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Original Article

The chemical evaluation of different dental graft materials by energy dispersive X-ray spectrometry technique

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Abstract

Aim: The physicochemical properties of dental graft materials are very important because they strongly influence the bone regeneration capabilities of biomaterials. The purpose of this study is to investigate the chemical composition and surface energies of white (WPTG) and black porous titanium granules (PTG), bovine bone graft, and equine-derived bone graft through energy dispersive X-ray spectrometry (EDX) analysis of the comparison.
Methodology: The surface chemical compositions of PTG, WPTG, bovine bone graft and equine-derived bone graft were measured by EDX analysis. All graft materials' morphologic characteristics, such as particle and granule dimension were evaluated with Scanning Electron Microscopy (SEM). The EDX measurement of samples was evaluated at between x85 to x50000 magnification.
Results: PTG grafts showed elements of sodium (%8.88±9.98), chlor (2.44±1.96) and aluminum (0.99±0.37) as well as titanium (90.06±11.34) molecule at x5000 magnification. In WPTG, titanium (%34.55±6.41) and oxygen (%65.44±6.42) molecules were detected. EDX analyses have detected the presence of sodium, calcium, and phosphorus in the equine-derived and bovine bone graft.
Conclusion: It has been found that the PTG surface was not made of pure titanium, it has different chemical molecules at larger magnifications and xenografts exhibited different organic material content. Cell culture and experimental studies are needed to establish a relationship between the different commercial dental grafts and their regenerative properties.

Keywords: Energy dispersive X-ray technique, surface composition, scanning electron microscopy, bone substitute

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Introduction

Bone substitutes have the potential for regeneration as long as there is sufficient space for bone formation. In regenerative periodontal and intraoral surgery, different bone grafts are used for the reconstruction of periodontal defects and the treatment of peri-implantitis, socket preservation, odontogenic cysts, and sinus lifting, as well as in bone augmentation techniques for horizontal and vertical alveolar ridge deficiency (1, 2). Bone grafts can be classified as allogeneic, xenogenic, or alloplastic. In addition, bone grafts may have osteoinductive or osteoconductive properties that increase bone cell production or preserve the bone defect cavity (3, 4).

It is thought that the physicochemical properties of biomaterials, such as the surface chemistry and particle morphology, may affect the macrophages in the adhesion, apoptosis, fusion, and cytokine production of target cells (5) and are among the most important factors affecting in vivo biomaterial performance (6, 7). Thus, accurately evaluating the clinical results requires knowing the characteristics of a bone substitute.

When selecting the ideal bone graft, the tissue biocompatibility, defect size, manipulation of the graft, price, biological properties, and risks of the complication must be considered. Each graft will have different advantages and disadvantages. For example, autogenous bone graft ultrastructurally shows a trabecular structure with serious osteoinductive potential, as well as a pore size of 1 mm and porosity of 90% (4, 8). The chemical composition, morphology, and microstructural properties of bone grafts are also important to the development and growth of scaffold-based bone tissue. In particular, the size of the pores is important for bone healing and cell migration (9).

By avoiding the main deficiencies of allografts and xenografts, more durable biomaterials can be produced (10). In the recent past, many calcium-to-phosphate (Ca/P) ratios have changed, and many calcium phosphate-based materials (e.g., hydroxyapatite, calcium tetraphosphate, and tricalcium phosphate) have been used in the manufacture of porous scaffolds (11). It has been shown that biomaterials with a low Ca/P ratio are absorbed faster and cannot support tissue mechanically and cellularly (11,12). For this reason, the Ca/P ratio must be controlled to create stronger biomaterials(12).

Allografts have several disadvantages, such as their reduced mechanical strength in the sterilization stages, risk of infection, and limited availability. In general, xenogenic bone materials can be bovine-, porcine-, or equine-derived and are used often because they are widely available and chemically and biologically similar to human bone (13).

