Research Article

ISSN 2639-9490

Oral Health & Dental Science

Deproteinized Bovine Bone versus Beta-Tricalcium Phosphate Bone Substitutes: A Clinical and Histological Assessment of Bone Regeneration

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Received: 20 January 2021; **Accepted:** 14 February 2021

Citation: Machibya FM, Zhuang Y, Chen J. Deproteinized Bovine Bone versus Beta-Tricalcium Phosphate Bone Substitutes: A Clinical and Histological Assessment of Bone Regeneration. Oral Health Dental Sci. 2021; 5(1); 1-6.

ABSTRACT

Introduction: *Bone regeneration has been extensively applied in contemporary dental practice: However, the material selection is a continuous challenge to practitioners due to increasing number of bone substitutes with different biological and physical properties which determine their osteogenesis capacity.*

Objective: *To compare the percentage area occupied by bone and residual regeneration material in bone defects treated with (Bio Oss) Deproteinized bovine bone (Bio Oss) or Beta-tricalcium phosphate.*

Methodology: *The study assessed clinical (local swelling, bleeding on gentle touch, pus discharge, bone substitute (BS) discharge and Membrane exposure) and histological (percentage area occupied by bone and residual BS) features of standardized bone defects treated by either beta-Tricalciumphospate (β-TCP) or Deproteinized bovine bone (Bio Oss) (experimental) and control defects treated without regeneration materials.*

Results: *All defects healed without pus or regeneration material discharge. Bleeding was observed in 9 (20.8%) of defects within three days post surgery. Some swelling of different size was evident on all surgical sites up to 5th day post operation. There were no statistically significant differences on clinical features assessed.*

There was higher percentage area occupied by bone tissue at 8 weeks compared to 4 weeks post treatment except in Bio Oss group which recorded relatively constant bone percentage at 4th and 8th weeks. The BS residual percentage areas were statistically significant different between groups at 4th and 8th week post treatment, except for experimental (β-TCP and Bio Oss) groups which recorded statistically insignificant difference (P = 0.78) at 4th week. The β-TCP group had the highest bone percentage at 4th and 8th weeks, while the lowest percentage of bone at 4th and 8th week was observed in control and Bio oss groups respectively.

Conclusion: *The type of regeneration mode had no effect on the local clinical features clinical assessed.*

The percentage area occupied by bone increased with time except in Bio Oss group which showed no difference between 4th and 8th weeks. The β-TCP group had the highest bone percentage at 4th and 8th weeks, while the lowest percentage of bone at 4th and 8th week was observed in control and Bio oss groups respectively.

Keywords
Bone graft, Bone substitutes, Guided bone regeneration.

Introduction
Bone regeneration has been extensively applied in contemporary dental practice. The goal of bone regeneration procedures is to stimulate or at least facilitate the growth of new bone into the augmented site. For many years the gold standard for bone grafting has been autogenous bone from intra- or extra oral donor sites [1-4]. The search for suitable bone substitute materials has intensified over the years due to the shortcomings of autografts, mainly donor site morbidity and limited available bone volume. A bone substitute material can be used instead of or in combination with an autograft to increase graft volume. Bone grafts or substitute biomaterials are commonly used therapeutic strategies for clinical bone surgery to fill the bone defects for reconstructing large bone segments. Irrespective of the use, the bone substitute must be biocompatible and should be attractive for proliferation of new blood vessels and ultimately the growth of new bone into the augmented area [5]. The ideal bone substitute must maintain this biological support during healing time followed by gradual replacement by the newly formed bone. In some clinical indications, however, a low substitution rate may be beneficial, during which the physical support from the graft material maintains the augmented lumen and prevents soft tissue collapse [5].

Bio-Osss is an organic bovine bone substitute with osteoconductive properties and high biocompatibility [6]. It has been tested in many randomized clinical trials and is thus one of the best documented biomaterials. Controversy remains, however, as to whether this graft source is truly resorbable [7]. Whereas, beta-tricalciumphosphate (β-TCP) is a porous synthetic material which has shown osteoconduction and a very favorable substitution rate in standardized bone defects [8]. Both Bio-Osss and β-TCP are presently in clinical use but have never been compared in standardized bone defects.

