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**Original Research Article**

# Calvarial bone defect regeneration using beta-tricalcium phosphate: a translational research study in rat animal model

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## ABSTRACT

**Background:** Guided bone regeneration (GBR) using osteoconductive graft materials has been used for osseous defect healing. The aim of this translational research study was to design and test a critical size calvarial defect (CSD) model in rats, to test GBR with beta-tricalcium phosphate (beta-TCP), using histology and micro computed tomography (micro-CT) assessment.
**Methods:** Female Wistar albino rats (n=10) weighing 300 grams and aged 6-weeks were used and full thickness CSD were created in calvaria following exposure under general anesthesia. CSD were randomly divided into two groups for treatment, based on defect filling material: control group (no graft placed in defect; n=5); and beta-TCP group (defect grafted with beta-TCP; n=5). Both defects were covered with collagen membrane. After 8-weeks of healing the animals were sacrificed and calvarial specimens were subjected to micro-CT and histological assessment.
**Results:** Based on micro-CT the new bone volume (NBV) was significantly higher in beta-TCP group (3.48±0.27 $mm^3$; $p<0.05$), than control group (2.88±0.33 $mm^3$). Similarly, new bone mineral density (NBMD) was significantly higher in beta-TCP group (0.426±0.018 $g/mm^3$; $p<0.01$), than control group (0.243±0.015 $g/mm^3$). Histology revealed greater new bone bridging the entire defect with interspersed graft particles in the beta-TCP group.
**Conclusions:** Within the limitations of the present study, GBR of rat calvarial CSD with beta-TCP and collagen membrane, results in significantly higher NBV and NBMD, and is a reliable and reproducible translational research model.

**Keywords:** Guided bone regeneration, Critical-size defect, Beta-tricalcium phosphate, Micro-computed tomography

## INTRODUCTION

One of the greatest challenges faced by oral and maxillofacial surgeons is the reconstruction of craniofacial bone lost due to trauma, pathology and ablative surgery.[1] Some of the earliest methods for reconstruction of craniofacial bone defects included reconstruction plates, meshes and free non-vascularized bone grafts. This was followed by the use of vascularized free bone grafts and more recently tissue engineered options using bone substitute materials (BSM) along with adjuncts such as growth factors and stem cells.[2] The use of tissue engineering based regenerative techniques such as guided tissue regeneration (GTR) have been used in the field of

dentistry and periodontology since 1988.[3] Basically, GTR involves placement of a barrier membrane around periodontal bony defects, with the aim of preventing fibroblasts and connective tissue migration into the defect and aiding in unhindered bone healing.[3,4] However, the absence of any bone substitute within the defects resulted in the GTR technique providing only favorable soft tissue outcomes, thereby leading to the development of guided bone regeneration (GBR).[5] Conventional GBR protocol includes placement of a BSM within the bony defect, which is then covered by barrier membrane. The GBR site is allowed to heal for a period of 6–9 months, depending on the size, anatomic site and post-treatment functional requirements.[6-8]

Over the last three decades, GBR has evolved as a reliable and reproducible clinical procedure for regeneration and healing of osseous defects in the dental and craniofacial region.[9] Based on a systematic review, Chatelet et al reported similar clinical outcomes with GBR and autologous bone block grafting for pre-implant augmentation of dentoalveolar bone defects.[10] It was further noted that the overall implant survival rate was greater than 95% for both the aforementioned bone augmentation techniques.[10] An advantage of GBR over autologous bone grafting is the ability to use particulate BSM from a variety of sources (allogenic, xenogenic and alloplastic), thereby negating the need for graft harvesting surgery and its associated morbidity.[2,10] Additionally, using an alloplastic BSM such as beta-tricalcium phosphate (beta-TCP) or hydroxyapatite (HA) in GBR eliminates any risk of hypersensitivity or disease transmission which may be associated with allografts and xenografts.[9,11]