Porous titanium granules (PTG) are obtained from Grade 4 pure titanium that is manufactured in two forms: black porous titanium granules (PTG) and oxidized white porous titanium granules (WPTG). PTG granules consist of irregularly shaped and highly porous granules with a diameter of 0.7–1.0 mm (14). PTG has been used in dentistry for sinus lifting, furcation defects, and regenerative treatments of peri-implantitis (15–18). In one study where the physical and cytotoxic properties of PTG were compared with bovine bone grafts and alloplastic materials, it was found that the mechanical strength properties of PTG were greater than in the other bone substitutes (19). In another study where the crystallinity and oxide layer thickness of surface-grafted TiO2-particles were changed to match the surface of PTG using the anodization method, it was reported that the bioactivity of the oxidized PTG developed (20).

To the best of the researchers' knowledge, no prior study has compared the surface chemical composition of PTG, WPTG, and different types of xenografts. The aim of this study is to compare the chemical components of PTG with xenografts obtained from different sources using the scanning electron microscope (SEM) and energy dispersive X-ray spectrometry (EDX) technique.

Materials and Methods

There is no need for an ethical committee since the physicochemical properties of synthetic biomaterials are used in this study.

This study analyzed the physical and chemical structures of bone grafts derived from three different sources: bovine-bone xenografts, equine-bone grafts, and black and white porous titanium granules. Large granule xenografts were included in the study for their compatibility with the PTG graft. The properties of the graft materials are described below:

Porous Titanium Granules (PTG) (Natix, Tigran Technologies AB, Malmö, Sweden) are composed of pure titanium. PTG has been shown to be more flexible than xenografts. The percent porosity of the granules is 55.8%, while the porosity is 24.6 μm. The granule is about 0.7–1 mm in size (21).

White Porous Titanium Granules (WPTG) (Natix, Tigran Technologies AB, Malmö, Sweden), which are synthetic bone graft materials, are derived from the heat treatment and oxidation of black PTG (21).

Apatos (OsteoBiol, Roen Dental Products, Pianezza, Italy) is a biocompatible, osteoconductive biomaterial of equine origin having properties similar to mineralized human bone. The natural microporous consistency of Apatos accelerates the process by facilitating the formation of new bone tissue in the bone defect area. Apatos nanocrystalline hydroxyapatite is a mixture of cortical and cancellous bone granules. An equine-derived bone graft granule is about 1000–2000 μm in diameter (22).

Bio-Oss (Geistlich Pharma AG, Wolhusen, Switzerland) xenogenic spongiosa granules are natural bone minerals derived from bovine bones. The granules are non-organic, formed by a special extraction process, and treated with strong alkali and organic solvents at temperatures of up to 300°C. The granules are 0.25–1 mm in size (23).

SEM and EDX analysis

All bone grafts were removed from their storage boxes as instructed by the supplier. The samples were placed on carbon discs and carbon plaster for fixation and then put inside the microscope chamber under vacuum conditions. Images of the samples were taken with a Nova NanoSEM 650 (FEI Company, Hillsboro), a field emission scanning electron microscope, at a working distance range of 6–15 mm and an acceleration voltage of 20 kV. Images were taken with magnifications ranging from ×85 to ×50 000.

Energy dispersive X-ray spectrometry analyses of all samples were performed three times. All specimens were also morphologically evaluated on SEM at ×85, ×250, and ×5000 magnifications. Chemical surface analyses with EDX were made on the same magnifications on all substitutes. The mean values of three different particles from all bone grafts were obtained, and the results were evaluated (Figures 1 and 2).

