

Comparative Histomorphometric Analysis Between α -TCP Cement and β -TCP/HA Granules in the Bone Repair of Rat Calvaria

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This study compared the effect of two bioceramics on the process of bone repair: α -tricalcium phosphate (α -TCP) cement and β -tricalcium phosphate hydroxyapatite particles (β -TCP/HA). Calvarial defects were created in 50 rats, divided into two groups (α and β /HA). Software was used at 7, 21, 60, 90 and 120 days to assess bone formation. Mean new bone formation rates were as follows: α group, 1.6% at 7 days, 5.24% at 21 days, 24% at 60 days, 30.21% at 90 days and 50.59% at 120 days; β /HA group, 1.94% at 7 days, 2.53% at 21 days, 12.47% at 60 days, 26.84% at 90 days and 38.82% at 120 days; control group, 0.15% at 7 days, 10.12% at 21 days, 15.10% at 60 days, 18.94% at 90 days, 48.50% at 120 days. Both materials are osteoconductive and biocompatible. Perhaps the larger rate of new bone formation observed in the α -TCP group, it also occurs in the β -TCP/HA group within a longer time period.

Keywords: bone defect, calcium phosphate

1. Introduction

Several studies have sought to develop or improve new and thriving biocompatible materials that favor the repair of bone defects in order to provide, restore and/or maintain bone volume and quality in regions with impaired anatomical structure. The advantages of alloplastic grafts include commercial availability, absence of donor-site morbidity, easy manipulation and conformation, in addition to the possibility of physical and/or chemical integration of the material into the grafted site^{1,2}. Bone substitutes should have characteristics such as biocompatibility, nontoxicity and resistance to deformation. Resistance or not to resorption varies according to the desired application, and if biomaterials are resorbable, they should be metabolized by the body or eliminated by a normal physiological path³⁻⁶.

Tricalcium phosphate (TCP) and hydroxyapatite (HA) are alloplastic bone substitutes that belong with the class of bioceramics. In recent years, they have been used in bone surgeries, and have shown numerous advantages in orthopedics and traumatology, such as biocompatibility and bioactivity, which allow for tissue osteoconduction and *in situ* hardening^{7,8}. Alpha and beta-TCP have been used separately, in combination with each other or combined with HA⁹. HA, α -TCP and β -TCP have similar chemical structures (α -Ca₃(PO₄)₂ and β -Ca₃(PO₄)₂), but they differ in their crystalline structure, which results in different absorption characteristics. The solubility of β -TCP is smaller than that of α -TCP, which, in turn, is higher than that of HA. This implies longer permanence of β -TCP and of HA in the body^{9,10}. TCPs are porous, resorbable, biocompatible

materials, provoke little if any inflammatory response, permit the ingrowth of cells and vessels and have a direct connection with bony structure^{26,27}.

According to Den Boer et al.¹¹, several types of hydroxyapatite compounds have been tested for osteoconduction, and have yielded similar results to those obtained from autogenous grafts. Calcium phosphates, in general, are materials that can be easily manufactured into different compositions, sizes and shapes, depending on their specific application. They are characterized by relatively low tensile strength and low fatigue resistance⁹. The higher the number of small pores for a given total porosity, the more resistant the material will be^{12,26}. Shima et al.¹³ reduced the size of cement granules in order to decrease pore size and thus increase mechanical strength of cement. Pores act as strength concentrators upon the mass, and the reduction in their size will also decrease the magnitude of the associated field of strength intensity¹⁴.

Calcium phosphate absorption rates are adjusted to various types of applications: filling, coating, bone cementing, and tissue regeneration. Solubility rates of calcium phosphate cannot be changed or modulated easily in a single phase, but if two phases of calcium phosphate are combined, these rates can be increased. Biphasic calcium phosphates, containing β -TCP and HA, have been intensively investigated due to their levels of bioactivity and osteoconductivity. The presence of calcium sulfate hemihydrate in the composition of α -TCP cement allows reducing setting time, increasing pH values and forming pores in the cement to make osteoconduction

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easier¹. According to Driessens et al.¹⁵, α -TCP plus calcium sulfate hemihydrate provide a pH value between 6.5 and 8.0, which allows for cellular activity. Among other properties, this cement does not swell or disintegrate when exposed to fluids, does not retract or expand, and does not release heat during the setting reaction^{6,27}.

Generally, calcium phosphates are resorbed due to osteoclastic activity, forming new bone tissue at the bone-cement interface, thus characterizing the process of osteotransductivity. The speed at which this process occurs depends, among other factors, on the type of calcium phosphate tested, on the implantation site, and on the age of the recipient individual¹⁶.