TCP is a bioceramic used as an osteoconductive BSM, for a very long time, in the field of orthopedics for filling bone defects either alone or in combination with autograft bone and also for vertebral fusion.[12-15] In its purest form TCP is biphasic and exists in a macroporous alpha form and microporous beta form.[16] Owing to its fine geometry and microarchitecture, particulate beta-TCP has increased surface area and enhanced wettability. Therefore, beta-TCP is easily bioresorbed by osteoclasts when grafted in a bone defect site, enabling "centripetal creeping substitution" of new bone matrix from the defect edges.[17-19] Histological studies, of healing bone defects grafted with beta-TCP, have shown osteoclastic resorption, osteoblastic migration, osteoid formation and neovascularization by 14 days and defect fill through integration of beta-TCP particles in new bone matrix by 12-16 weeks.[8,17,18,20,21] Nevertheless, these studies provide no information as to when actual remineralization of the healing defect occurs and how well a BSM such as beta-TCP contributes to the remineralization process. Considering the fact that beta-TCP undergoes accelerated bioresorption, leading to early and enhanced availability of calcium and phosphorus ions, it would be interesting to study remineralization of osseous defects treated with beta-TCP. In this context, micro computed tomography (micro-CT) based animal models for bone regeneration have become a crucial translational research tool, as they allow assessment of regenerated bone at varying time points from graft placement until healing, something not plausible in the actual clinical scenario.[1,9]

Based on preexisting evidences available in the literature, the present study was envisaged with the intention of designing a critical sized calvarial defect model in rats for evaluating GBR using beta-TCP.[22] Moreover, this study also surmised that evaluating GBR at an early stage using not only histology, but also micro-CT assessment would provide insights about the remineralization potential of beta-TCP. Therefore, the primary aim of this translational research study was to design and test a critical size calvarial defect model in rats, to test GBR with beta-TCP, using histology and micro-CT assessment. The study further aimed to quantify, using micro-CT, the degree of bone formation and mineralization after 8 weeks of defect healing in terms of newly formed bone volume and its mineral density.

## METHODS

Following institutional ethical approval, the present study was conducted in accordance with NIH-guidelines for care and use of laboratory animals (NIH publication #85-23 Rev.1985). Ten female Wistar albino rats (n=10), weighing approximately 300 grams and aged about 8–10 weeks, were included in the study and were housed under veterinary supervision, in separate plastic cages. The animals were acclimatized prior to the experiment through 12 hourly light and dark cycles, in a controlled laboratory environment, for a period of 10 days and had free access to food and water throughout the study period.

### *Anesthesia and surgical protocol*

General anesthesia was administered under veterinary supervision through intraperitoneal injection of xylazine (6-9 mg/kg, xylaxin 20 mg/ml injection, Pharmika India Pvt. Ltd., Delhi, India) and ketamine (60-80 mg/kg, ketaset 100 mg/ml, Zoetis, Parsipanny, NJ, USA). General anesthesia was confirmed when the animal became unconscious and when a blunted or absent corneal reflex was elicited. Afterwards, the skin over the scalp was shaved and disinfected with povidone iodine solution (7.5%). A mid sagittal incision extending from the frontal region to the external occipital protuberance was marked and full-thickness incision was made using a no.15 Bard-Parker surgical knife. The skin and the periosteum were reflected to expose the calvarial bone. After identification of the sagittal, coronal and lambdoid sutures a calvarial critical size defect (CSD) was created on the left parietal bone using a bone trephine (KLS Martin and Mondeal Medical Systems, Tuttlingen, Germany. 4.2 mm external diameter and 3.3 mm internal diameter) on a rotary hand piece under saline irrigation. Care was exercised to avoid injury to the dura mater while preparation of the CSD. The animals were randomly allotted to one of two groups

depending the bone regeneration method used in the defect (Figure 1).

Group 1 (control group) (n=5) – CSD was allowed to fill with blood coagulum and covered by collagen membrane (CM) (Periocol-GTR, Eucare Pharmaceuticals Pvt. Ltd., Chennai, Tamil Nadu, India).