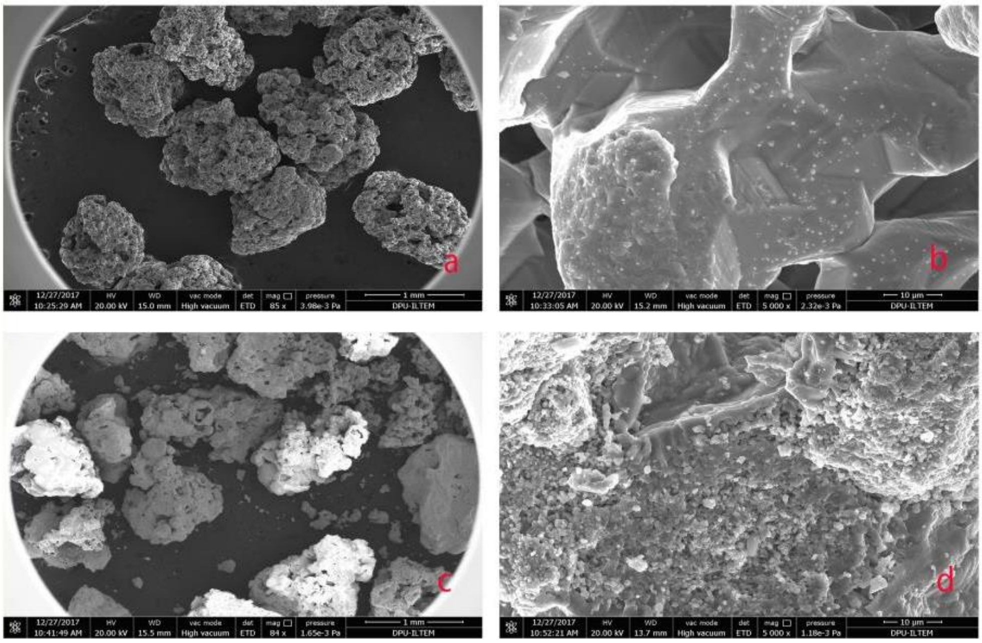

Figure 1. Porous titanium granules SEM images a) PTG images at x85 magnification, b) PTG images at x5000 magnification, c) White PTG images at x85 magnification, d) White PTG images at x5000 magnification.

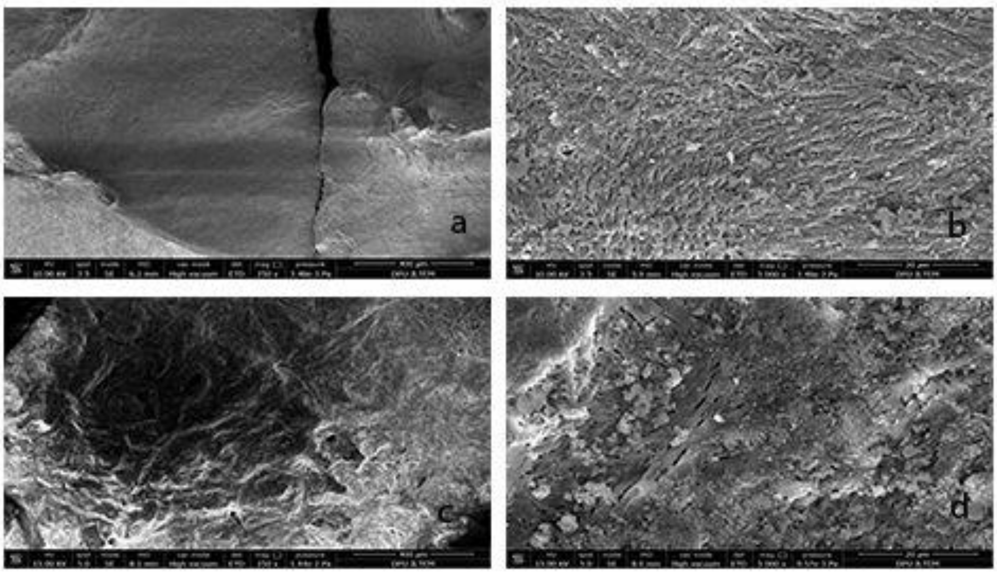

Figure 2. SEM images of Xenografts a) Bovine bone graft particules images at x250 magnification, b) Bovine bone graft particules images at x5000 magnification, c) Equine bone graft particules images at x250 magnification, d) Equine bone graft particules images at x5000 magnification.

