Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: May 31, 2023

**Citation:** Bali P, Mahesh L, Shukla S, et al., 2023. Histologic comparison of three different alloplastic bone graft substitutes – a split-mouth study. Medical Research Archives, [online] 11(5). https://doi.org/10.18103/mra.v11i5.3925



**DOI:** https://doi.org/10.18103/mra.v11i5.3925

ISSN: 2375-1924

RESEARCH ARTICLE

# Histologic comparison of three different alloplastic bone graft substitutes – a split-mouth study.

**Praful Bali[1], Lanka Mahesh[1], Sagrika Shukla[1]*, Anshi Jain[2]**

[1]Private Practice, New Delhi, India

[2]Reader department of Oral Pathology, ITS Dental College, UP, India

*shukla.sagrika@gmail.com

## Abstract

**Objective:** To histologically compare three different alloplasts and assess bone regeneration

**Study Design:** Bone core of three different bone grafts was procured from a grafted site after a period of 6 months and implants were placed. The bone cores were studied histologically at higher magnification and bone formation was analyzed.

**Results:** All the grafts showed good results clinically, however histologically β-TCP had shown better results and more bone formation as compared to others.

**Conclusion:** Chronologically, Powerbone® shows the maximum formation of mature bone followed by Adbone® and then Novabone®

**Key words:** Bone graft, bone regeneration, Alloplasts, synthetic bone.

## Introduction:

Dental implants have become a common treatment to replace the missing tooth/teeth, however a predictable treatment depends upon many factors, out of which the quality of the bone is important. According to contemporary trends, the ideal characteristics of a bone-substitute material include volume maintenance, pre-specification of the desired anatomical form, support to the periosteum, acceleration of bone remodelling, osteoconductive guidance, carrier function for antibiotics, growth factors or gene therapy approaches or scaffolds for tissue engineering[1-3]. It may be too optimistic to expect that a single grafting material will fulfil all these functions and will be suitable for all indications. Still, a huge variety of bone graft materials are available which can produce predictable results and can be categorized in four groups: (A) autogenous bone graft (B) allografts, from another individual within the same species; (C) xenografts, from another species; or (D) alloplastic, synthetically produced grafts. Each graft has its own merits and demerits and its use primarily depends upon the clinician, still autogenous bone graft is considered the gold standard in bone regeneration procedures due to their inherent osteoinductivity[4]. However, donor site morbidity, unpredictable resorption, limited quantities available, and the need to include additional surgical sites are autografts-related drawbacks that have intensified the search for suitable alternatives[1].

Allografts are sourced from the same species, hence its regenerative potential is majorly donor dependent, the older the batch, the less the regeneration expected from the bone graft[5-6]. Xenografts on the other hand are sourced from animals and provide an unlimited supplies of the material and resorption is very slow[7]. So this latter is a disadvantage where mineralised bone is required. Also, there is risk of transmission of bovine spongiform encephalopathy causing Creutzfeldt-Jakob disease (CJD) in humans, xenograft of porcine (pig) origin can cause Porcine endogenous retroviruses infection in humans, (however no case of any disease transmission has been reported far)[7,8]. Hence, the use of newer materials as bone graft-substitute has increased for bone augmentation procedures to overcome limitations of aforementioned grafts, synthetic bone grafts are available[1]. An alloplast is a inorganic synthetic bone grafting material which gives the manufacturer the freedom to design the graft in such a manner that it is biocompatible and osteoconductive in nature. Thus, its fate is primarily dependent on its chemical composition, structure and physical properties[8]. And that is what we have studies histologically in the study, two different alloplasts, Powerbone® (TCP) and Novabone® both in granules form.

