Published in final edited form as:

Mater Sci Eng C Mater Biol Appl. 2010 June 15; 30(5): 763–769. doi:10.1016/j.msec.2010.03.012.

Maxillary sinus floor augmentation on humans: Packing simulations and 8 months histomorphometric comparative study of anorganic bone matrix and β -tricalcium phosphate particles as grafting materials

A. Martinez^{a,*}, J. Franco^b, E. Saiz^{b,1}, and F. Guitian^c

^a Facultad de Medicina y Odontología, University of Santiago de Compostela, Rua San Francisco s/n; 15782 Santiago de Compostela, Spain

^b Lawrence Berkeley National Laboratory, 1 Cyclotron Rd Mail stop 62R0203 Berkeley, CA 94720, USA

^c Instituto de Ceramica de Galicia, Av Mestre Mateo s/n, 15782 Santiago de Compostela, Spain

Abstract

The present study compares the behaviour of an anorganic bone matrix material and a synthetic β -Tricalcium phosphate employed as grafting materials in a sinus floor augmentation two step protocol in humans. In order to estimate the initial occupation level for the two materials, an 'in vitro' simulation has been performed to analyse macroporosity created due to particle packing in terms of porosity and interparticle distances. Grafting in the sinus floor augmentation was performed by filling the defects only with pure grafting materials without autogenous bone addition. The new-bone generated is 100% based on the osteoconductive properties of the grafted materials in contact with physiological fluids. The implants were placed 8 months after the grafting procedure. All the implanted positions were biopsied and embedded in methacrylate resin. Histomorphometric analyses were done over thin film undecalcified sections. Packing simulations allow establishing a comparison of the resorbed volumes related to the initial occupancy of the grafting materials inside the defect. The nature of this interconnected pore network is very alike for either material so new-bone generated was similar (~35 vol.%).

Keywords

Grafting particulates; Calcium phosphates; Histomorphometry; Packing simulations; Sinus floor augmentation

1. Introduction

The human body possesses intrinsic mechanisms which allow self healing, but 'restitutio ad integrum' is not frequent. Biomaterials can enhance the natural capability of healing and can be successfully used to restore some body functions [1]. Dental implants have become a satisfactory option to restore masticatory functions in patients with edentulous spaces [2]. Long term survival and success of dental implants require primary stability and appropriate bone volume [3]. The scarring process in the upper maxilla usually entails atrophic alveolar

ridges, that lack the volume for a predictable fixture placement [4]. The employment of guided bone regeneration procedures allows the improving of the bone crest volume. This increasing of bone volume in the posterior upper maxilla has been achieved by combining various procedures and materials [5–7]. Sinus augmentation is one of the most predictable procedures to increase the amount of bone in posterior edentulous maxillae [8]. In cases where alveolar ridge height is less than 4 mm, a two step protocol is recommended because it improves osteointegration and long term success of the implants [9,10]. In a first surgical procedure a sinus floor elevation is performed and the graft is placed. The material implanted under the Schneiderian membrane starts to promote bone formation and is replaced by the bone. In a second surgery, implants are placed in this medium, surrounded by the new formed bone and partially resorbed grafting material [11].

Autogenous bone grafts are still considered the gold standard for bone regeneration in implant dentistry [12]; they have the best behaviour in terms of osteogenesis but they also have significant disadvantages. There is limited bone supply in the oral cavity and frequently bone should be harvested from an extraoral site. This procedure might require the employment of general anaesthesia and is frequently associated with morbidity at the donor site, and patient discomfort [13]. Different materials have been proposed as bone substitutes; those materials are used as a scaffold that allows development of new-bone by maintaining an initial volume which is progressively replaced by new-bone. Anorganic bone grafts (ABG) are different xenogenic materials (mainly from bovine origin) from which all organic components have been removed [5,14–19]. This material and human cancellous bone are very alike in structure, having 75% to 80% porosity and a crystal size of approximately 10 nm [5]. The interconnecting pore system of macropores, mesopores and micropores facilitates the vascular colonization and the osteoblasts appositional growth [5,16]. ABG show high biocompatibility and osteoconductivity when used in sinus elevation procedures [16,17,20–22]. However, there is some controversy regarding the kinetics of resorption and new-bone formation. Many authors consider that due to its slow resorption ABG is a long lasting material [5,13,23,24]. Consequently the final result of an ABG implanted site is a composite formed by new-bone and the remaining material with controversial mechanical implications [21,25]. On the other hand some authors think that this long term stability avoids undesirable bone volume resorption due to the bone remodelling process [22,26]. Long term volumetric stability in sinus graft procedures has confirmed long lasting presence of ABG particles [19]. Beta-tricalcium Phosphate (TCP) particles have been employed successfully as alloplastic grafting materials in sinus elevation procedures [27–30]. These particles exhibit a micro porous surface which facilitates the anchorage of proteins and cells to the surface of the graft [31], have good osteoconductive properties and presumably resorb faster than ABG [20]. Particulate grafts exhibit a good behaviour due to the growth and development of a vascular network within the interparticle voids and have been successfully used in bone replacement procedures with or without seeding stem cells [32]. In contrast, graft blocks, might be rejected due to poor revascularization [8,33] and have to be adapted, prior to implantation, to the defect, whereas particulate grafts can easily fill the defect.