Statistical analysis

The data were analyzed using the software program SPSS for Windows V20.0 (IBM SPSS Inc., Armonk, NY, USA). All parameters were analyzed using the Kolmogorov-Smirnov test to determine the normality distribution. All descriptive values were recorded as mean ± SD and percentages. A p-value less than 0.05 was considered statistically significant.

Results

The surfaces of the PTG were composed of Na (8.88 ± 9.98%), Cl (2.44 ± 1.96%), Al (0.99 ± 0.37%), and Ti (90.06 ± 11.34%) molecules.

The surfaces of the WPTG granules appeared to be composed of O (65.44 ± 6.42%) and Ti (34.55 ± 6.41%) molecules.

The equine bone graft was composed of O (49.11±1.88%), Na (1.11±0.7%), Ca (28.82±0.72%), P (19.25±0.54%), N (3.45±0.01%), and Mg (0.55±0.025%) molecules.

The bovine bone graft appeared to be composed of O (18.85±0.71%), Na (0.6±0.71%), Ca (54.6±0.79%), P (20.72±0.014%), N (4.84±0.4%), and Mg (0.39±0.15%) molecules (Table 1).

The Ca/P ratio was 2.63 for the bovine bone graft and 1.49 for the equine bone graft. While these two xenograft P ratios were similar, the Ca ratios were higher in the bovine-bone graft. When the O values were compared, the highest percentage was found in the WPTG, while the lowest percentage was found in the bovine bone graft. The Ti ratio in the PTG was higher than in the WPTG.

When the O/Ti ratios of the titanium granules were evaluated, the values were found to be 0.01 for the PTG and 1.89 for the WPTG.

PTG and WPTG grafts have osteoconductive properties and, morphologically, have a more porous structure than xenografts.

Table 1. Chemical composition of all graft materials (atomic weighted%)

	Ti(%)	O(%)	Na(%)	Cl(%)	Ca (%)	Al(%)	P(%)	N(%)	Mg(%)
Bovine Bone graft	-	18,85±0,71	0,6±0,71	-	54,6±0,79	-	20,72±0,014	4,84±0,4	0,39±0,15
Equine bone graft	-	49,11±1,88	1,11±0,7	-	28,82±0,72	-	19,25±0,54	3,45±0,01	0,55±0,025
Porous Titanium Granules	90,06±11,34	-	8,88±9,98	2,44±1,96	-	0,99±0,37	-	-	-
White Porous Titanium Granules	34,55±6,41	65,44±6,42	-	-	-	-	-	-	-

Discussion

The physicochemical surface properties of bone substitutes play a key role in cell response and interactions at the organic-inorganic interface (24). This study investigated the physical and chemical structure of bone grafts obtained from three different sources. In this study, two different bone xenografts were selected together with two PTG grafts. In the literature, the two most preferred bone grafts for regenerative treatment are bovine- and equine-derived bone grafts (25). In the studies presented, it was reported that the granules of PTG are very similar in structure to human bone (26).

In the study by Karaji et al., Ti granules were treated with the anodization method, and it was reported that the ratio of O in the TiO2 layer increased on the surface of the granules (20). In addition, in an analysis of EDS, the PTG showed high-intensity peaks of Ti and low-intensity peaks of O, and the O/Ti ratio was found to be 0.53 (20). In the present study, while the O/Ti value was 0.01 in the PTG, it was found to be 1.89 in the WPTG. According to the anodization method, it can be concluded that oxidation occurred in the WPTG as the manufacturing was stronger, but comparative studies at the cell culture level are required to confirm this conclusion.

Studies have reported that the osteoinductive potential of CaP-containing biomaterials mostly depends on their physicochemical properties, but that increases in the amount of CaP, especially in tricalcium phosphate-containing biomaterials negatively affects their osteoinductive potential (27, 28). In one study, it was reported that the microporous structure, in addition to the chemical surface, of CaP-containing biomaterials increases the affinity and osteoinduction of bone cells (29).