## Material and method:

Patient with decayed tooth or teeth which needed extraction and placement of implants were chosen for the study, wherein a split mouth placement of the graft would be possible. Accordingly, 20 sites were divided into two groups based on the following inclusion and exclusion criteria.

| | | |
|---|---|---|
| Group A | 10 sockets to be grafted | Sites with Novabone® (morsels) bone graft |
| Group B | 10 sockets to be grafted | Sites with Powerbone® (β-TCP) |
| Group C | 10 sockets to be grafted | Sites with Medbone (Adbone® - β-TCP) bone graft. |

INCLUSION CRITERIA: -

- Patients from the age of 20 -80 years of age.
- Having decayed tooth/teeth ready to be extracted and socket grafted in any of the 3 quadrants.
- Compliant patients.

EXCLUSION CRITERIA: -

- Patients with systemic diseases and/or presence of infections contraindicating periodontal surgery.
- Systemic antibiotic therapy in the preceding 3 months.
- Patients on medication known to interfere with periodontal tissue health and healing.
- Pregnant or lactating females.
- Patients with known habit of smoking and tobacco chewing.
- Patients allergic to any product or to any of the medications used in the study.
- Patients with parafunctional habits.

Once the patients participating in the study were enrolled, they signed a consent form to willingly participate in the study and were treated by keeping Helsinki declaration[52] (2019) into consideration. The procedure was conducted under local anesthesia and after tooth extraction, socket grafting was done. In one patient minimum three or more than three sites for socket grafting were considered in any three separate quadrants. In such a manner, 10 sockets were grafted with Novabone® morsels bone graft and 10 with (β-TCP) Powerbone® and rest 10 sockets with Adbone® (β-TCP). Sockets were covered with a CollaPlug (Zimmer, SA.) and 3-0 silk sutures were used to close the sockets. Implants (Bioner) of 4 mm in diameter were placed after 6 months. This is the same time when a core was obtained for histological purpose with the help of trephine with the diameter of 3.2. after the procedure was completed 3-0 silk sutures were again placed if there were more that 2 sites adjoining to each other, wherein a flap was elevated. Post-operatively, Patients were advised to maintain proper oral hygiene for which they were prescribed Clohex-plus mouthwash twice daily and medications were prescribed.

### Histological analysis:

3.2 mm trephine core biopsies were obtained and fixed immediately in neutral buffered formalin solution for 24-48 hours. The specimens were processed after decalcifying in mild decalcifying agent (10% EDTA, pH 7.4). The tissues were processed using standard tissue processing laboratory protocol, clearing and infiltration with paraffin wax. Embedding and tissue block preparation was done with paraffin wax. 4-micron thick sections were stained with Hematoxylin and Eosin stains. The slides thus obtained were viewed in research microscope (Olympus

BX53) and digital images were captured in low and high magnification (Olympus EPL3).

**Results:**

Histological inference of Novabone® bone graft:

As can be seen in the histological images, image (A) at low magnification Shows the presence of Surface epithelium and Thick bundles of collagen fibers with minimum inflammation (figure 1) (B) Shows the presence of calcified material (figure 2). (C) Shows the presence of irregular bony trabeculae with varying stages of mineralization and entrapped osteocytes and presence of giant cells and (figure 3) (D) Under Masson trichrome varying stages of mineralization is highlighted through different stains by mature bone shows more red color whereas immature bone shows more green and entrapped osteocytes. (figure 4)