To facilitate bone growth into a porous scaffold, macropores should be bigger than 50 μm with an optimum value over 300 μm to achieve good osteogenesis [34]. Particle size affects the effective packing of the graft inside the defect; when employing particulate materials macroporosity is dictated by particle packing [35]. Small particles might diminish the size of the interparticulate voids and should not be smaller than 10 μm in order to avoid inflammatory reactions from the multinucleated giant foreign cells macrophagic activity [19]. On the other hand, large particles may retard the graft substitution and the formation of bone bridges [22]. When these particulate grafts are used as scaffolds for bone regeneration, *de novo bone formation* process occurs as a consequence of a biological cascade of events during the initiation of bone formation by newly differentiating population of osteogenic

cells, over particulate surfaces [36]. In order to provide the best environment possible for this process to take place, an effective particle packing should create the macroporosity required to allow the growth of vascular buds and eventually basic multicellular units (BMU) inside the material [37].

Although many different graft materials have been proposed we often lack systematic ‘in vivo’ studies that will define and assess quantitatively the key parameters which determine the performance of the graft. It is necessary to determine what these parameters are and relate their evolution to the physicochemical characteristics of the material (composition, resorbability, micro and macro porosity and chemistry...). The aim of this study is to compare the resorption and bone generation of two different particulate grafting materials at opposite extremes of the resorbability spectrum, 8 months after a sinus floor elevation procedure. ABG are considered long lasting materials whereas TCP is more resorbable. Both materials were physically and chemically characterized. As long as biopsies are only harvested at a single time of 8 months after implantation it would not be possible to talk about resorption times. In order to establish a time zero situation of the materials when packed inside the defect, packing simulations have been performed to analyse ‘in vitro’ particle arrangement and therefore their resulting porosity. Data obtained from packing simulations allow estimating the resorption volumes for the grafted materials in combination with the results obtained from the histomorphometric measurements. This simulation also gives information about the amount and distribution of macroporosity available for new-bone growth.

2. Materials and methods

2.1. Graft characterization

There are many available commercial bone graft particulates either for ABG and TCP (Table 1). Amongst all the materials the choice of two particular brands was made in terms of the same particle size interval and their ease of availability. The calcium phosphate grafting materials employed in the maxillary sinus floor augmentation are an anorganic bovine-bone derived (ABB) (Bio-Oss 200–1000 μm , Geistlich, Switzerland) and synthetic 100% β -Tricalcium Phosphate (TCP) (KeraOs, 200–1000 μm , Keramat, Spain). To guarantee enough material volume for physicochemical characterization and packing simulation procedures, 4 commercial batches of each material were mixed and homogenized. No sieving or any kind of classification was performed so the evaluated and grafted materials were the same. The characterization was made by Scanning Electron Microscopy (S-4300SE/N, Hitachi America Ltd., USA), X-ray Diffraction (XRD, Cu K α 1 $\lambda=1.5406$ Å, 30 mA/40 kV. Siemens D-5000, Germany), Differential thermal and gravimetric analysis (DTA-TG, PL-1640 STA, Polymer Labs, UK), Specific surface area with the Brunauer, Emmett and Teller method (BET, Gemini 2360, Micromeritics Instrument Corporation, GA, USA) and Mercury porosimetry (Autopore II 9220, Micromeritics Instrument Corporation, GA, USA). The Ca/P molar ratio was determined by quantitative X-ray diffraction analysis [38] of ‘as received’ material for the synthetic material, and over 1000 °C 1 h thermally treated ABB. In order to determine what is the effective packing porosity, particles of both materials were introduced inside 5 mm diameter glass test tubes. The tubes were filled with epoxy resin (Bepox 1159, Gairesa, Spain) under vacuum and remaining trapped air bubbles were eliminated by centrifugation at 3000 rpm for 15 min (H-900, Kokusan, Japan). Neither particle breakage nor packing improvements have been detected because of the centrifugation process; the initial level of the materials inside the test tubes remained constant and SEM examination did not find evidence of particle breakage. Twenty axial slices were cut from the epoxy-infiltrated specimens and polished (Isocut, Ecomet 4, Buehler LTD Lake Bluff, IL. USA). The effective packing porosity was measured using image analysis (Image J v. 1.40 g, NIH, USA) over

backscattered electron (BSE) images. Interparticle void image analysis has also been done over BSE images measuring distances between particles along the lines of an overlaid grid.