Group 2 (test group – β-TCP + CM) (n=5) – CSD was filled with a mixture of β-TCP (Sybograf-T, Eucare Pharmaceuticals Pvt. Ltd., Chennai, Tamil Nadu, India) and normal saline, and covered by CM (Periocol-GTR, Eucare Pharmaceuticals Pvt. Ltd., Chennai, Tamil Nadu, India).

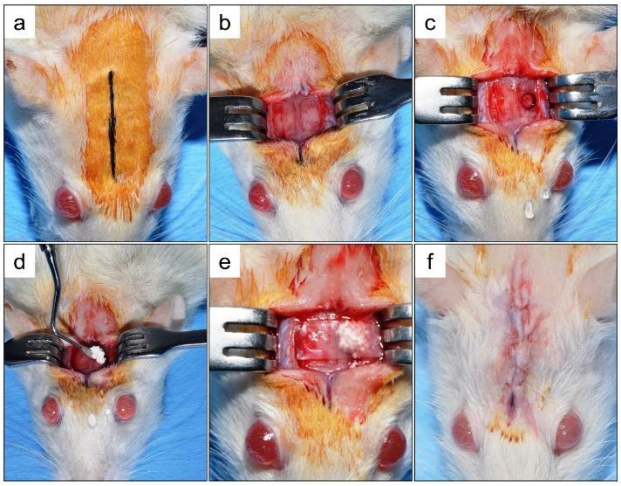


**Figure 1: Surgical procedure (a) marking of midline skin incision on the scalp; (b) exposure of the parietal calvarial bone; (c) critical size defect created using a trephine on the left parietal calvarial bone; (d) placement of beta-tricalcium phosphate in the critical size calvarial defect; (e) coverage of the grafted defect with a collagen barrier membrane; and (f) primary closure of the surgical site.**

Following placement of bio-materials in the CSD, the surgical site was closed primarily using 4-0 resorbable sutures (ethicon coated vicryl, Johnson and Johnson Pvt. Ltd., Mumbai, Maharashtra, India). Throughout the surgical procedure the heart rate and breathing of the animal were monitored by a veterinarian. Post-operatively the surgical site was disinfected and a single dose of amoxicillin trihydrate (30 mg/kg) was administered to all the animals.

***Study animal sacrifice, radiographic and histological evaluation***

Eight weeks after the surgical procedure, all animals were sacrificed by placing them in a closed ether fumigation chamber for 5 minutes. After euthanasia the entire skull was harvested along with the periosteum and excluding the soft-tissue envelope. The harvested specimen was fixed in 10% neutral buffered formaldehyde solution. The formalin fixed specimens were radiographically analyzed in a "high resolution in vitro micro-CT scanner" (Skyscan 1172, Skyscan, Kontich, Belgium). The parameters for scanning for all the specimens were uniformly set at 65kV/385 µA/1 mm aluminium filtration. Calibrations for Hounsfield unit (HU) and bone mineral density were applied to the acquired data based on µCT scan data of calcium phantoms of pre-determined mineral density scanned using similar parameters. The entire CSD was considered as the region of interest. The volume of new bone in each section were calculated by multiplying the new bone area and slice thickness. The new bone volume in each section was summed up to provide total volume of newly formed bone (NBV). Similarly, mineral density of the new bone (NBMD) was obtained by calculating the average of NBMD in all scan sections.

After micro-CT scanning, the parietal bone encompassing the CSD, along with the superficial periosteum and cranial dura, was separated from the skull specimens. These calvarial bone specimens were decalcified in 0.5M EDTA solution at pH 8 for 4 weeks. Decalcified specimens were embedded in paraffin and 4 µm sections were prepared with an orientation paralleling the sagittal suture before being mounted on slides and stained with hematoxylin and eosin for qualitative histological analysis. The histological sections were viewed under light microscopy (OLYMPUS CX41, Olympus Corporation, Tokyo, Japan) at x4 and x10 magnifications. Images of the histological sections were obtained using a live view digital SLR camera (Olympus E330, Olympus Corporation, Tokyo, Japan).

