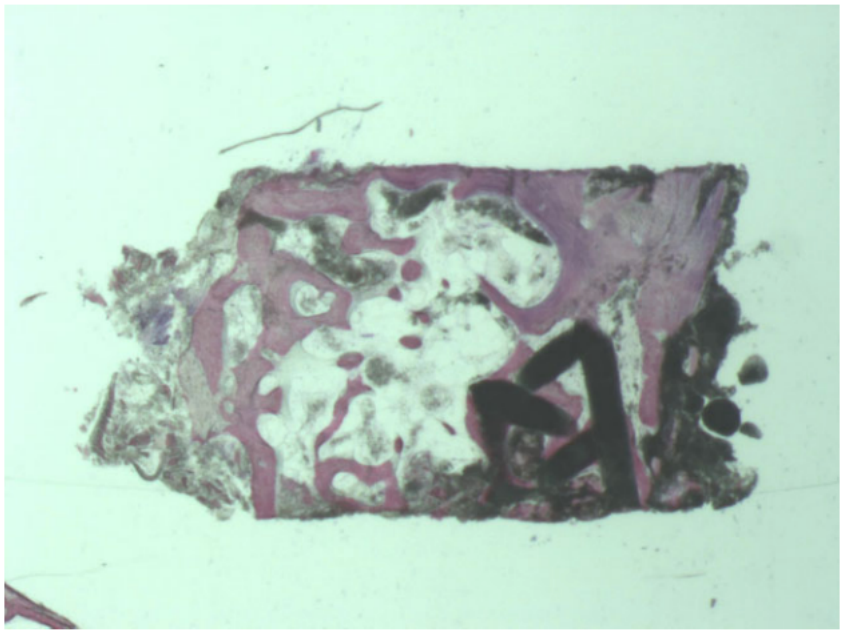

Figure 4 Trabecular bone with marrow spaces and residual particles of biomaterials mainly located in the apical portion of the samples can be observed. Acid fuchsin-toluidine blue 12×.

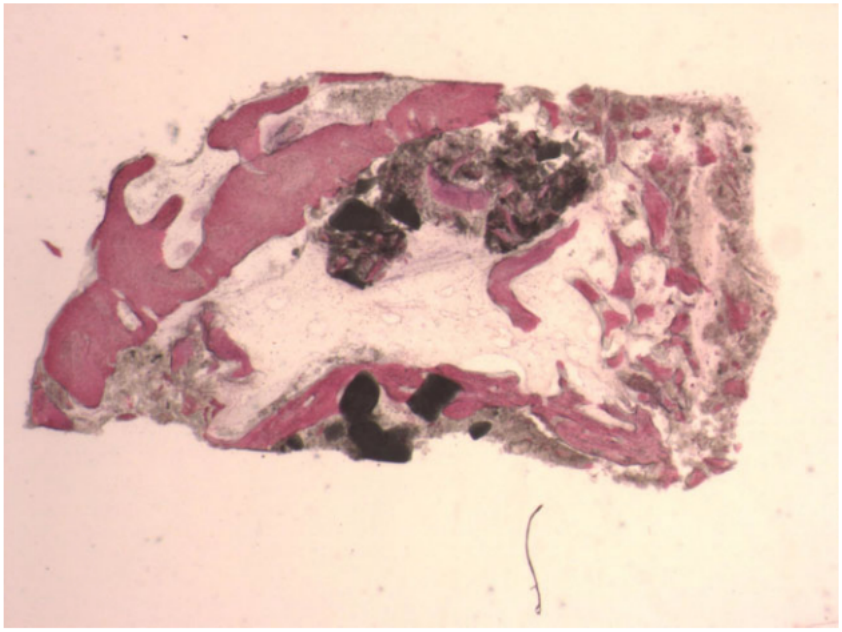

Figure 5 Trabecular bone with wide marrow spaces bridging residual biomaterial particles can be observed. Acid fuchsin-toluidine blue 12×.