2.2. Patient selection

The selected patients were partially edentulous in the upper post-canine region. Previous exclusion criteria are shown in Table 2. A total amount of 20 sinus augmentations were performed in 16 patients with severely resorbed alveolar process (range 1–5 mm) and a mean of 3.8 mm of remaining bone. The mean age of patients was 49.5 years (range 38–67). Because of the dissimilarities in the alveolar ridge defects, each sinus was considered independently (even in those patients who underwent a bilateral elevation) and the choice of the graft nature was randomized. All the sinus elevation procedures were required in order to obtain enough amount of bone to allow the implant placement. All patients were carefully informed about the surgical procedure, the bone substitute materials and the implant prosthetic solutions. Full informed consent was obtained from all the patients. Panoramic radiographs were taken before and after the sinus augmentation, and after implant placement. The University of Santiago/Institute Ethics Committee approved the study.

2.3. Sinus floor elevation procedure

The sinuses were randomly distributed into two groups; 10 sinuses were filled with ABB and 10 with TCP. Both materials were mixed with physiologic serum to facilitate manipulation. The surgical protocol employed with both materials was the same. All the patients were under antibiotic prophylaxis before sinus elevation procedure: 2 g of Amoxicillin 1h before surgery. Surgery was performed on all patients under local anaesthesia (Ultracain 05 epinefrin, Normon, Spain). The technique was very strict avoiding any damage to soft and hard tissues. The surgical procedure is well described in the literature [8]. The protocol employed was a two-stage delayed approach. In all the cases a two-stage lateral approach was used. Longitudinal mucosal incision was displaced palatally following the variation of overlapped flap, distal releasing incision was made buccally, and the mesial releasing incision was avoided if possible. Sinus elevation procedure was carried out following the antero-lateral approach. Sinus was filled up with particulate grafts to a 13.1 ± 1.8 mm height in order to bridge buccal and palatine walls. The average volume of graft particulates was 1.6 ± 0.4 cm³. Integrity of Schneiderian membrane was tested in all patients and in none of the studied cases did perforation of the membrane occur. Anthrotomy window was closed, in all cases, with a resorbable collagen membrane (Bio-Guide, Geistlich, Switzerland) covering the packed material. Surgical wounds were closed with tension free sutures, releasing periostium, employing PGA (Monosin, Braum, Germany) and Gore-Tex sutures (W.L. Gore & Associates, Flagstaff, USA). The following post-operative protocol was common to all patients: Amoxicillin 1 g/8 h for 7 days to avoid infection, Ibuprofen-Arginine 600 mg/8 h for 4 days, to reduce pain and swelling and Rinobanedit (Bayer, Barcelona, Spain) with endonasal application as a decongestive to facilitate the sinus drainage and epithelisation. A 0.5 mm plastic vacuum-formed maxillary splint (Henry Schein, UK) was prepared at the end of the surgical procedure, for each patient, and applied in their upper maxilla to promote the mucous healing and protect the palatal sutures from the tongue, establishing an effective barrier between the sinus and the mouth. Sutures and splint were permanently removed 2 weeks after surgery. Post-operative complications were limited to local swelling. Postsurgical visits were scheduled at monthly intervals to check the healing process.

2.4. Biopsy retrieval and implants placement

After a healing period of 8 months, and at the time that implants were placed in each patient, alveolar bone samples were retrieved. The surgical procedure was made under local anaesthesia by doing small releasing incisions and with a supracrestal longitudinal incision.

Biopsies were taken using a trephine (outer diameter 2.8 mm, and inner diameter 1.9 mm. Hu-Friedy Mfg. Co. Chicago, USA) under copious 4–5 °C sterile saline irrigation. A biopsy has been obtained for every implanted position. The depth of the biopsy was approximately 10 mm. The biopsies were harvested in the site where the implants had to be placed. The biopsy specimens were washed in sterile saline solution and immediately immersed in 10% phosphate buffered formalin solution, and kept refrigerated at 7 °C. The implants (MK III, Nobel Biocare, SE) were placed following the specific protocol. Implants were covered again suturing the mucoperiosteal flap.