The knowledge of physical, chemical and biological characteristics of bone substitutes (BSs) available for clinical practice is important in material selection for optimum clinical outcome. While some bone substitutes undergo almost immediate biodegradation and resorption, others can be detected on the implant site for several years [7,9]: Some materials are reported to have better osteointegration with host bone [10]. Ideally, osteogenesis, osteoinduction and osteoconduction are the three essential elements of bone regeneration along with the bonding between host bones and grafting material which is called osteointegration [11]. For optimal bone regeneration, the bone substitutes should meet some specific characteristics, such as biocompatibility, osteoinductive, bioresorbable, osteoconductive, structurally similar to bone, porous, mechanically resistant, easy to use, safe, and cost-effective [12]. Deproteinized bovine bone (DBB) is a bone substitute of natural origin extracted from bovine bone that undergoes a low heat (300°C) chemical process which removes all organic components, but maintains the natural architecture of bone Calcium-phosphate (CaP) crystals known as hydroxyapatite with morphological and structural properties similar to that of human bone hydroxyapatite [13]. The material has demonstrated osteoconductive properties in vitro and in vivo studies, that improve bone regeneration [14]. Beta-Calciumphosphates are synthetic bone substitutes with different ratios of hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP). Variation of HA/TCP ratio determines the patterns of BCP biodegradation and osteoconduction [15,16].

Methodology
Ethical considerations
The study was approved by the Ethics Committee of Fujian Medical University. All animal handling and surgical procedures were conducted according to the Institutional Review Board (IRB) guide lines for the use and care of laboratory animals.

Animal experiments
The study included six male beagle dogs aged 18 months with a mean weight of 11.8 Kg. The sample size was determined by assuming clinical significant difference of 2 mm bone height with 1 mm standard deviation at the power of 0.9 and 0.05 significant levels. The data were collected by intraoral clinical examination computed tomography (CT) scan image and immunoassay analyses. Twenty-four alveolar bone defects were created by extending the first pre-molar extraction socket. The experimental defects were treated by GBR using synthetic β-TCP (Bio-lu Biomaterials Co., Ltd. Shanghai, China) or xenograft BioOss® (Geistlich, Wolhusen, Switzerland) regeneration materials, whereas the control defects were left empty. Resorbable collagen membranes Bio-Gide® (Geistlich, Wolhusen, Switzerland) were used in both experimental and control defects. The regeneration materials were equally allocated to the maxillary right and left (UR and UL) as well as to the mandibular right and left (LR and LL) defects by randomizing three pre-determined sets of defect managements to the six experimental animals (i.e set 1: UR- β-TCP, UL-Bio Oss, LR-Control and LL- β-TCP; set 2: UR-Bio Oss, UL- β-TCP, LR-Bio Oss and LL Control; set 3: UR-Control, LR-βTCP, UL-Control and LL Bio Oss). Every set was randomly assigned to two dogs; consequently, the three GBR groups (β-TCP, Bio Oss and Control) were equally distributed to the right and left of maxillary and mandibular jaws. The set randomization also allowed for every GBR group to be assigned to eight defects.

Surgical procedure
Under general anesthesia, the maxillary and mandibular first premolar extraction sockets were extended medially from the second premolar using cylindrical tungsten bur to create standardized artificial defects measuring 5 mm deep, 7 mm long (mesial-distal) and 5 mm wide on each quadrant of the animal's jaws. Depending on the GBR group allocation, the defects were filled with β-TCP or Bio Oss mixed with animal's blood collected during defect preparation. The mixture was packed into the artificial defects to the natural alveolar height level whereas; the control defects were left empty. The filled experimental and the

empty control defects were all covered by resorbable collagen membranes Bio Gide® followed by wound closure using 3/0 nylon sutures which remained in the site for two weeks.

Clinical assessment

A standardized clinical data sheet was used to collect clinical features of all defects during two weeks healing stage. The features clinically observed at this stage included local swelling, bleeding on gentle touch, pus discharge, BRM discharge and Membrane exposure. The assessment was done on second, third, fifth, seventh, tenth- and fourteenth-day post-operative under general anesthesia.