Another study evaluated the chemical compositions of three bone substitutes with a hydroxyapatite structure and one bone substitute with a calcium carbonate structure, both of which are frequently used in dentistry (7). In the grafts with similar chemical properties, significant differences were detected in terms of particle size, crystallinity, porosity, pore size distribution, surface area, and mineral content (7).

In another study, the physicochemical properties of biomaterials with different CaP content were evaluated, and the results showed that Bio-Oss had lower crystallinity properties, fewer nanoscale crystal grains, and a nanoscale surface architecture for the nanoscale microporosities (24). The same study also reported that Bio-Oss had a low CaP release and showed a high degree of protein adsorption (24). In the present study, general SEM images were used to evaluate Bio-

Oss, Apatos, PTG, and WPTG. However, mercury intrusion data would be required for a microporosity evaluation. Therefore, evaluations of the mechanical properties, such as microporosity and micropore size, and in vitro comparative studies are needed in the future.

Ca and P ions released from CaP-containing biomaterials may have positive effects on osteoblastic activities. One study has shown that mesenchymal stem cells have an effect on the differentiation of osteoblastic activity (30), but there are also studies reporting that bone formation is negatively affected in some bone substitutes that release more CaP directions (24). In a study by Desterro, the Ca rate was found to be 33.52±1.20 for a bovine bone graft (Bio-Oss), and the P ratio was found to be 15.74±1.41 (31). In a report presented HA, a bovine bone graft was shown to be 39.9% calcium (Ca) and 18.5% phosphorus (P) in the mineralized phase of the bones (32). In the present study, two bone grafts with a hydroxyapatite structure from different sources were evaluated, and the Ca and P surface values were obtained. In addition, when the Ca and P values of the hydroxyapatite bone grafts were evaluated as an atomic weight percentage, the Ca and P values for the bovine bone were (54.6 ± 0.79%) and (20.72 ± 0.014%), respectively; for the equine-derived bone graft, these values were recorded as (28.82 ± 0.72%) and (19.25 ± 0.54%), respectively. However, in the future, studies comparing the CaP release and osteogenic detection capacities are needed.

Ca is important for enhancing the osteoconductive properties and tissue integration of biomaterials (33). Studies have reported that higher calcium concentrations are more prone to resorption and help to compensate for the negative charges of phosphate (34). One study reported that the Ca/P ratios were 1.35 ± 0.07 for porcine-derived xenografts and 1.75 ± 0.16 for hydroxyapatite calcium phosphates (7). In an experimental study where a porcine-derived bone graft was used for socket healing after the repair of small and large defects, the Ca/P ratios detected by EDX analysis in the eighth week were reported to be 4.11 ± 0.71 in the small defects and 3.67 ± 0.57 in the large defects (35). Another study reported that the average Ca/P ratios of the two materials were 1.72 ± 0.07 (DBB) and 1.63 ± 0.02 (BCP), respectively (36). In this study, the Ca/P ratios were found to be 2.63 for Bio-Oss and 1.49 for the equine-derived bone graft.

In another study, a surface chemistry analysis of Bio-Oss was performed, showing the granules to be approximately 0.4% Na and 0.5% Mg, with the beta-tricalcium phosphate consisting entirely of CaP (36). In the present study, Mg and Na were detected in both of the hydroxyapatite bone grafts compatible with this study. However, in contrast to the previously mentioned study, N and O molecules were also detected. The equine-derived bone graft was found to be (49.11 ± 1.88%) O molecules, while the bovine bone graft was found to be (18.85 ± 0.71%).