2.5. Histology

The biopsy specimens were processed immediately to obtain undecalcified thin ground sections, following Donath's method [39]. The preparations were dyed with Harris Hematoxiline (Papanicolaou, Merck, Germany) and Wheatley's modification of trichromatic stain (Chromotrope 2R, Newcomersupply, USA) and preserved with Canada balsam solution (Fluka Biochemika, USA). Preparations were examined by using a transmitted light microscope (Optiphot, Nikon, Japan) equipped with a digital Camera (DP-12, Olympus, Japan). Morphometric study was carried out using Image J software. The retrieved biopsies were divided into three zones, apical, central and occlusive. The amount of bone, remaining material and bone marrow was evaluated in the middle third of the biopsies. The most apical third was rejected because of a more disaggregated appearance of the granulate materials and the occlusive one because it contained mature cortical bone.

2.6. Statistics

KS test was employed to verify the normality of the samples. Two tails *T*-test was employed using statistical software package (SPSS. V17, Chicago, USA). Values of $P < 0.05$ were considered significant.

3. Results

Table 3 summarizes the physicochemical properties of the grafts. Two main weight losses can be observed in the DTA-TG analysis of ABB. The first one (5.1 wt.%) occurs from 100 °C to 777 °C. The second (1.2 wt.%) takes place between 800 °C and 940 °C. The weight loss of TCP is less than 0.1 wt.% in the whole range from 100 °C to 1000 °C. The microporosity of the ABB granules measured using mercury porosimetry is 56.3 vol.% with an average pore size diameter of ~20 nm. The TCP particles exhibit much lower microporosity (17.8 vol.%) with an average pore size of ~1 µm. The obtained microporosities are consistent with the SEM observations (Fig. 1) and specific surface areas measured by BET. The only detectable phase by XRD in the TCP synthetic material is β -TCP (Fig. 2). Two broad diffraction peaks in the 31.7°–32.7° range can be observed in the XRD diffractograms of ABB. These peaks match the two main peaks of Hydroxyapatite (HA, JCPDS file 9–432). After heating at 1000 °C for 1 h the ABB graft consists mostly of well-crystallized hydroxyapatite with a small content of β -TCP (Fig. 2). The Ca/P molar ratio measured using XRD is 1.5 for TCP (only β -TCP phase present) and 1.66 for ABB (XRD quantitative analysis) [38]. The average interparticle space is bigger for TCP (382 µm) than for ABB (340 µm) with a significant difference ($P < 0.04$). However, the interparticle distance distribution is very alike for either material (Fig. 3). In both cases more than 97% of measured interparticle distances are bigger than 50 µm (lower limit for macroporosity [34]). Due to the small population of pores, smaller than 50 µm, all the interparticle surface measurements are associated to macroporosity. These measurements gave as a result from packing 'in vitro' simulations that macroporosity is larger for ABB (58.4±3.9 vol.%) than for TCP (49.9±4.3 vol.%). The difference between the groups is

statistically significant ($P < 0.001$). Fig. 4 shows a BSE micrograph comparing ABB and TCP effective packing.

Monthly check-ups and radiographic examination before implant placement have shown that there is no evidence of particle migration or volume loss in any of the grafted positions. Well differentiated bone surrounding and connecting remaining particles from both materials were observed. Around 35 vol.% of new-bone was found for both ABB and TCP. The proportion of lamellar bone was 40.7 ± 15.1 vol.% for TCP group and 34.9 ± 7.1 vol.% for ABB (Fig. 5). The difference between the groups is not significant ($P > 0.1$). The remaining presence of TCP particles was $32.6 \pm 6.2\%$ and 34.8 ± 10.5 vol.% for ABB without significant differences ($P > 0.5$). The proportion of bone marrow is larger and more evenly distributed in the ABB group (30.4 ± 14.2 vol.%) than in the TCP group (18.1 ± 8.2 vol.%), with a significant difference ($P < 0.01$). The new-bone formed was more homogeneously distributed in the ABB group. No gaps between the material and bone were observed in any group. There was an intimate apposition of bone in contact with particles in both materials (Fig. 6); neither of the materials were encapsulated by connective or fibrous tissue, nor exhibited chronic or acute cell infiltration. Frequently osteoblasts or extra cellular matrix (ECM) were found in both groups in intimate contact with the material surface. Those osteoblast-like cells are surrounded by a matrix that shows progressive mineralization. In the TCP samples BMU were observed colonizing the material surface, showing scattered multinucleated cells related to the material degradation. These cells are next to groups of osteoblast-like cells related to ECM formation (Fig. 7), as described by the modelling and remodelling of the cancellous bone [40]. Small fragments of TCP are usually incorporated into the mineralized ECM of new formed bone that grows all around without any kind of gap (Fig. 8). Fragments of TCP have been separated from the main particles and become surrounded by rich-cell mesenchymal matrix. Particles of remaining ABB show less affinity to the dye and seem to be tightly integrated into the new formed bone. Some peripheral graft-bone lacunae of the ABB particles are colonized by cells from the host (Fig. 9).