***Statistical analysis***

The quantitative results of micro-CT (NBV and NBMD) were analyzed statistically using statistical package for the social sciences (SPSS) software program (version 18, IBM Statistics, Chicago, IL, USA). Mean values of the above quantitative variables were compared using t-test, assuming 95% significance level, i.e. a p-value <0.05 was considered statistically significant.

## RESULTS

***New bone volume and mineral density***

Based on micro-CT findings, there was new bone formed within the CSD in both control and beta-TCP groups. While there was no complete regeneration of the defect in the control group, the defect was filled with new bone and remaining beta-TCP particles were interspersed within the new bone matrix. Statistically, NBV in the control group (2.88±0.33 $mm^3$) was significantly lower ($p<0.05$) than the NBV in the beta-TCP group (3.48±0.27 $mm^3$) (mean difference - 0.6, 95% C.I.=0.1603 to 1.0397, p=0.0137). Comparing the mineral densities of the new bone in the control and beta-TCP groups, it was found that the NBMD was significantly higher ($p<0.01$) in the beta-TCP group (0.426±0.018 $g/mm^3$), than in the control group

(0.243±0.015 g/mm$^3$) (mean difference – 0.1830, 95% C.I. - 0.1588 to 0.2072, p=0.0028) (Figures 2 and 3).

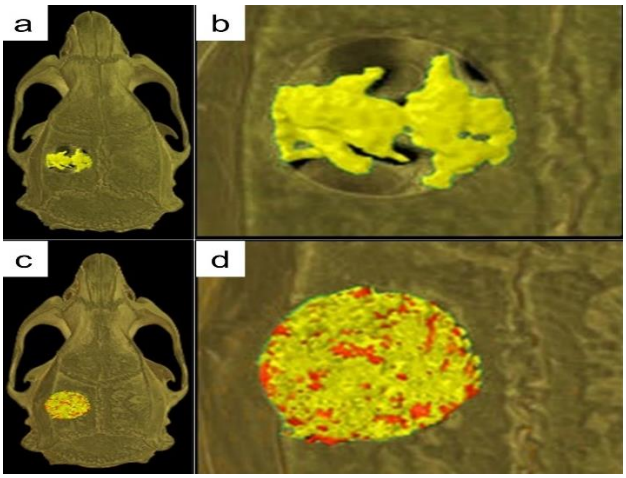


**Figure 2: Micro CT imaging of the critical size calvarial defect in the left parietal bone, in the control group- (a) micro CT image of the entire skull showing new bone formation (yellow color) within the defect; (b) close-up micro-CT image of the defect showing new bone formation (yellow color) from the edges of the defect and incomplete bone regeneration within the defect; (c) micro CT image of the entire skull showing new bone formation (yellow color) within the defect and interspersed beta-tricalcium phosphate particles (orange color); and (d) close-up micro-CT image of the defect showing new bone formation (yellow color) resulting in complete bone regeneration of the defect along with the interspersed beta-tricalcium phosphate particles (orange color).**

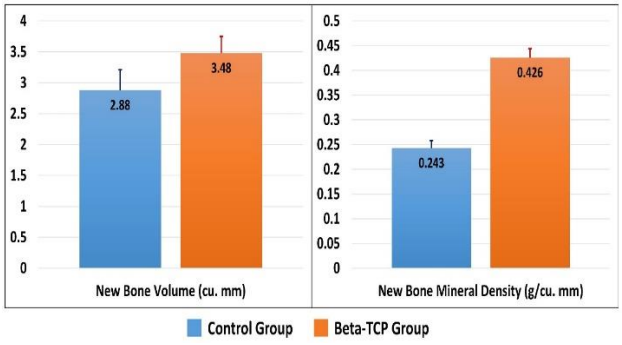


**Figure 3: Bar graphs with standard error bars showing the quantitative values of new bone volume and new bone mineral density, based on micro computed tomography imaging, in the control and beta-tricalcium phosphate groups.**

### *Histological findings*

Histological analysis of the specimens in both groups revealed the presence of new bone within the CSD, evidenced by the presence of unorganized lamellar matrix with irregularly placed osteocytes and blood vessels. Light microscopic analysis of the control group specimens revealed new bone only at the edges of the defect along with fibrous connective tissue bridging the entire defect. On the contrary, histological analysis of the specimens in the beta-TCP group showed new bone not only in the edges of the defect, but also bridging the defect center. A layer of connective tissue was seen covering the new bone and extending up to the periosteum on the edges of the defect. Furthermore, in the beta-TCP group, the remnant graft particles were seen cranial to the newly formed bone as a granular crystalline material surrounded by inflammatory infiltrate. In both groups, collagen barrier membrane remnants were seen as a homogeneous acellular acidophilic material (Figure 4).