Bone grafts with mechanical integrity and good structural support should be used due to their osteoconductive properties. The TiO_2 molecule has been proven to possess biocompatibility, increase bone formation, improve vascular growth, and limit the bacteriostatic effect (19). The penetration of phosphorous and calcium to the Ti structure could induce apatite formation and improve bone-to-implant contact (37). In this study, black PTG was evaluated at low magnification and found to consist of 100% titanium; the ×2000 and ×50 000 magnifications showed that Na, Cl, and Al were also present. This content is thought to form during the production of PTG.

This study has evaluated the physicochemical properties and inflammatory responses of porcine-derived (Osteobiol) and hydroxyapatite bone grafts. The granules of porcine-derived bone grafts have been reported to be more irregular and spicular but less inflammatory than hydroxyapatite alloplast biomaterials (38). In this study, PTG and WPTG were also found to be more irregular and pore-like than hydroxyapatite xenografts. However, in vivo experimental studies should be conducted to determine the effects of morphological and chemical properties on the inflammatory response.

Through EDX analysis, the present study found that WPTG had just two molecules: titanium and oxygen. A prior study showed that the cell viability of fabricated TiO2 was two times greater than that of black PTG. When the cell proliferation was evaluated, no significant difference was found between the fabricated TiO2 and black PTG (19).

Due to its surface properties, pore widths, and porosity, PTG facilitates the affinity, binding, and migration of osteoblasts to the graft surface. However, no appropriate pore size or pore diameter assessment of PTG has been performed for osteoblasts (39, 40). Both PTG and WPTG are irregular, and further studies on how osteoblast biological effects occur in cases where the crystallinity and physicochemical surfaces are regulated and standardized are needed.

Conclusions

According to this study, the physical and chemical properties of bone substitutes may affect the biological response. Porous titanium granule (PTG) and white porous titanium granule (WPTG) grafts have unrestrained osteoconductive properties and a more porous structure than xenografts. However, xenografts can be resorbed despite their osteoconductive properties, show a flatter structure, and may be reinforced over a longer period during equine-bone grafting by modifying their calcium-to-phosphate (Ca/P) ratios. Further cell culture studies are needed on this subject. It should be noted that there are no standardized studies in the literature since biomaterials have very different physical and surface chemistries for their crystallinity, microporosity, and porosity. Therefore, controlled in vivo and in vitro studies for each feature of a biomaterial are required.