The present study provided a histomorphometric analysis of the effect of α -TCP cement and β -TCP/HA granules on the bone repair of critical calvarial defects in rats, with regard to osteoconductivity, quantifying and comparing the area of newly formed bone in both groups. The comparison between these materials is justified because they have the same indication as bone substitutes, but they have different physical structure, α -TCP is cement developed in UFRGS Materials Engineering and β -TCP/HA are granules commercialized by Encobio SA. The presence of HA in β -TCP and its absence in α -TCP is a factor to analysis in this article.

2. Materials and Methods

2.1. Sample

The sample consisted of 50 male adult rats (*Rattus norvegicus albinus*, Wistar), weighing on average 300 to 400 g, randomly distributed into two groups of 25 animals: Group- α and Group- β , according to the material inserted into the experimental cavity. The protocol was approved by the Animal Care and Use Committee of Pontifícia Universidade Católica do Rio Grande do Sul.

2.2. Surgical procedure

The animals were submitted to general anesthesia with ketamine chlorhydrate 50 mg.kg⁻¹ (0.05 mL/100 g) and xylazine chlorhydrate 5 mg.kg⁻¹ (0.025 mL/100 g), given intramuscularly. After hair removal from the upper region of the head, lidocaine chlorhydrate 2% with norepinephrine 1:50.000 was injected subcutaneously at the site in order to provide hemostasis and additional intraoperative analgesia, in addition to helping with pain management in the immediate postoperative period.

Surgical access was obtained by a linear coronal dermoperiosteal incision measuring 1.5 cm in length, between the outer auricles. The periosteum was divulsed and pulled apart along with the adjacent soft tissues, exposing the surface of parietal bones. Two cavities measuring 4 mm in diameter, one on each side of the sagittal suture, were made by using a cylindrical multiple blade bur, rupturing the outer and inner cortical, under profuse irrigation with physiological saline 0.9%. Alpha-TCP cement and β -TCP/HA granules were inserted into the cavities on the left side, in their respective experimental group. Control cavities, bored into the right side of the calvarium, were not filled with material, but with blood from the surgical site (Figures 1 and 2). After repositioning of the soft tissues, including the repositioning of the periosteum over the cavities, the suture was made on a single plane using a 4.0 nylon thread (Mononylon®, Ethicom).

Alpha-TCP cement (manufactured by Labiomat, Laboratory of Biomaterials of the School of Materials Engineering of Universidade Federal do Rio Grande do Sul, SANTOS, 2002) consists of a powder (75% of α -TCP and 15% of calcium sulfate hemihydrate, a composition with no similar description in the international literature), with addition of a solution containing accelerator (2.5% of Na₂HPO₄) until a cement consistency was obtained, being then inserted into

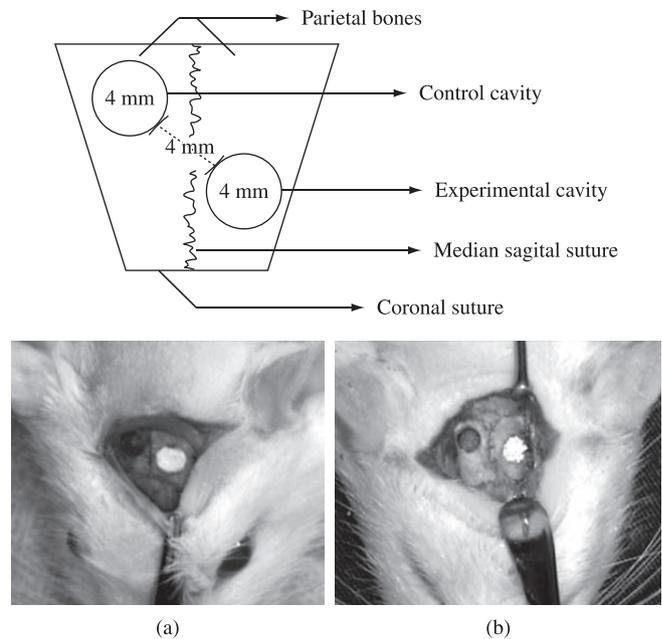


Figure 1 Schematic representation of the rat calvarium. Bone cavities (experimental and control) bored into the parietal bones. Defects induced in the calvarium. Control cavity filled with blood clot in both groups. In a) experimental cavity α , filled with α -TCP cement. In b) experimental cavity β , filled with β -TCP/HA granules.