4. Discussion

According to the analysis, ABB are bone derived particles without remaining organic matter mainly precedent from crushed cancellous bone. The analysed batches were composed by mature bone with small quantities of carbonated species that decompose during heating between 700 °C and 1000 °C. The elevated BET values are consistent with the removal of the organic matter from the bone structure and a remaining microporosity with average pore sizes of 20 nm. In the case of TCP particles, the micropores have a median pore size of 1 µm and a BET value $< 1 \text{ m}^2/\text{g}$. The irregular shape of both graft materials allows the creation of an internal macroporosity between particles when packed inside a defect. From our 'in vitro' simulations, this macro porosity is 10 vol.% bigger for ABB particles. Nevertheless, as shown in Fig. 3, in both cases there are gaps larger than 100 µm and their average interparticle distance measured by image analysis is bigger than 300 µm. This value has been pointed out as adequate for good osteogenesis because it is the critical size where capillaries can be observed [34]. Although the overall macroporosity is larger for ABB particles, the macropore systems are very alike for both materials (Fig. 3). Because of this similarity in the pore system, the main difference in the host response to the grafting materials should be attributed to the nature of the materials itself, in terms of chemical solubility and physical morphology. Thorough characterization of grafting particulate materials is of vital importance for the determination of accurate interplays between their properties and biological response [30].

Besides longitudinal studies [19], the absence of data for the initial occupation of the grating material makes it impossible to discuss resorption percentages from the histomorphometric

measurements. According to 'in vitro' simulation, the initial occupation volumes were 50.1 vol.% and 42.6 vol.% for TCP and ABB respectively. Raw analysis of the remaining graft presence makes no difference between TCP and ABB. In this work we hypothesize that the occupancy of the grafting materials obtained from the packing simulations can be taken as a zero time situation. If we divide the remaining percentage of TCP and ABB by the initial occupancy percentages (TCP=50.1 vol.%, ABB=42.6 vol.%), then the remaining graft percentages would transform into 65.1 vol.% and 81.6 vol.% for TCP and ABB respectively (Fig. 5). These remaining percentages mean that TCP resorption is 34.9 vol.% and ABB resorption is 18.4 vol.%. These resorption percentages are consistent with the chemical composition of the materials; ABB particles due to their major content in HA are less soluble than TCP particles [41], therefore it is reasonable that TCP resorption percentage be higher.

The present study agrees with previous ones showing that both materials are osteoconductive and allow the formation of bone without inflammatory infiltrate even without harvesting autogenous bone [20,29]. After an 8 month grafting period, more than 30 vol.% of new-bone has been created in the two groups. This new-bone volume will provide the required stability for the implants placed in a second step. Although TCP has resorbed more than ABB, there are no significant differences in the new-bone amount between sites grafted with ABB or TCP particles. In both cases, the volume percentage of new-bone is always smaller than the initial macroporosity between particles and there is enough bone volume for the right placement of the implants, even without the addition of autologous bone with the grafted materials. This availability of space and the similarity in the interparticle distances might explain why the bone volume created is statistically the same for ABB and TCP. The interconnected macroporosity allows a rapid interaction with the media creating an early clot rich in growth factors (GF) and proteins. The releasing of those GF and proteins will colonize the surface of both materials and will start the formation of new-bone [42].

The main goal in a maxillary sinus floor augmentation procedure is to obtain enough bone volume to place osseointegrated fixations. It is also very important that this new generated bone be able to exhibit a long term favourable biomechanical response. ABB and TCP have shown excellent biocompatibility and osteoconductive capabilities [5,14,17,19,27–30]. The main difference between ABB and TCP particles response might be attributable mainly to their different solubility 'in vivo'. The resorption of ABB particles is slower than TCP so new-bone is mainly generated over the surface of the ABB particles. Considering the zero time hypothesis, in the case of TCP particles the amount of resorbed material is almost double that of ABB. Data reported in the literature shows that after 6 months the resorption of ABB and its eventual substitution by bone slows down and the material may last for years [17,18,43]. The resulting structure is a composite formed by new-bone and residual ABB particles [44]. This composite seems to have an efficient biomechanical response adequate to the implant mechanical demands [25]. Although further biomechanical assessments are necessary, it is reasonable to assume that the residual ABB particles are accepted as a structural element in the bone remodelling process, and bone grows surrounding them [44]. We have found the presence of cells from the host inside the lacunae of the ABB, mainly in the peripheral portions showing the full tolerance and compatibility of this graft material. Osteoblast-like cells have been found directly attached to the surface of both materials with the formation of osteoid matrix. It is important to highlight that after an 8 month grafting period, the interface between ABB and new-bone is stable, whereas for TCP there seems to be a dynamic resorption front where the material is progressively replaced by new-bone. The interface stability of ABB is supported by a slow solubility of ABB particles and the use of the empty structures by the host cells (Fig. 9) as already reported by Mangano et al. [5]. The osteoconductive capability of β -TCP is well known [45–47] and the results we have