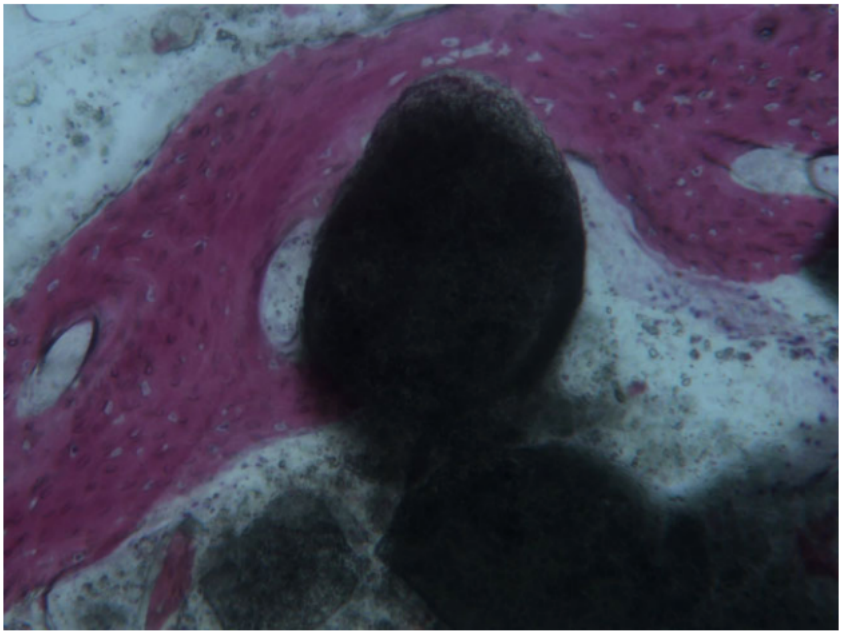

Figure 6 At higher magnification, biomaterial residual particles in tight contact to newly formed bone, with no gaps at the interface, were present. Acid fuchsin-toluidine blue 100×.

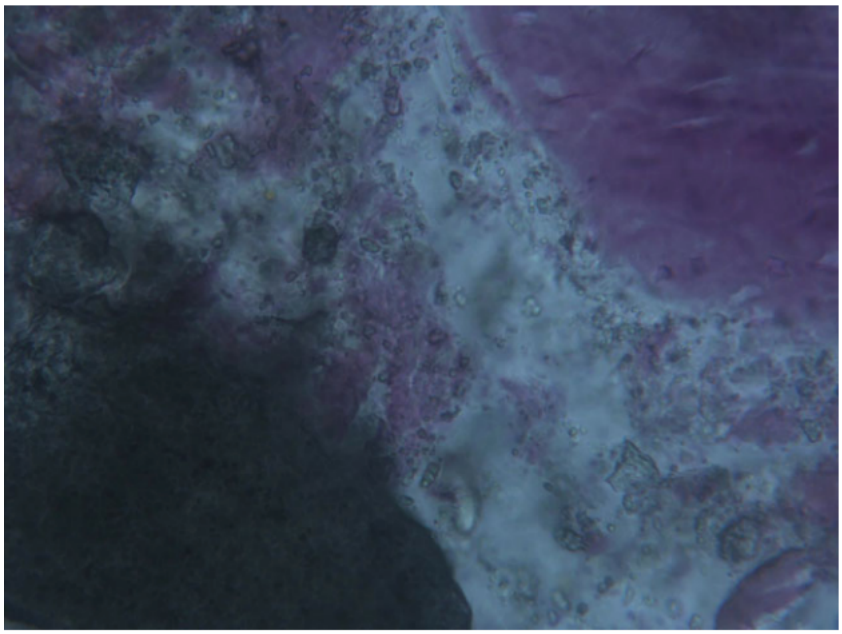

Figure 7 Residual biomaterial particles scattered and mixed with newly formed bone chips. Acid fuchsin-toluidine blue 200×.

At low power magnification, in all the specimens, many grafted particles bridged by newly formed b one could be detected (Figure 5). In some portions of the specimens, the graft appeared to be lined by newly formed bone. No gaps were present at the bonebiomaterial interface and the bone was always in tight contact with the bone substitute material (Figure 6). In same fields, the residual biomaterial particles seemed to be scattered and mixed with newly formed bone chips, mainly in the areas where particles are located in the vicinity of marrow spaces (Figure 7). No inflammatory cells and multinucleated giant cells were present around the biomaterial or at the interface with bone. The histomorphometric analysis revealed 26 1 2% of residual grafted biomaterial, 29 1 3% of newly formed bone and 45 1 2% of marrow spaces.

Radiographic Analysis At radiographic evaluation, after a 6-month healing period, the bone gain was on average 6.85 1 0.60 mm. Specifically, the bone crest height after sinus augmentation and vertical height gain obtained in the CT scans at baseline and 6 months after the maxillary sinus graft augmentation using HA-beta TCP 30/70 are shown in Table 1.

TABLE 1 Average (mm) of Vertical Bone Gain, Obtained in the CT Scans 6 Months after Maxillary Sinus Graft Augmentation Using Biphasic HA/TCP 30/70

	Residual Bone Crest Height (mm)			Crestal Height after Augmentation (mm)			Vertical Bone Gain (mm)		
	Anterior	Medial	Posterior	Anterior	Medial	Posterior	Anterior	Medial	Posterior
Mean	4.893333	4.685	3.868333	12.4175	11.41083	10.1975	7.524167	6.725833	6.329167
Standard deviation	2.062736	1.744662	1.533166	2.350494	2.871694	2.803171	1.545059	1.726981	2.064683

CT = computed tomography; HA = hydroxyapatite; TCP = tricalcium phosphate.