Non decalcified Histological Section procedure

The dogs were sacrificed either at 4 or 8 weeks after surgery with an overdose of sodium thiopental. Maxillary and mandibular block sections were dissected and fixed in 10% neutral buffered formalin. After fixation, the specimens were ground in sagittal direction and cut with a microtom into 250 μm thick sections. The sections were further reduced to 15 μm and polished. The Paraffin embedded samples were cut by a rotary microtome to provide slides. Each sample provided multiple mesio-distal slides of 5 µm thicknesses each. The thin sections were subsequently rinsed in distilled water and stained with Hematoxylin-eosin (HE) solution. Examination of the histological sections were made using an ECLIPSE Ci-E microscope (Nikon, Tokyo, Japan) and the images were captured using a Canon EOS M50 Camera (Kyushu, Japan) and saved as tagged image format files with the Adobe Photoshop CS software.

Histometric analysis

Three sagital sections, about 100 µm apart, representing the most central portion of the defect site, were selected for histological measurements. The defects' percentage areas occupied by new bone and BS remnants selected by magic wand were measured using Photoshop CS3 (Adobe) measurement log function. The mean of the measured values from the three specimens was calculated and labeled as representative data of each defect.

The means and standard deviations were calculated for each group. The data showed a normal distribution tendency; hence, parametric statistical analysis was applied with the level of statistical significance set at $P < .05$. One-way analysis of variance (ANOVA) was used to evaluate the differences between the groups. The statistical package for social sciences (SPSS) software version 19.0 (IBM SPSS Inc, Chicago, IL, USA) was used.

Results

Clinical features assessment

All defects healed without pus or regeneration material discharge. However, bleeding was observed in 7 (20.8%) of defects within three days post surgery. Some swelling of different size was evident on all surgical sites on the up to 5th day post operation. On day 10, the swelling had subsided from all defects. The differences observed on clinical assessment were not statistically significant.

Qualitative histology evaluation

At 4 weeks post surgery, the center of the defects was mostly occupied with immature trabecular woven bone around the BS particles and surrounded by vascularized stroma among Bio Oss sites: (Figure 1a): Whereas, extensive bone trabeculae occupied the center of the defects with particles surrounded by woven bone for βTCP (Figure 1b). The amount of material remnants was significantly low in βTCP compared to Bio Oss group. Control Defects were occupied by granulation tissue and mesenchymal stroma with small scattered areas of bone formation (Figure 1c).
At 8 weeks post surgery, the center of the Bio Oss defects showed woven bone growth on the material surface with areas of mature bone marrow and mesenchymal stroma (Figure 2a).

Beta-tricalcium phosphate sites had mostly mature bone marrow with some trabecular bone growing on the surface of the material particles (Figure 1b). Among the Control sites, the defect centers were mainly occupied by newly formed bone with areas of mature bone marrow (Figure 1b).

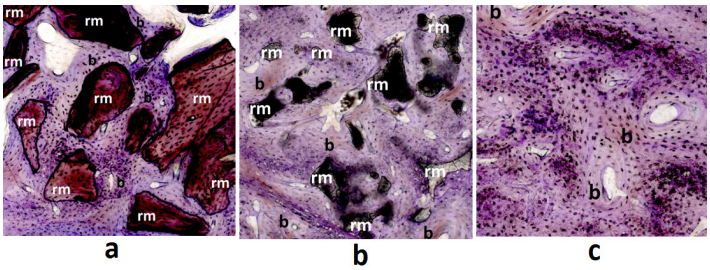


Figure 1: Histological images of healing bone defects 4 weeks after surgery (a) Bio-Oss, (b) □-TCP and (c) Control defects. "**rm**" shows remnants of bone regeneration material (Bio-Oss or □-TCP) at different stage of resorption; "**b**" indicates bone tissue at different stage of maturations.

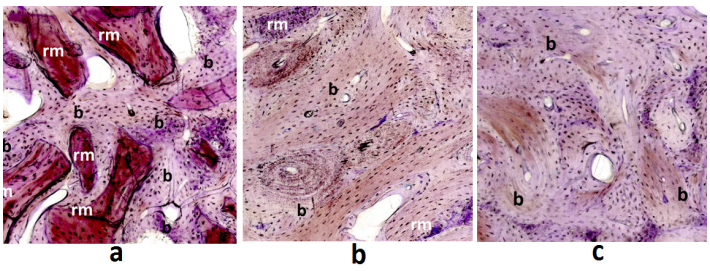


Figure 2: Histological images of healing bone defects 8 weeks after surgery (a) Bio-Oss, (b) β-TCP and (c) Control defects. "**rm**" shows remnants of bone regeneration material (Bio-Oss or β-TCP) at different stage of resorption; "**b**" indicates bone tissue at different stage of maturations.