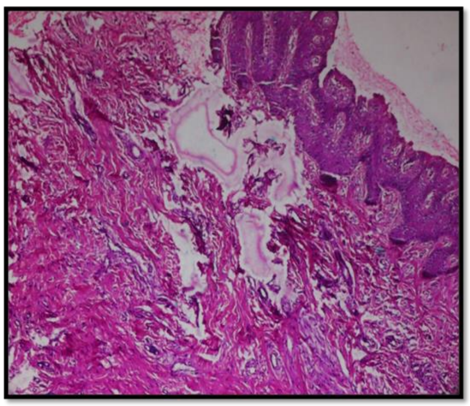

Figure 1: Histological section for Novabone® morsels at low magnification

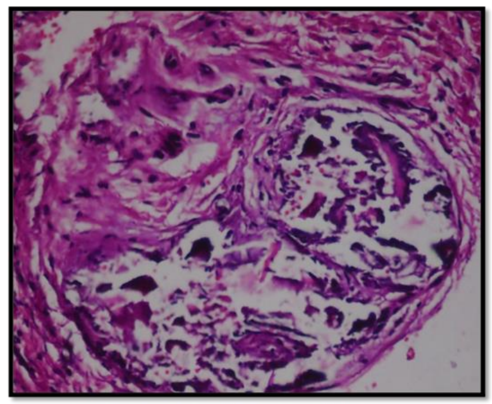

Figure 2: Histology for Novabone® morsels showing the presence of calcified material

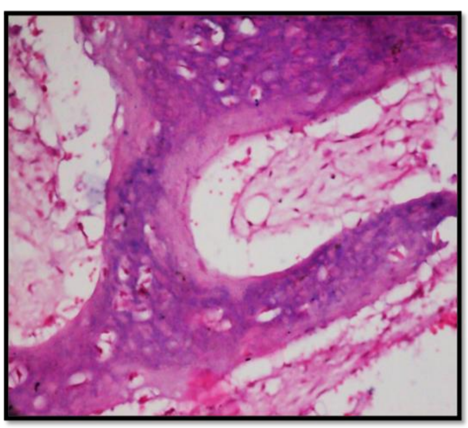

Figure 3: Histology for Novabone® morsels showing varying stages of mineralization

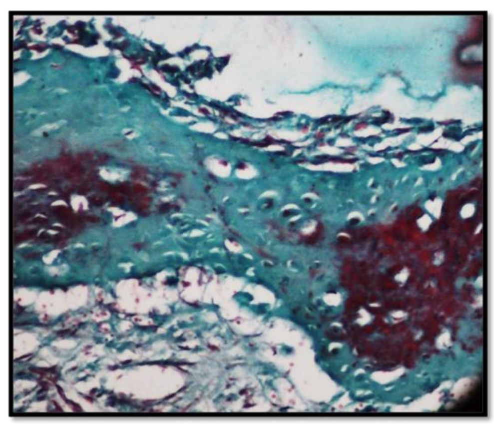

Figure 4: Histology for Novabone® morsels showing mineralization at higher magnification

Histological results for Powerbone® bone graft:

Here the results (A) At low magnification (10x) shows the presence of residual graft material which is intermingled with mature bony trabeculae formation in center and periphery of graft material as well. Along with new irregular bony trabeculae at varying stages of mineralization and entrapped osteocytes is highly appreciated. Inset at 40 x magnification shows new bone formation around the graft material (figure 5). (B) To highlight the presence of residual graft material at bone graft interface special stains were used. Under Masson Trichrome staining section is showing the presence of residual graft material and new bony trabeculae formation depicting in red color around the graft material (yellow color arrow) (figure 6). (C, D) Under Picrosirus Red stain similar picture is highlighted showing red colour shows the presence of mature bone. Minimum amount of inflammation is seen in the section indicating the acceptance of graft material by the body and minimum vascularity is seen.(figure 7 and 8)

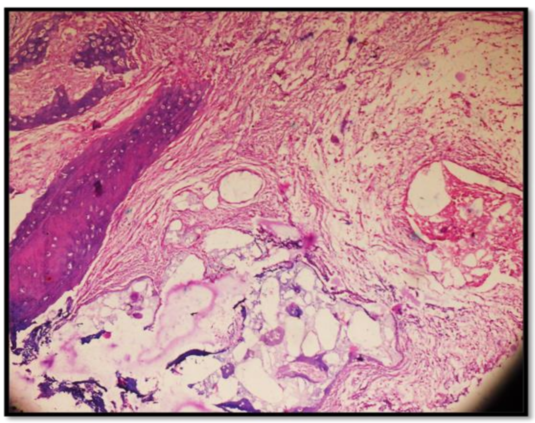

Figure 5: Histology for Powerbone® showing various stages of mineralization at low magnification (10x)