obtained agree with other author's findings when working with similar β -TCP grafts in maxillary sinus floor augmentation procedures [27–30]. β -TCP is a resorbable material but there are still doubts about how this process takes place. We have hardly found reticuloendothelial system (RES) cells in the interface. This might suggest that osteoclasts or macrophagic cells do not play an important role in the resorption process of β -TCP grafts as previously reported by Knabe [29] and Zerbo [27]. The mobile resorption front does not affect the growth of new-bone, and the bone remodelling process takes place because of a dissolution process of the material and eventually the surface colonization by capillaries and the consequent BMU development.

5. Conclusions

The performance of packing simulations in order to establish the time zero stage for grafting materials helps to evaluate the resorption percentage of the grafted materials from the carrying out of a biopsy when the implants are placed. The standardization of this packing analysis procedure would avoid the ethical controversy of the execution of multiple biopsies on humans at different times. The ABB and TCP evaluated seem to work as a good scaffold for bone regeneration, allowing the migration and formation of new-bone over their surfaces. The use of these materials in sinus augmentation without addition of autogenous bone seems to bring similar results. No implant failures were reported during the follow-up period 2–3.5 years after placement. Eight months after grafting, the amount of new-bone is more than 1/3 of the total volume for either material, and presumably will increase with time. This agrees with our evaluation that shows that the differences in resorption rates do not have significant effects on the kinetics of new-bone formation. In this respect, the kind of macroporosity (similar in both materials) seems to be the key parameter. The behavioural differences are attributable to the chemical composition and microporosity of each grafting material. However, in order to fully assess how these differences affect the performance of the graft, the next step will be to perform a systematic biomechanical analysis of the regenerated bone and the bone–particle construct.

Acknowledgments

This work was partially supported by the National Institutes of Health (NIH) under Grant no. 5R01 DE015633 and the Ministry of Industry and Energy of Spain project number PROFIT300100-2006/73.

References

1. Ratner BD, Bryant SJ. 2004
2. Belser UC, Mericske-Stern R, Bernard JP, Taylor TD. Clin Oral Implants Res. 2000; 11(Suppl 1): 126. [PubMed: 11168262]
3. Sennerby L, Roos J. Int J Prosthodont. 1998; 11:408. [PubMed: 9922733]
4. Cawood JI, Howell RA. Int J Oral Maxillofac Surg. 1988; 17:232. [PubMed: 3139793]
5. Mangano C, Scarano A, Perrotti V, Iezzi G, Piattelli A. Int J Oral Maxillofac Implants. 2007; 22:980. [PubMed: 18271380]
6. Moy PK, Lundgren S, Holmes RE. J Oral Maxillofac Surg. 1993; 51:857. [PubMed: 8393101]
7. Zitzmann NU, Scharer P. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998; 85:8. [PubMed: 9474608]
8. Jensen OT, Shulman LB, Block MS, Iacono VJ. Int J Oral Maxillofac Implants. 1998; 1311(Suppl)
9. Lundgren, S.; Sennerby, L. Bone reformation: contemporary bone augmentation procedures in oral and maxillofacial implant surgery. Quintessence Books; London: 2008. p. 62
10. Jensen, OT. The sinus bone graft. Quintessence Pub. Co; Chicago: 1999. p. 52
11. Lundgren S, Nyström E, Nilson H, Gunne J, Lindhagen O. Int J Oral Maxillofac Surg. 1997; 26:428. [PubMed: 9418144]