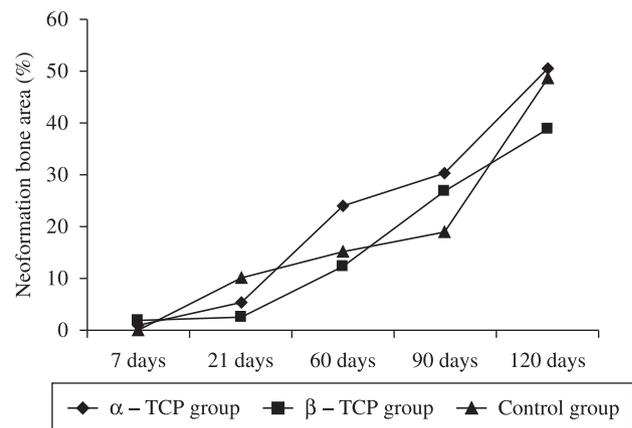


Figure 2 New bone formation in groups α , β and control in the observation periods.

experimental cavity α . In group β /HA, the cavity was filled with porous granules (mesh of 60-80), containing 65% of HA and 35% of β -TCP (Osteosynt®, EINCO Biomaterial Ltda.). The material was inserted into experimental cavity β /HA, where it was mixed with blood from the surgical site.

The animals were killed postoperatively on days 7, 21, 60, 90 and 120 by isoflurane inhalation in a chamber and had their calvaria removed by osteotomy with a diamond blade under irrigation with physiological solution 0.9%. After local macroscopic analysis, the surgical specimens were stored in neutral buffered formalin at 10% for over 24 hours and less than 72 hours.

2.3. Slide preparation

The surgical specimens were submitted to decalcification and routine histological processing for slide preparation, being then

embedded in paraffin blocks. Thereafter, they were sectioned at a 6 μ m thickness in a microtome using the largest diameter of the defect, and stained with hematoxylin and eosin (HE) and analyzed under a light microscope.

2.4. Slide analysis

The histological images were captured from the microscope by the computer using 40 \times magnification. The slides were analyzed using Image Pro Plus, version 6.2[®], which allowed measuring the total area of each bone defect and the area of newly formed bone inside the defect. The measurement of the area (in micrometers) of newly formed bone was converted into a percentage value representing the total area of the defect. These percentage values were submitted to statistical analysis.

It was used completely randomized design, with two treatments applied in five periods, with three repetitions and one control group for each treatment and for each period. After to verify estimated to variance analysis (ANOVA) and being significant, the data had been compared by the Holm-Sidak test with $p < 0,05$. The results were evaluated for each treatment, compared with control, referring with application time and the treatments between itself had been compared. As the data had presented significance for ANOVA and to the test of comparison of averages, linear regression analysis in each type of treatment was applied in accordance with the application time.

3. Results

3.1. Structure

Alpha-TCP cement consists of a powder composed of 75% of α -TCP (α -Ca₃(PO₄)₂) and 15% of calcium sulfate hemihydrates (CaSO₄H₂O), with addition of a solution containing accelerator (2.5% of Na₂HPO₄) until a cement consistency is obtained. Porous granules of β /HA contain 65% of HA (Ca₃(PO₄)₃OH) and 35% of β -TCP (β -Ca₃(PO₄)₂).

3.2. Properties

The percentage values of the mean new bone formation in the repair process in groups α and β are shown in Table 1. Note that there was a gradual increase in new bone formation in both groups throughout the five observation periods. New bone formation was higher in group α than in group β , at all observation times, with a statistically significant difference at 21 days and at 120 days. At the end of the experiment, the rate of new bone formation in group α amounted to 50.59%, being higher than in the control group (48.50%), which, in turn, had a larger area of new bone formation than group β (38.8%).

The process of new bone formation is shown in Figure 3 in microscopic sections stained with HE, analyzed under a light

microscope, at a 40 \times magnification, showing the comparison between groups α and β .

3.3. Applications

β -TCP/HA granules are resorbed more slowly than α -TCP and seem to maintain the volume in the grafted region; therefore, their application can be indicated in cases of bone grafting to restore bone volume. Due to its high solubility rates, α -TCP is indicated to replace grafts that precede titanium dental implants, in which the biomaterial needs to be quickly replaced with the newly formed bone.

4. Discussion

The characteristics observed in both phosphates corroborate the properties that are conceptually necessary, since they did not produce exacerbated inflammatory reaction, were not encapsulated or rejected by the body and allowed for osteoconduction^{1,18-20}.

The comparison between α -TCP cement and β -TCP/HA granules over time is important as it allows for predictability in the manipulation of the newly formed bone.