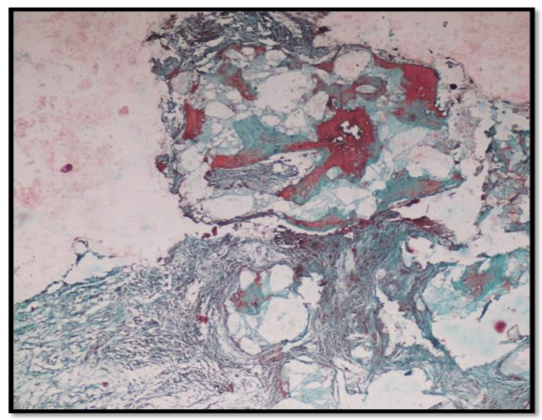

Figure 6: Highlighted histological image of Powerbone® showing bone formation

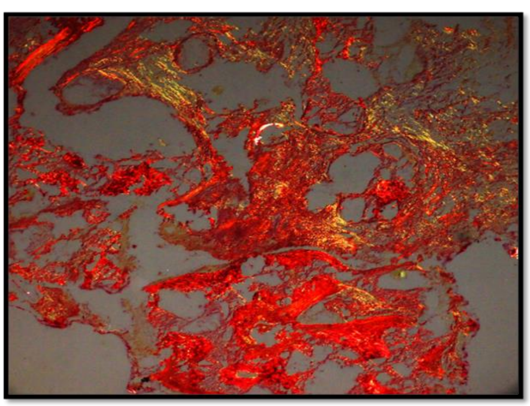

Figure 7: Highlighted histological image of Powerbone® showing presence of mature bone

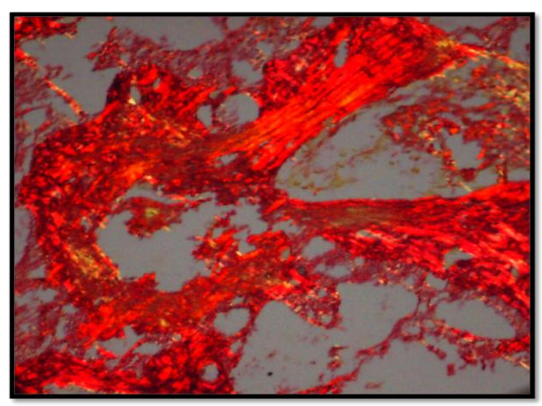

Figure 8: Different histological section of Powerbone® showing presence of mature bone.

Histological results for Adbone® bone graft: Here, (A)At low magnification shows the presence of Irregular bony trabeculae with entrapped osteocytes surrounded by fibrous connective tissue stroma and (figure 9) (B) Under Masson Trichrome Stain red color indicates mature bone (figure 10).

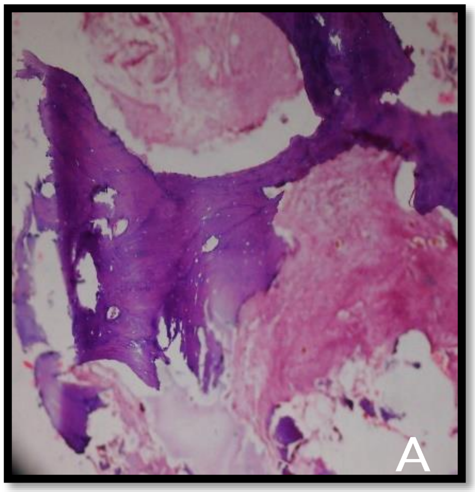


Figure 9: Histological image of Adbone® at low magnification.