12. Gerressen M, Hermanns-Sachweh B, Riediger D, Hilgers RD, Spiekermann H, Ghassemi A. Clin Oral Impl Res. 2009; 20:109.
13. Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA. Clin Orthop Relat Res. 1996;300. [PubMed: 8769465]
14. Valentini P, Abensur D. Int J Periodontics Restorative Dent. 1997; 17:232. [PubMed: 9497715]
15. Hallman M, Sennerby L, Lundgren S. Int J Oral Maxillofac Implants. 2002; 17:635. [PubMed: 12381063]
16. Yildirim M, Spiekermann H, Biesterfeld S, Edelhoff D. Clin Oral Implants Res. 2000; 11:217. [PubMed: 11168213]
17. Piattelli M, Favero GA, Scarano A, Orsini G, Piattelli A. Int J Oral Maxillofac Implants. 1999; 14:835. [PubMed: 10612920]
18. Pinholt EM, Bang G, Haanaes HR. Scand J Dent Res. 1991; 99:154. [PubMed: 1828906]
19. Sartori S, Silvestri M, Forni F, Icaro Cornaglia A, Tesei P, Cattaneo V. Clin Oral Impl Res. 2003; 14:369.
20. Artzi Z, Nemcovsky CE, Dayan D. Clin Oral Impl Res. 2002; 13:420.
21. Iezzi G, Degidi M, Scarano A, Petrone G, Piattelli A. J Periodontol. 2007; 78:2057. [PubMed: 18062129]
22. Froum, SJ.; Wallace, SS.; Cho, SC.; Tarnow, DP. The sinus bone graft. Quintessence Pub. Co; 2006. Use of Xenografts for sinus augmentation; p. 211
23. Haas R, Mailath G, Dortbudak O, Watzek G. Clin Oral Impl Res. 1998; 9:117.
24. Carmagnola D, Berglundh T, Lindhe J. J Clin Periodontol. 2002; 29:377. [PubMed: 12060419]
25. Ito K, Yamada Y, Nagasaka T, Baba S, Ueda M. J Biomed Mater Res. 2005; 73A:63.
26. Schwartz Z, Weesner T, Van Dijk S, Cochran DL, Mellonig JT, Lohmann CH, Carnes DL, Goldstein M, Dean DD, Boyan BD. J Periodontol. 2000; 71:1258. [PubMed: 10972641]
27. Zerbo IR, Bronckers A, Lange G, Burger EH. Biomaterials. 2005; 26:1445. [PubMed: 15482833]
28. Szabo G, Huys L, Coulthard P, Maiorana C, Garagiola U, Barabas J, Nemeth Z, Hrabak K, Suba Z. Int J Oral Maxillofac Implants. 2005; 20:371. [PubMed: 15973948]
29. Knabe C, Koch C, Rack A, Stiller M. Biomaterials. 2008; 29(14):2249. [PubMed: 18289665]
30. Coelho PG, Coimbra ME, Ribeiro C, Fancio E, Higa O, Suzuki M, Marincola M. Mater Sci Eng C. 2009; 29:2085.
31. De Groot K. Ann N Y Acad Sci. 1988; 523:227. [PubMed: 3382123]
32. Mankani MH, Kuznetsov SA, Marshall GW, Robey PG. Tissue Engineering Part A.
33. Davis WH, Martinoff JT, Kaminishi RM. J Oral Maxillofac Surg. 1984; 42:606. [PubMed: 6590811]
34. Karageorgiou V, Kaplan D. Biomaterials. 2005; 26:5474. [PubMed: 15860204]
35. Artzi Z, Weinreb M, Carmeli G, Lev-Dor R, Dard M, Nemcovsky CE. Clin Oral Impl Res. 2008; 19:686.
36. Davies, JE.; Hosseini, MM. Hystodynamics of endosseous wound healing. In: Davies, JE., editor. Bone Engineering. Vol. 1. EM Squared, Inc; Toronto: 2000. p. 1
37. Frost HM. Calcif Tiss Intl. 1969; 3:211.
38. Ginebra MP, Fernandez E, De Maeyerl EAP, Verbeeckl RMH, Boltong MG, Ginebra J, Driessens FCM, Planell JA. J Dent Res. 1997; 76(4):905. [PubMed: 9126187]
39. Donath, K. Norderstedt. Exakt-Kulzer; Germany: 1995. p. 1-16.
40. Legros R, Balmain N, Bonel G. Calcif Tiss Intl. 1987; 41:137.
41. Lu J, Descamps M, Dejoui J, Koubi G, Hardouin P, Lemaitre J, Proust JP. J Biomed Mater Res (Appl Biomater). 2002; 63:408.
42. Lieberman JR, Daluiski A, Einhorn TA. The role of growth factors in the repair of bone biology and clinical applications. JBJS. 2002; 84:1032.
43. Sartori S, Silvestri M, Forni F, Icaro Cornaglia A, Tesei P, Cattaneo V. Clin Oral Impl Res. 2003; 14:369.
44. Artzi Z, Weinreb M, Givol N, Rohrer MD, Nemcovsky CE, Prasad HS, Tal H. Int J Oral Maxillofac Implants. 2004; 19:357. [PubMed: 15214219]

45. Hotz G, Herr G. Int J Oral Maxillofac Surg. 1994; 23:413. [PubMed: 7890986]
46. Ducheyne P, Qiu Q. Biomaterials. 1999; 20:2287. [PubMed: 10614935]
47. Ogose A, Kondo N, Umezu H, Hotta T, Kawashima H, Tokunaga K, Ito T, Kudo N, Hoshino M, Gu W. Biomaterials. 2006; 27:1542. [PubMed: 16165205]

Fig. 1.