The results obtained in this study reveal a larger percentage of newly formed bone in cavities in which α -TCP was used, with statistical significance. The α -TCP block degraded significantly more than the β -TCP block²¹. Hirakata²² and Li et al.¹⁰ asserted that the solubility and/or absorption rate of α -TCP are higher than those of β -TCP. When the β -TCP crystal, in the cement phase, is mixed with water, it requires longer time to turn into calcium-deficient hydroxyapatite, therefore, remaining in the tissue and favoring osteoconduction. Perhaps this larger rate of new bone formation observed in the α -TCP group also occurs in the β -TCP/HA group within a longer time period. According to Driessens et al.¹⁶, CFCs do not act as permanent bone substitutes, but they are slowly replaced.

By comparing the defects filled with α -TCP cement with control defects, there was higher new bone formation in experimental cavities at 90 days, confirming the studies conducted by Jansen et al.²³ and Kessler et al.²⁴ In this same observation period, the mean rate of new bone formation of 26.84% in the β -TCP/HA group was higher than in the control group (18.94%), and this difference was statistically significant; and smaller than in the α -TCP group (30.32%), without statistical significance. Alpha-tricalcium phosphate (alpha-TCP) ceramic is a bioresorbable material that degrades in bone tissue after implantation, since it exhibits higher solubility than beta-tricalcium phosphate (beta-TCP) ceramics. The high solubility of alpha-TCP in an aqueous solution causes its transformation into hydroxyapatite (HA) through hydrolysis²⁸. To Nilen and Richter²⁹ HA/beta-TCP ratio dominantly determines the rate and extent of BCP resorption in vivo, the possible thermal decomposition of HA during BCP synthesis must be considered, particularly if high temperature treatments are involved.

Table 1. Comparison of the area of new bone formation (mean and standard deviation, in %) between groups α and β , according to observation periods.

Observation period	Group α		Group β		p	Control group	
	Mean (%)	SD (%)	Mean (%)	SD (%)		Mean (%)	SD (%)
7 days	1.06	2.37	1.94	3.10	0.629	0.15	0.33
21 days	5.24	5.58	2.53	1.19	0.004*	10.12	8.01
60 days	24	14.5	12.47	3.87	0.159	15.10	11.70
90 days	30.32	6.84	26.84	2.8	0.354	18.94	8.58
120 days	50.59	5.42	38.82	8.76	0.043*	48.50	14.40

SD = Standard deviation. * Statistically significant difference between groups α and β ($p < 0.05$).