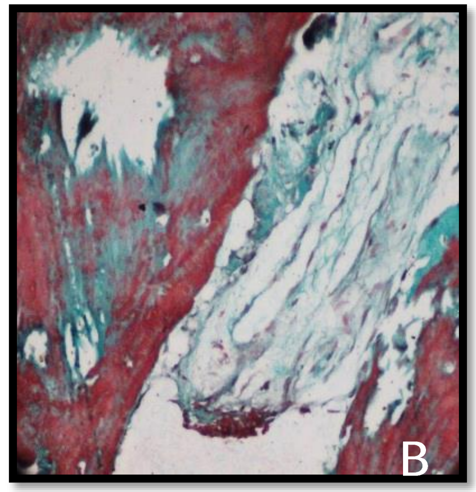


Figure 10: Histological image of Adbone® showing mature bone.

Discussion:

Bone is continuously modelled and remodelled, and reveals a unique potential for regeneration however, this healing capacity of the bone has limitations and bone formation beyond the skeletal envelope require therapeutic intervention and one such classical example is tooth extraction. Time and again various studies have shown that once a tooth is extracted, alveolar bone undergoes resorption horizontally and vertically[9] which makes the use of bone graft paramount for implant placement. The evolution of bone grafts from 1920s, has come a long way in the manner of their procurement and understanding the predictability of results, hence, today, Alloplasts give manufacturer the choice and freedom to provide predictable bone regeneration. Nonetheless, of the choice of the graft material used, bone regeneration should be achievable keeping the properties of the graft into consideration. All the three different synthetic grafts used, claim to form bone in their respective manner and demonstrate an excellent adaptation clinically, thus it becomes difficult to say which amongst all is a better bone graft. But when it comes to histological demonstration, based on bone formation, differentiation becomes easy.

NovaBone® Dental Morsels combines the properties of a xenograft and allograft, it provides macroporosity and ionic release (osteostimulation) for bone formation at the site. It is a crystalline composite composed of oxides of silicon, calcium, sodium, and phosphorous in a silica base[10]. These properties are based on intimate contact with the bone which then forms Si-rich layer, a crucial step in bone bonding, which acts as a template for calcium phosphate precipitation. The calcium phosphate layer then directs new bone formation together with absorbing proteins[11]. Company brochure states that histologically, NovaBone Dental Morsels demonstrate consistent resorption characteristics. The sheep study used as an example below demonstrates good bone regeneration at both the 6 & 12 week time periods. Histology sections at both time period show graft particles surrounded by bone. At 6 weeks, the residual graft content is higher as corroborated in histomorphometric data.

When it comes to TCP, recent literature shows that β-tricalcium phosphate (β-TCP) is not only osteoconductive, but osteoinductive as well. It is one of the most widely used bone graft material and has the ability to be readily replaced by new bone[12-13]. Chung et al[14] reported 55% of complete resorption in 12 months. When it comes to osteoconductivity, it does not fit the concept of bioactivity according to Kokubo[15], since no apatite layer was detected between bone and β-TCP[16-17]. But β-TCP is osteoconductive according to Kitsugi et al[18] who detected significantly higher bone bonding strength for β-TCP at 10 weeks of implantation. Also, there is more bone formation when osteoclastic resorption is more intense which is seen in case of β-TCP at the microscale[19]. also, the calcium ions released during resorption are used for bone formation[18]. But when it comes to osteoinduction, not all β-TCP show this property. Only after autoclaving β-TCP becomes osteoinductive as it triggers formation of apatite layer which can then grow right after implantation[20]. The only disadvantage of this synthetic graft is the inconsistent resorption rate. Altermatt[22] et al mentioned that "even 7 years after implantation, the radiographic evaluation showed no evidence of biodegradation". A lot of this depends upon the porosity of the biomaterial. It has been stated that high porosity is not suitable for bone regeneration. In study Wang et al[23] achieved higher bone fusion with 60% porous β-TCP granules than 75% porous β-TCP granules. In this study, β-TCP has shown good bone formation, in the authors opinion the material used was not autoclaved, in spite of that mature bone formation is on the higher side as compared to NovaBone®.