SEM micrographs of the starting particulates A. TCP particles show globular aggregated aspect with concavities and convexities. The spaces between aggregates give as a result the formation of grooves. B. ABB particles have sharp edges presumably derived from grinding cancellous bone structures. C. Microporosity of TCP; the TCP particles are formed by micron-sized grains sintered together and exhibit a microscopic porosity consistent with Hg porosimetry readings. D. ABB particles exhibit a submicron porosity resulting from the removal of the collagen fibers from the bone matrix during the deproteinization process. This sub micron porosity is responsible for the large specific surface of ABB.

Fig. 2. XRD spectra. A). TCP; there are no detectable secondary phases. B). As received AMB; The small sizes of the apatite needles present in the sample are responsible for the broadening of the peaks on the 31.7°–32.7° range. C). ABB after 1 h at 1000 °C; there is a small peak which matches the β -TCP main peak. The Ca/P is 1.5 for β -TCP and 1.66 for ABB.

Fig. 3.

Inter particle distance distribution. Besides the region <100 μ m where ABB has a higher percentage than TCP, the macropores created due to particle packing are very alike for both materials.

Fig. 4. BSE micrographs. Polished surface of ABB and TCP particles embedded in epoxy resin. The black zone corresponds to the graft free area. Interparticle distances were measured along the lines of the overlaid grid. The overall macroporosity is higher for ABB but the inter particle voids are similar for both materials. The scale bar is 2 mm.

Fig. 5.

Histomorphometric results for bone, marrow and remaining grafting material. When remaining material presence results are normalized using the zero time hypothesis to the initial occupation volume, it shows that TCP particles have been significantly more resorbed than ABB ones.

Fig. 6. Optical transmitted light microphotography of undecalcified thin sections dyed with Harris Hematoxylin/Chromotrope 2R stain. There is new-bone (NB) in direct apposition to the surface of both materials. Particles are surrounded by new formed bone. The ABB particles are easily recognizable because of less affinity to the dye, probably due to the lack of collagen inside the particles.

Fig. 7. Transmitted light microphotography of undecalcified thin sections dyed with Harris Hematoxylin/Chromotrope 2R stain. Bone metabolic unit colonizing the TCP graft interface. Inside this BMU there are multinucleated cells invading the TCP surface (inside black circle) and in the opposite pole an osteoblast-like cell (inside black square) is being included in the extracellular matrix. Note the new-bone (NB) lacunae occupied by osteocites.

Fig. 8. Transmitted light microphotography of undecalcified thin sections dyed with Harris Hematoxylin/Chromotrope 2R stain. Detail of a small TCP fragment included in the bone extracellular matrix (ECM) with close apposition to osteocytes inside the lacunae surrounding it. The new-bone (NB) ECM different mineralization gradation could be observed: Pink to purple low mineralized bone. Bluish high mineralized bone.

Fig. 9. Transmitted light microphotography of undecalcified thin sections dyed with Harris Hematoxylin/Chromotrope 2R stain. ABB-bone interface detail. Note the osteocyte like cells colonizing the lacunae of an ABB particle (highlighted with white ellipses). NB (new-bone).

Table 1

Commercial bone graft particulates.

Product	Composition	Origin	Particle size interval
Cerasorb® (Curasan, Germany)	β -TCP	Synthetic	100–500 μm 500–1000 μm 1000–2000 μm
Vitoss® (Orthovita, USA)	β -TCP	Synthetic	100–1000 μm
Ceros® granules (Mathys AG Bettlach, Switzerland)	β -TCP	Synthetic	500–700 μm 700–1400 μm
KeraOs® (Keramit, Spain)	β -TCP	Synthetic	250–1000 μm
R.T.R® (septodont, UK)	β -TCP	Synthetic	500–1000 μm
Bio-Oss® (Geistlich, Switzerland)	Anorganic bone matrix	Bovine	250–1000 μm
Osteobiol®Apatos (Tecnooss Dental, Italy)	Anorganic bone matrix	Porcine	600–1000 μm
Gen-ox® Inorganic (Baumer, Brazil)	Anorganic bone matrix	Bovine	250–1000 μm
Biogen® (Biotech, Italy)	Anorganic bone matrix	horse	500–1000 μm
Endobon® (Biomet, Switzerland)	Anorganic bone matrix	Bovine	500–1000 μm

Table 2

Patient exclusion criteria.

Exclusion criteria
Drug abuse or any significant systemic disease
Affection of previous pathology in maxillary sinus
Smokers
Under biphosphonates treatment
Active periodontitis

Table 3

Physicochemical properties of the grafted materials.

Material	BET (m ² /g)	Porosity (%)	Mean Pore size (μm)	Weight loss (1000C) (%)	Ca/P	Effective packing (%)
ABM	63.33	56.3	0.02	6.33	1.66	42.6±3.9
TCP	0.37	17.8	1.09	0.1	1.5	50.1±4.3