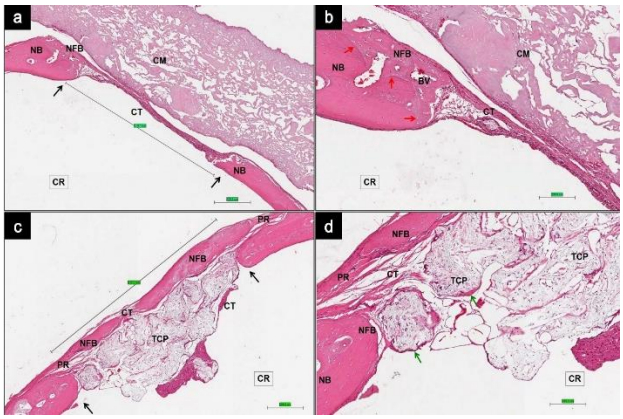


**Figure 4: Histological assessment: (a) control group (HE original magnification x4) – showing edges of the defect (black arrows) bridged by fibrous CT. NFB is seen close to the edge of the defect, adjoining the NB. CM remnants are seen as an acellular acidophilic layer, superficial to the defect (CR side of the defect); (b) control group (HE original magnification x10) – showing NFB with irregular lamellae, osteocytes and BV and fibrous CT extending from the NFB towards the center of the defect. The interface between the NB and NFB is clearly discernible (red arrows); (c) beta-TCP group (HE original magnification x4) – showing edges of the defect (black arrows) bridged by a continuous layer of PR, NFB, and fibrous CT. CR to the layer of NFB, beta-TCP remnants are seen as a layer of granular crystalline material; (d) beta-TCP group (HE original magnification x10) – showing NB with concentric lamellar at the edge of the defect and NFB with irregular lamellae and osteocytes formed towards the center of the defect, beta-TCP remnants are seen encapsulated by infiltrate of inflammatory cells (green arrows), on the CR aspect of the defect.** Connective tissue (CT), new bone (NFB), native bone (NB), collagen membrane (CM), blood vessels (BV), periosteum (PR), cranial (CR).

## DISCUSSION

The findings from this study revealed new bone formation within the defect in both control and beta-TCP groups after 8-weeks of bone grafting. Both, micro-CT and histological analysis revealed new bone formation which did not

completely fill the defect, albeit in the beta-TCP group near complete defect fill was achieved through new bone interspersed with graft particles (Figure 2). The concept of critical size defect is imperative for testing bone regeneration, wherein a defect is considered critical when it does not heal spontaneously within the study endpoint or in the life time of the animal.[9,22,23] Bone is naturally bestowed with the ability to heal by formation of new bone, except when a defect is greater than a particular dimension. For rat models, a calvarial CSD is defined as any full thickness defect greater than 3-4 mm in diameter, and such kind of defects have not been shown to heal even after 12-16 weeks of healing.[22] In the current study we employed a full thickness defect measuring 4.2 mm in diameter and found no spontaneous healing or defect fill after 8 weeks, as evidenced in the control group (Figure 2). However, the fact that the use of beta-TCP led to near total defect fill, reiterates the advantage of grafting bone defect sites for early healing and functional rehabilitation.[1,9] Moreover, the choice of animal model for the present study was based on the versatility of the rat calvarial CSD, which is an easily approachable, reproducible, and orthotopic site for studying bone tissue engineering, and allows several modalities of analysis.[22] While the use of micro-CT has routinely been reported in the literature for studying bone regeneration, the present study used both micro-CT and histology to have a reliable comparison of the outcomes.[24] In effect, the use of micro-CT in this study enabled both quantitative and qualitative evaluation of new bone in terms of its volume and mineral density, which would not have been possible with conventional histological assessment.