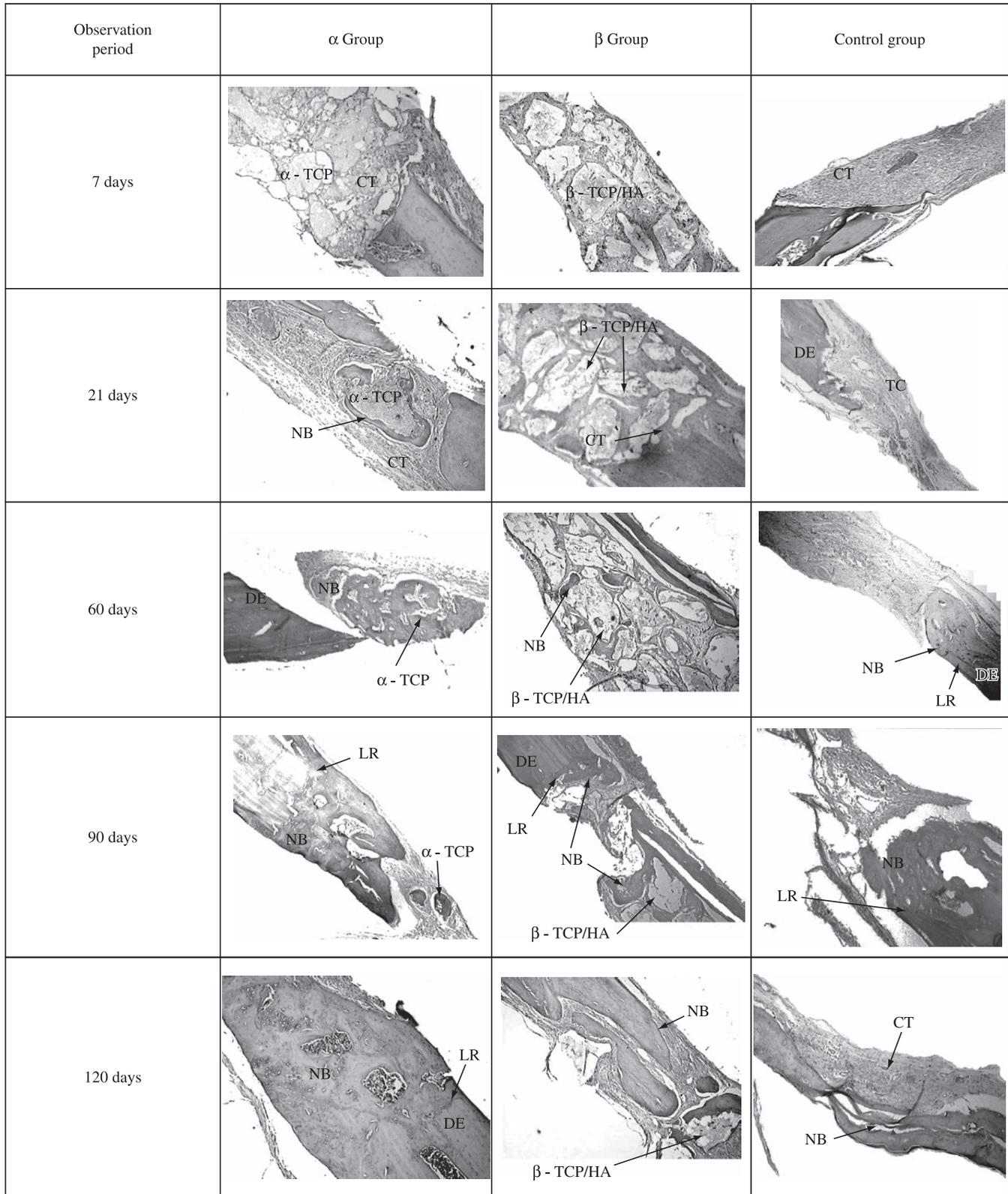


Figure 3. Comparison of the repair process between groups α , β and control: 7 days – In all groups: inflammatory infiltrated cells. In test groups: there is material involved in conjunctive tissue (CT). 21 days – The standard of conjunctive tissue is more organized in all groups, showing new bone formation (NB) around materials granules in test groups. 60 days – α group: increase in neoformation bone area close to defect edges (DE) and around the material granules. β -TCP/HA group: more neoformation around the material granules. Control group: neoformation only in the defect edge. 90 days – Groups α and control: more neoformation in the edges, reversion line (RL) apparent. β -TCP/HA group: more neoformation around the granules. 120 days – α group: majority of defect is formed by new bone tissue, there is no granules of material. β -TCP/HA group: granules of material involved by new bone tissue and fibrous conjunctive tissue areas. Control group: band of new bone tissue filling part of the defect, but keeping fibrous conjunctive tissue in 2/3 of the defect.

In the present study, after 120 days, a larger amount of material was found in the defect in the β -TCP/HA group. Chow⁹ and Li et al.¹⁰ demonstrated that β -TCP has low solubility rates. According to information provided by the manufacturer of β -TCP/HA granules, hydroxyapatite was inserted in order to reduce the solubility of the bioceramics²⁵. The analysis of the defects induced in the present study reveals that the filling volume of granule-containing cavities was maintained, but that did not occur in the control cavities filled with blood clot.

This study showed higher mean rates of new bone formation in the α -TCP group. These results are due to the cement structure, since its crystals have an open structure, with pore formation caused by calcium sulfate hemihydrate¹, which allows for the formation of the osteoid matrix amidst the crystals. On the other hand, β -TCP/HA granules, as they have a structure in which the crystals are larger and less soluble²², were kept for a longer time inside the defect. This promoted new bone formation, although in a smaller amount than in the former group, and maintained tissue volume inside the defect. Observations in periods longer than 120 days would be necessary for elucidating the effect of the materials analyzed here on bone repair.

Based on the results obtained and on literature reports, both materials are believed to have the necessary properties for clinical use. In cases in which a higher bone volume is required, such as in the correction of defects with esthetic involvement, β -TCP/HA may be indicated. However, in cases in which earlier formation of bone tissue is necessary, for instance in grafts that precede dental implants, α -TCP cement provides quicker new bone formation. These materials have specific indications depending on their solubility into the tissue and on the speed at which they promote osteoconduction.

Alpha-TCP cement and β -TCP/HA granules used promote osteoconduction and are biocompatible. Alpha-TCP cement is more soluble and allows for a larger new bone formation area, compared to β -TCP/HA granules and to the blood clot (control). Beta-TCP/HA granules are resorbed more slowly and seem to maintain the volume in the grafted region.

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