Histometry assessment outcome

The percentage of defect occupied by bone and residues of BS

The assessment of defect showed significant higher percentage areas occupied by bone tissue at 8 weeks compared to 4 weeks post treatment among beta tricalciumphosphate and control groups, while the amount of bone in Bio Oss group remained relatively constant between 4- and 8-weeks post treatment (Figure 3 and

Table 1).

Table 1: The percentage areas occupied by bone and Residual regeneration materials according to Guided Bone Regeneration Modes at 4th and 8th weeks after surgery.

Type of bone regeneration materials and time	4 Weeks		8 Weeks		
Percentage area occupied by bone	***Mean***		***Mean***		***P Value***
Bio Oss	40.5242	.059	40.5441	.061	.817
Beta Tricalciumphosphate	48.024	.064	50.024	.060	.000
Control	34.015	0.61	45.015	0.58	.000
Percentage area occupied by residual of bone regeneration material	***Mean***		***Mean***		***P Value***
Bio Oss	30.502	.037	27.4720	.035	.000
Beta Tricalciumphosphate	31.4020	.035	20.6020	.041	.000

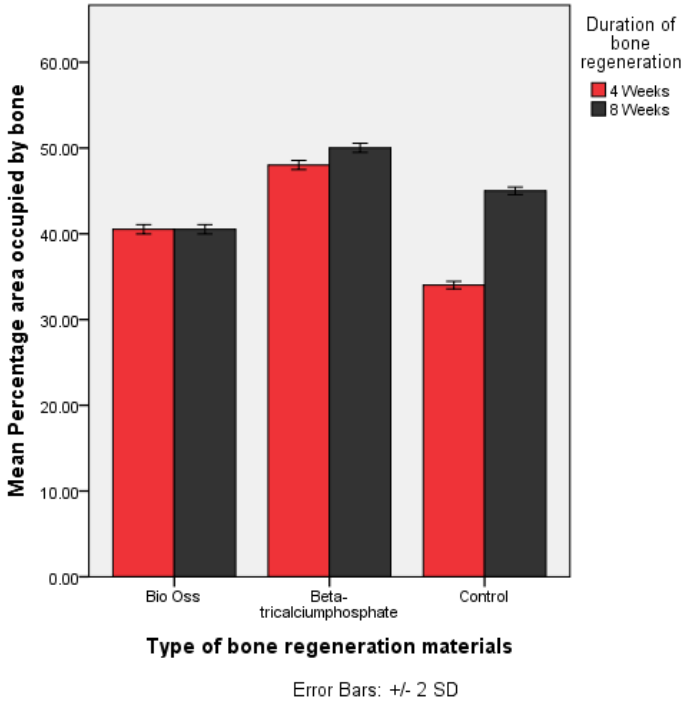
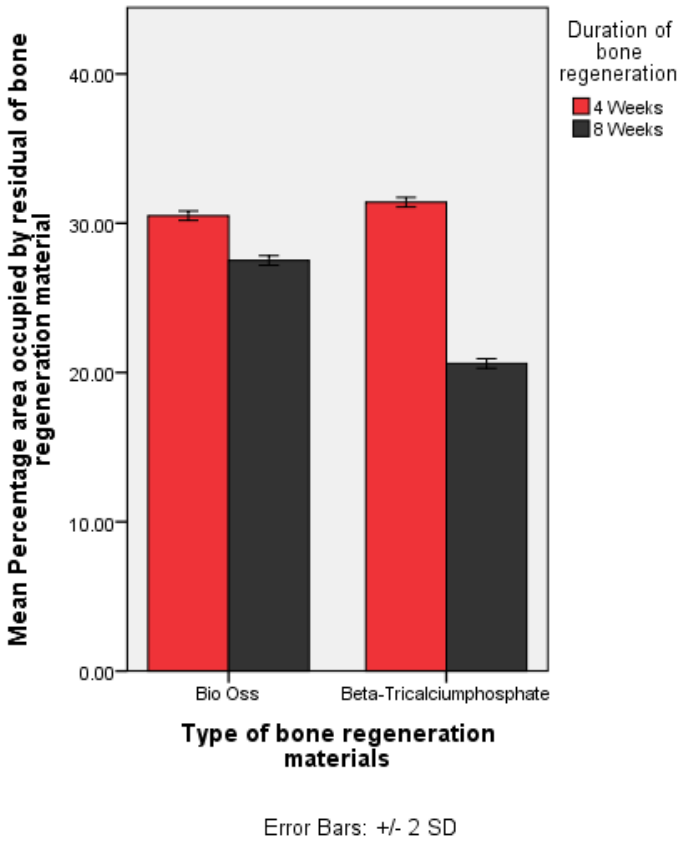