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References

1. Esposito M, Grusovin MG, Felice P, Karatzopoulos G, Worthington HV, Coulthard P. The efficacy of horizontal and vertical bone augmentation procedures for dental implants - a Cochrane systematic review. Eur J Oral Implantol. 2009;2(3):167-184.
2. Schwarz F, Sahm N, Bieling K, Becker J. Surgical regenerative treatment of peri-implantitis lesions using a nanocrystalline hydroxyapatite or a natural bone mineral in combination with a collagen membrane: a four-year clinical follow-up report. J Clin Periodontol. 2009;36(9):807-814. (Crossref)
3. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. Eur Spine J. 2001;10 Suppl 2:S96-S101. (Crossref)
4. Dimitriou R, Jones E, McGonagle D, Giannoudis PV. Bone regeneration: current concepts and future directions. BMC Med. 2011;9:66. (Crossref)
5. Sheikh Z, Brooks PJ, Barzilay O, Fine N, Glogauer M. Macrophages, Foreign Body Giant Cells and Their Response to Implantable Biomaterials. Materials (Basel). 2015;8(9):5671-5701. (Crossref)
6. Williams DF. On the mechanisms of biocompatibility. Biomaterials. 2008;29(20):2941-2953. (Crossref)
7. Figueiredo M, Henriques J, Martins G, Guerra F, Judas F, Figueiredo H. Physicochemical characterization of biomaterials commonly used in dentistry as bone substitutes--comparison with human bone. J Biomed Mater Res B Appl Biomater. 2010;92(2):409-419. (Crossref)
8. Cooper DM, Thomas CD, Clement JG, Turinsky AL, Sensen CW, Hallgrímsson B. Age-dependent change in the 3D structure of cortical porosity at the human femoral midshaft. Bone. 2007;40(4):957-965. (Crossref)
9. Fabbri M, Celotti GC, Ravaglioli A. Hydroxyapatite-based porous aggregates: physico-chemical nature, structure, texture and architecture. Biomaterials. 1995;16(3):225-228. (Crossref)
10. Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials. 2006;27(18):3413-3431. (Crossref)
11. Rabiee SM, Moztarzadeh F, Salimi-Kenari H,Solati-Hashjin M. Preparation and properties of a porous calcium phosphate bone graft substitute. Mater. Sci. Poland. 2007;25(4):1019-1027.
12. Daculsi G, CorreP, Malard O, LeGeros RZ, Goyenvalle E. Performance for bone ingrowth of biphasic calcium phosphate ceramic versus bovine bone substitute. Trans Tech Publications Ltd. 2006;309-311:1379-1382. (Crossref)
13. Glowacki J. A review of osteoinductive testing methods and sterilization processes for demineralized bone. Cell Tissue Bank, 2005;6(1):3-12. (Crossref)
14. 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. (Crossref)
15. Guler B, Uraz A, Yalım M, Bozkaya S. The Comparison of Porous Titanium Granule and Xenograft in the Surgical Treatment of Peri-Implantitis: A Prospective Clinical Study. Clin Implant Dent Relat Res. 2017;19(2):316-327. (Crossref)
16. Dursun CK, Dursun E, Eratalay K, et al. Effect of Porous Titanium Granules on Bone Regeneration and Primary Stability in Maxillary Sinus: A Human Clinical, Histomorphometric, and Microcomputed Tomography Analyses. J Craniofac Surg. 2016;27(2):391-397. (Crossref)
17. Wohlfahrt JC, Lyngstadaas SP, Heijl L, Aass AM. Porous titanium granules in the treatment of mandibular Class II furcation defects: a consecutive case series. J Periodontol. 2012;83(1):61-69. (Crossref)
18. Andersen H, Aass AM, Wohlfahrt JC. Porous titanium granules in the treatment of peri-implant osseous defects-a 7-year follow-up study. Int J Implant Dent. 2017;3(1):50. (Crossref)
19. Sabetrasekh R, Tiainen H, Lyngstadaas SP, Reseland J, Haugen H. A novel ultra-porous titanium dioxide ceramic with excellent biocompatibility. J Biomater Appl. 2011;25(6):559-580. (Crossref)
20. Karaji ZG, Houshmand B, Faghihi S. Surface Modification of Porous Titanium Granules for Improving Bioactivity. Int J Oral Maxillofac Implants. 2016;31(6):1274-1280. (Crossref)
21. Verket A, Lyngstadaas SP, Rønold HJ, Wohlfahrt JC. Osseointegration of dental implants in extraction sockets preserved with porous titanium granules - an experimental study. Clin Oral Implants Res. 2014;25(2):e100-e108. (Crossref)
22. Scarano A, Piattelli A, Perrotti V, Manzon L, Iezzi G. Maxillary sinus augmentation in humans using cortical porcine bone: a histological and histomorphometrical evaluation after 4 and 6 months. Clin Implant Dent Relat Res. 2011;13(1):13-18. (Crossref)
23. Schmitt CM, Doering H, Schmidt T, Lutz R, Neukam FW, Schlegel KA. Histological results after maxillary sinus augmentation with Straumann® BoneCeramic, Bio-Oss®, Puros®, and autologous bone. A randomized controlled clinical trial. Clin Oral Implants Res. 2013;24(5):576-585. (Crossref)
24. Duan R, Barbieri D, Luo X, et al. Variation of the bone forming ability with the physicochemical properties of calcium phosphate bone substitutes. Biomater Sci. 2017;6(1):136-145. (Crossref)
25. Zizzari VL, Zara S, Tetè G, Vinci R, Gherlone E, Cataldi A. Biologic and clinical aspects of integration of different bone substitutes in oral surgery: a literature review. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(4):392-402. (Crossref)
26. Pepelassi E, Perrea D, Dontas I, Ulm C, Vrotsos I, Tangl S. Porous Titanium Granules in comparison with Autogenous Bone Graft in Femoral Osseous Defects: A Histomorphometric Study of Bone Regeneration and Osseointegration in Rabbits. Biomed Res Int. 2019;2019:8105351. (Crossref)
27. Fellah BH, Gauthier O, Weiss P, Chappard D, Layrolle P. Osteogenicity of biphasic calcium phosphate ceramics and bone autograft in a goat model. Biomaterials. 2008;29(9):1177-1188. doi:10.1016/j.biomaterials.2007.11.034. (Crossref)
28. Chan O, Coathup MJ, Nesbitt A, et al. The effects of microporosity on osteoinduction of calcium phosphate