Although several authors have reported the successful clinical outcomes following use of β-TCP for socket preservation, alveolar bone grafting and for GBR in periodontal bone defects, the use of animal models helps compare the degree of bone regeneration with respect to time of healing, graft used and adjuncts applied.[25-29] In the current study, placement of beta-TCP in the rat calvarial CSD and covered by collagen membrane resulted in significantly greater new bone formation, as recorded quantitatively by micro-CT and qualitatively through histological assessment (Figures 2-4). The current micro-CT and histology results are comparable to findings reported by Murai et al, who reported new bone formation and mineralization as early as 4-weeks with beta-TCP grafting in rabbit calvarial defects.[30] Additionally, both micro-CT and histological findings of this study showed incorporation of beta-TCP particles within the new bone matrix. This is in coherence to what was documented by Ogose et al, wherein histological findings revealed osteoclastic resorption of beta-TCP by 2-weeks and new bone deposition in close proximity to the graft particles by 4-weeks.[17] Yet another important aspect of studying bone regeneration, is to evaluate the degree of mineralization as it directly correlates with the ability of the bone to withstand functional loads such as dental implants.[9] GBR of rat calvarial CSD with beta-TCP, in the present study, resulted in significantly higher NBMD by 8 weeks (Figure 3). Interestingly, the degradation characteristics of beta-TCP could be the reason behind the aforementioned improvement in NBMD. Beta-TCP particles undergo rapid ceramolysis from the grafted site within 4-weeks, following which it becomes progressively slower and thereby helping in the mineralization of new bone matrix with an initial burst phase and delayed sustenance phase.[16] These quantitative findings pertaining to the volume and mineral density of newly formed bone within the defect emphasize the translational value of the current study for its clinical applications in craniofacial osseous defect reconstruction, using beta-TCP.

In addition to its quantitative value, micro-CT provided qualitative information which aided as a visual representation of bone defect healing. This was further reiterated through the histological findings confirming the presence of newly formed bone with osteocytes in the lamellae and neovascularization, in both the control and beta-TCP groups (Figure 4). Moreover, the histological images revealed the presence of remnant collagen barrier membrane, indicating unhindered bone formation within the defect, even after 8-weeks of defect healing. In addition to preventing ingrowth of fibrous tissue into healing bony defects, collagen membrane is known to stimulate hemostasis, promote chemotaxis of fibroblasts and osteoblasts, and act as a scaffold for neovascularization.[31-33]

Based on an in vivo study, it has further been postulated that GBR using collagen membrane and beta-TCP enables retention of the graft material within the defect, thereby promoting early new bone formation and mineralization.[9] Although the present study provided quantitative and qualitative, micro-CT and histological data regarding GBR of rat calvarial CSD with beta-TCP, it was only obtained at a single point in time, after 8-weeks of defect healing. The use of longitudinal data collection shall further enhance our understanding of the biology behind osseous defect healing with alloplastic, osteoconductive grafts like beta-TCP. Furthermore, the addition of a positive control group, wherein CSD healing with autologous bone graft was used as a gold standard of comparison, would have enhanced the robustness of the presented outcome data.

## CONCLUSION

Within the limitations of the present study, GBR of rat calvarial CSD with beta-TCP and collagen membrane, results in significantly higher new bone formation and greater mineral density, than when the CSD is not grafted at all. The used of a translational rat calvarial CSD model to study GBR using micro-CT and histology is a reliable and reproducible technique, the findings of which shall form the basis for understanding osseous defect regeneration and planning large scale clinical trials.