Figure 3: Bar chart displaying the percentage area occupied by bone tissue according to BR modes at four and eight months post GBR.

Regarding residual of BSs, both experimental (B-TCP and Bio Oss) groups showed significant lower percentage at 8th week compared with 4th week (Figure 4 and Table 1).

Across the regeneration mode (B-TCP, Bio Oss and control), there were statistically significant differences between groups at 4th and 8th week post treatment, except for experimental (B-TCP and Bio Oss) groups which recorded statistically insignificant difference (P = 0.78) on amount of BS residuals at 4th week. The Beta tricalciumphosphate group had the highest bone percentage at 4th and 8th weeks, while the lowest percentage of bone at 4th and 8th week was observed in control and Bio oss groups respectively (Table 1). At 4th week, the percentage of BS was relatively similar in both β-TCP, and Bio Oss; however, β-TCP showed greater reduction (10.6%) than Bio oss (3.03%): Thus Bio Oss group had



significantly higher BS residual at 8th week than β-TCP (Table 1).

Figure 4: Bar chart displaying the percentage area occupied by residue of bone substitutes according to BR modes at four and eight months post GBR.

Discussion

In the current study, the clinical examinations of surgical area showed good healing progress without significant differences between regeneration modes. This is in agreement with previous studies [17] supporting the effective use of xenographs and allographs in bone regeneration. The percentage area assessed at 4th and 8th week post surgery, showed significant increase in bone formation with time in all regeneration modes. It is a known phenomenon [7,9,15,16] that regeneration materials undergo resorption with time, though at different rates. Hence, it is expected to have more bone tissue with time elapses post surgery. The results of residual resorption leave more space for new bone formation. The differences in amount of bone between regeneration modes can be attributed to the differences in resorption rates of BS.

Many studies have reported higher rate of β-TCP resorption compared to Bio Oss; thus, the higher bone percentage observed in β-TCP can be explained by their different rates of resorption Although there was an increase in bone percentage at 8th week in Bio oss group, the BS percentage was relatively constant at 4th and 8th week post-surgery.

Some researchers have reported presence of Bio oss residuals in surgical areas up to one year post operation [7,9]: Therefore, the

lack of differences between 4th and 8th weeks assessment in the current study may be due to the slower rate of resorption of Bio Oss materials.

Beta tricalciumphosphate had the highest bone percentage at 4th and 8th weeks. The finding is in line with previous studies' reports. Beta-tricalciumphosphate is among the regeneration materials with high facilitation of bone regeneration and the material is reported to outperform or equals the gold standard (autotransplant) by some studies [18] the lowest percentage of bone at 4th week was observed in control group. Similar findings have been reported by previous studies [19]. However at 8th week Bio oss groups had the lowest percentage of new bone formation. The fact that Bio Oss remains on surgical site for log time may be the reason for the group to register smaller percentage of bone compared to β-TCP and control groups. The percentage of BS was relatively similar in both β-TCP, and Bio Oss at 4th week; however, β-TCP showed greater reduction (10.6%) than Bio oss (3.03%). Thus, Bio Oss group had significantly higher BS residual at 8th week than β-TCP. The reported relatively higher rate of resorption of β-TCP [20,21] is the most probable reason for the observation.

Conclusions

The type of regeneration mode had no effect on the local clinical features assessed.

The percentage area occupied by bone increased with time except in Bio Oss group which showed no difference between 4th and 8th weeks. The β-TCP group had the highest bone percentage at 4th and 8th weeks; while the control and Bio oss groups registered the lowest percentage of bone at 4th and 8th weeks respectively.

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