bone graft substitute biomaterials. Acta Biomater. 2012;8(7):2788-2794. (Crossref)

29. Gao C, Peng S, Feng P, Shuai C. Bone biomaterials and interactions with stem cells. Bone Res. 2017;5:17059. (Crossref)
30. Shih YR, Hwang Y, Phadke A, et al. Calcium phosphate-bearing matrices induce osteogenic differentiation of stem cells through adenosine signaling. Proc Natl Acad Sci U S A. 2014;111(3):990-995. (Crossref)
31. do Desterro Fde P, Sader MS, Soares GD, Vidigal GM Jr. Can inorganic bovine bone grafts present distinct properties?. Braz Dent J. 2014;25(4):282-288. (Crossref)
32. McDowell H, Gregory TM, Brown WE., Solubility of Ca5 (P04) 3OH in the System Ca (OH) 2-H3P04-H20 at 5, 15, 25, and 37 C. J Res Natl Bur Stand Sec A, 1977;81:273-781. (Crossref)
33. Ripamonti U, Klar RM. Regenerative frontiers in craniofacial reconstruction: grand challenges and opportunities for the mammalian transforming growth factor-β proteins. Front Physiol. 2010;1:143. (Crossref)
34. LeGeros RZ. Calcium phosphate-based osteoinductive materials. Chem Rev. 2008;108(11):4742-4753. (Crossref)
35. Lozano-Carrascal N, Satorres-Nieto M, Delgado-Ruiz R, et al. Scanning electron microscopy study of new bone formation following small and large defects preserved with xenografts supplemented with pamidronate-A pilot study in Fox-Hound dogs at 4 and 8 weeks. Ann Anat. 2017;209:61-68. (Crossref)
36. Lindgren C, Hallman M, Sennerby L, Sammons R. Back-scattered electron imaging and elemental analysis of retrieved bone tissue following sinus augmentation with deproteinized bovine bone or biphasic calcium phosphate. Clin Oral Implants Res. 2010;21(9):924-930. (Crossref)
37. Vanzillotta PS, Sader MS, Bastos IN, Soares Gde A. Improvement of in vitro titanium bioactivity by three different surface treatments. Dent Mater. 2006;22(3):275-282. (Crossref)
38. Figueiredo A, Coimbra P, Cabrita A, Guerra F, Figueiredo M. Comparison of a xenogeneic and an alloplastic material used in dental implants in terms of physico-chemical characteristics and in vivo inflammatory response. Mater Sci Eng C Mater Biol Appl. 2013;33(6):3506-3513. (Crossref)
39. Sirak SV, Giesenhagen B, Kozhel IV, et al. Osteogenic Potential of Porous Titanium. An Experimental Study in Sheep. J Natl Med Assoc. 2019;111(3):310-319. (Crossref)
40. Carvalho AL, Faria PE, Grisi MF, et al. Effects of granule size on the osteoconductivity of bovine and synthetic hydroxyapatite: a histologic and histometric study in dogs. J Oral Implantol. 2007;33(5):267-276. (Crossref)