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## REFERENCES


1. Badwelan M, Alkindi M, Alghamdi O, Ahmed A, Ramalingam S, Alrahlah A. Bone Regeneration Using PEVAV/β-Tricalcium Phosphate Composite Scaffolds in Standardized Calvarial Defects: Micro-Computed Tomographic Experiment in Rats. Materials (Basel, Switzerland). 2021;14(9).
2. Alkindi M, Ramalingam S, Alghamdi O, Alomran OM, Binsalah MA, Badwelan M. Guided bone regeneration with osteoconductive grafts and PDGF: A tissue engineering option for segmental bone defect reconstruction. J Appl Biomaterials Functional Materials. 2021;19:2280800020987405.
3. Dahlin C, Linde A, Gottlow J, Nyman S. Healing of bone defects by guided tissue regeneration. Plast Reconstr Surg. 1988;81(5):672-6.
4. Dahlin C, Sennerby L, Lekholm U, Linde A, Nyman S. Generation of new bone around titanium implants using a membrane technique: an experimental study in rabbits. Int J Oral Maxillofac Implants. 1989;4(1):19-25.
5. Colangelo P, Piattelli A, Barrucci S, Trisi P, Formisano G, Caiazza S. Bone regeneration guided by resorbable collagen membranes in rabbits: a pilot study. Implant Dent. 1993;2(2):101-5.
6. Hammerle CH, Jung RE, Yaman D, Lang NP. Ridge augmentation by applying bioresorbable membranes and deproteinized bovine bone mineral: a report of twelve consecutive cases. Clin Oral Implants Res. 2008;19(1):19-25.
7. Talreja PS, Gayathri GV, Mehta DS. Treatment of an early failing implant by guided bone regeneration using resorbable collagen membrane and bioactive glass. J Indian Soc Periodontol. 2013;17(1):131-6.
8. Koyama Y, Kikuchi M, Edamura K, et al. Reconstruction of bone fenestration on mandiblar by the guided bone regeneration methods with beta-TCP/PLGC membranes. J Nanosci Nanotechnol. 2007;7(3):859-61.
9. Ramalingam S, Al-Rasheed A, ArRejaie A, Nooh N, Al-Kindi M, Al-Hezaimi K. Guided bone regeneration in standardized calvarial defects using beta-tricalcium phosphate and collagen membrane: a real-time in vivo micro-computed tomographic experiment in rats. Odontology. 2016;104(2):199-210.
10. Chatelet M, Afota F, Savoldelli C. Review of bone graft and implant survival rate : A comparison between autogenous bone block versus guided bone regeneration. J Stomatol Oral Maxillofac Surg. 2022;123(2):222-7.
11. Ramalingam S, Alrayyes YF, Almutairi KB, Bello IO. Lateral Periodontal Cyst Treated with Enucleation and Guided Bone Regeneration: A Report of a Case and a Review of Pertinent Literature. Case Rep Dentistry. 2019;4591019.
12. Ayoub MA, El-Rosasy MA. Hybrid grafting of post-traumatic bone defects using beta-tricalcium phosphate and demineralized bone matrix. Eur J Orthop Surg Traumatol. 2013;24(5):663-70.
13. Dai LY, Jiang LS. Anterior cervical fusion with interbody cage containing beta-tricalcium phosphate augmented with plate fixation: a prospective randomized study with 2-year follow-up. Eur Spine J. 2008;17(5):698-705.
14. Epstein NE. Efficacy of posterior cervical fusions utilizing an artificial bone graft expander, beta tricalcium phosphate. Surg Neurol Int. 2011;2:15.
15. Hirata M, Murata H, Takeshita H, Sakabe T, Tsuji Y, Kubo T. Use of purified beta-tricalcium phosphate for filling defects after curettage of benign bone tumours. Int Orthop. 2006;30(6):510-3.
16. Wiltfang J, Merten HA, Schlegel KA, et al. Degradation characteristics of alpha and beta tri-calcium-phosphate (TCP) in minipigs. J Biomed Mater Res. 2002;63(2):115-21.
17. Ogose A, Kondo N, Umezu H, et al. Histological assessment in grafts of highly purified beta-tricalcium phosphate (OSferion) in human bones. Biomaterials. 2006;27(8):1542-9.
18. Zerbo IR, Bronckers AL, de Lange G, Burger EH. Localisation of osteogenic and osteoclastic cells in porous beta-tricalcium phosphate particles used for human maxillary sinus floor elevation. Biomaterials. 2005;26(12):1445-51.
19. Zerbo IR, Zijderveld SA, de Boer A, et al. Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clin Oral Implants Res. 2004;15(6):724-32.
20. Walsh WR, Vizesi F, Michael D, et al. Beta-TCP bone graft substitutes in a bilateral rabbit tibial defect model. Biomaterials. 2008;29(3):266-71.
21. Chopra PM, Johnson M, Nagy TR, Lemons JE. Micro-computed tomographic analysis of bone healing subsequent to graft placement. J Biomed Mater Res B Appl Biomater. 2009;88(2):611-8.
22. Spicer PP, Kretlow JD, Young S, Jansen JA, Kasper FK, Mikos AG. Evaluation of bone regeneration using the rat critical size calvarial defect. Nat Protoc. 2012;7(10):1918-29.
23. Badwelan M, Alkindi M, Ramalingam S, Nooh N, Al Hezaimi K. The Efficacy of Recombinant Platelet-Derived Growth Factor on Beta-Tricalcium Phosphate to Regenerate Femoral Critical Sized Segmental Defects: Longitudinal In Vivo Micro-CT Study in a Rat Model. J Invest Surg. 2020;33(5):476-88.
24. Kochi G, Sato S, Ebihara H, Hirano J, Arai Y, Ito K. A comparative study of microfocus CT and histomorphometry in the evaluation of bone augmentation in rat calvarium. J Oral Sci. 2010;52(2):203-11.
25. Brkovic BM, Prasad HS, Konandreas G, et al. Simple preservation of a maxillary extraction socket using beta-tricalcium phosphate with type I collagen:

preliminary clinical and histomorphometric observations. J Can Dent Assoc. 2008;74(6):523-8.
26. Weijs WL, Siebers TJ, Kuijpers-Jagtman AM, Berge SJ, Meijer GJ, Borstlap WA. Early secondary closure of alveolar clefts with mandibular symphyseal bone grafts and beta-tri calcium phosphate (beta-TCP). Int J Oral Maxillofac Surg. 2010;39(5):424-9.
27. Kaushick BT, Jayakumar ND, Padmalatha O, Varghese S. Treatment of human periodontal infrabony defects with hydroxyapatite + beta tricalcium phosphate bone graft alone and in combination with platelet rich plasma: a randomized clinical trial. Indian J Dent Res. 2011;22(4):505-10.
28. Kishore DT, Bandiwadekar T, Padma R, Debunath S, Profulla, Reddy A. Evaluation of relative efficacy of beta-tricalcium phosphate with and without type I resorbable collagen membrane in periodontal infrabony defects: a clinical and radiographic study. J Contemp Dent Pract. 2013;14(2):193-201.
29. Saini N, Sikri P, Gupta H. Evaluation of the relative efficacy of autologous platelet-rich plasma in combination with beta-tricalcium phosphate alloplast versus an alloplast alone in the treatment of human periodontal infrabony defects: a clinical and radiological study. Indian J Dent Res. 2011;22(1):107-15.
30. Murai M, Sato S, Fukase Y, Yamada Y, Komiyama K, Ito K. Effects of different sizes of beta-tricalcium phosphate particles on bone augmentation within a titanium cap in rabbit calvarium. Dent Mater J. 2006;25(1):87-96.
31. Takata T, Wang HL, Miyauchi M. Migration of osteoblastic cells on various guided bone regeneration membranes. Clin Oral Implants Res. 2001;12(4):332-8.
32. Wang HL, Carroll MJ. Guided bone regeneration using bone grafts and collagen membranes. Quintessence Int. 2001;32(7):504-15.
33. Prathap S, Hegde S, Kashyap R, Prathap MS, Arunkumar MS. Clinical evaluation of porous hydroxyapatite bone graft (Periobone G) with and without collagen membrane (Periocol) in the treatment of bilateral grade II furcation defects in mandibular first permanent molars. J Indian Soc Periodontol. 2013;17(2):228-34.

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