DISCUSSION

In sinus floor augmentation technique, bone grafting material selection is one of the crucial factors that affect the final outcome.33 Indeed, its choice influences the increase of the vertical dimension of the bone crest and therefore, the achievement of implants primary stability and the success of the rehabilitation over the long term. Among bone substitutes, biphasic HA has been demonstrated to be biocompatible and osteoconductive, but its osteoinductive properties remain to be fully characterized.34,35 In HA/TCP compounding of CaP biomaterial scaffolds, referred to as BCP biomaterials, HA acts as a scaffold and TCP as the resorbable component.36 In an experimental study on sheep ribs, it was showed that HA/TCP compounding was able to support new bone formation by acting as a scaffold and maintaining its osteoconductive properties until degradation. In addition, by comparing different HA/TCP ratio, 30/70 versus 50/50, there were no statistical significant differences, but the 30/70 ratio resulted better integrated into the host bone.37 Characteristics, such as HA/beta-TCP ratio, micro porosity and macro porosity, have a strong impact on bone formation and biological behavior of these materials. The scaffold used in the present study shows a reticular HA/beta-TCP 30/70 structure, manufactured by a rapid prototyping (RP) technique. This technique is based on the plotting of three-dimensional scaffold from computer-aided design files, and offers a tool for precise control of the overall geometry and the properties of the porous structure, specifically the porosity, poring design, and external shape of a specific Ha/TCP ratio ceramic bone substitute. In the last years, a number of techniques have been employed to manufacture ceramic bone substitutes meeting all these requirements, including RP. In addition, various direct RP methods, like ink-jet and three-dimensional printing38–40 or dispense-plotting41,42 are used to manufacture complexshaped scaffolds from biocompatible materials like CaP ceramics or degradable polymers. The RP technique used for the present scaffold is based on CAD data, medical imaging techniques, and it could be also used as data source, establishing the possibility to manufacture patient-specific bone grafts or bone tissue engineering constructs. Thereby, it gives the possibility to adapt the mechanical properties of the implant by variation of phase composition and geometrical design to the individual requirements.43 Indeed, besides material composition, an important characteristic of scaffolds that shall promote tissue ingrowth and new bone formation is their macrostructure. Different studies have shown a better osteogenic response in scaffolds with pores of 300 μm, while the minimum recommended pore size is 100 μm.44–49 The present scaffold has rod diameters and pore sizes between the rods within the ranges recommended by several authors for bone tissue engineering.47 A recent study on sheep demonstrated a prompt and complete tissue integration already detectable 45 days after the sinus augmentation procedures. To our best knowledge, there are no articles in the

literature reporting on the 30/70 HA/beta-TCP ratio used as biomaterial grafts in sinus augmentation procedure in humans. There is only one study, on a animal model, comparing two different HA/TCP ratios: 30/70 versus 50/50.37 The authors concluded that the 30/70 scaffold was better integrated into physiological bone remodeling, although no significant difference was detected.37 The 30/70 HA/beta-TCP material tested on sheep was in granules,37 while the one investigated in the present study is represented by blocks; of course, the different formulations can affect the behavior of the materials. The present histomorphometric analysis, 6 months after sinus augmentation, supported the biocompatibility of the examined material as in the regenerated sinuses no inflammatory cell infiltrate was detected. In addition, the HA/TCP 30/70 scaffold was well integrated into the new bone with no gap at the interface, indicating its osteoconductivity. Specifically, the data of the present study are in agreement with a recent study evaluating the clinical and histological aspects of bone formation in maxillary sinus augmentation using macroporous biphasic CaP ceramics (MBCP), a mixture of 60%, HA and 40% beta-TCP, after a healing period of 6 months. The histomorphometrical evaluation demonstrated similar values of newly formed bone, residual grafted material, and marrow spaces.36 In another study on the use of BCP in combination with autogenous bone chips as grafts in sinus augmentation, the authors concluded that biphasic HA/TCP was biocompatible and showed osteoconductive properties and, in association with autogenous bone, promotes newly bone formation, with an increase of its fraction along an extended healing period.37 The radiographic evaluation proved the efficacy of the 30/70 HA/beta-TCP to support a vertical bone gain suitable for implant insertion 6 months after sinus augmentation. No other studies are available in the literature investigating vertical bone gain with the same HA/TCP proportions and it would be difficult to make comparison as different ratios may strongly affect the bone regeneration and materials replacement behavior.

In conclusion, within the limitation of the small sample size, the present results indicate histologically the high biocompatibility and osteoconductivity of HA-beta-TCP 30/70, and clinically its successful use for sinus augmentation procedures.

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