In the current histological study, both Powerbone® and Adbone® are β-TCP, however the former has a polygonal granules of particle size ranging from and 0.25-7mm, which promotes interlocking and improves mechanical stability. Hence, because of this micro and macro-porosity, particles stick enhancing cell attachment and development[24]. Whereas, with the latter, the porosity is 60% with particle size ranging from 300-500 microns. In spite of both the grafts being β-TCP, histologically there is marked difference between the both, Powerbone® shows more mature bone as compared to Adbone®, which can be attributed to the difference in particle size and porosity[25]. Between Novabone® and β-TCP grafts, the regenerative potential can be appreciated when the mature bone is visible in red, former shows more mature bone as compared to the latter when both the graft were stained with Masson Trichrome. Chronologically, Powerbone® shows the maximum formation of mature bone followed by Adbone® and then Novabone®

## Conclusion:

Histological analysis of bone graft materials has shown that even two bone grafts comprising of β-TCP can vary in terms of bone formation as it depends upon the porosity, graft particle size and as aforementioned upon the graft's manufacturing. All the three different synthetic grafts used, demonstrate excellent adaptation clinically, however differ histologically in bone formation. Chronologically, Powerbone® shows the maximum formation of mature bone followed by Adbone® and then Novabone®

**Corresponding author:**
Sagrika Shukla Mds,
Private Practice, New Delhi, India.
E-Mail: shukla.sagrika@gmail.com

**Acknowledgement:**
None.

**Conflict of interest:**
None

**Funding source:**
None

References:

1. Tatum H jr. Maxillary and sinus implant reconstruction. Dent Clin N Am. 1986;30(2):207-229. DOI.org/10.1016/S0011-8532(22)02107-3.

2. Fischer J, Kolk A, Wolfart S, Pautke C, Warnke PH, Plank C. Future of local bone regeneration – Protein versus gene therapy. J Craniomaxillofac Surg. 2011;39(1):54–64. doi: 10.1016/j.jcms.2010.03.016.

3. Naujoks C, Langenbach F, Berr K, Depprich R, Kubler N, Meyer U et al. Biocompatibility of osteogenic predifferentiated human cord blood stem cells with biomaterials and the influence of the biomaterial on the process of differentiation. J Biomater Appl. 2011;25(5):497–512. doi: 10.1177/0885328209358631.

4. Klijn RJ, Meijer GJ, Bronkhorst EM, Jansen JA. A meta-analysis of histomorphometric results and graft healing time of various biomaterials compared to autologous bone used as sinus floor augmentation material in humans. Tissue Eng Part B Rev. 2010; 16(5):493–507. doi: 10.1089/ten.TEB.2010.0035.

5. Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng. 2012; 40(5):363-408. doi: 10.1615/critrevbiomedeng.v40.i5.10.

6. Sanchez AR, Sheridan PJ, Kupp LI. Is platelet rich plasma perfect enhancement factor? A current review. Int J Oral Maxillofac Implants. 2003;18(1):93-103.

7. Dumitrescu AL. Bone Grafts and Bone Graft Substitutes in, Dumitrescu AL, in Chemicals in surgical periodontal therapy, 1st edition, Berlin Heidelberg, Springer-Verlag, 2011 Pp 73-144. DOI: 10.1007/978-3-642-18225-9_2

8. Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL. Regeneration of periodontal tissue: Bone replacement grafts. Dent Clin North Am. 2010;54(1):55–71. Doi.org/1001016/j.cden.2009.09.003

9. Mahesh L, Guirado JLC, Shukla S, Kumar VR, Kumar YR. Clinical and radiographic findings without the use of bone substitute materials in extraction sockets and delayed implant placement- A case series. J Oral Bio Craniofax Res. 2020;10(2):141-45. doi: 10.1016/j.jobcr.2020.03.011.

10. Shukla S, Chug A, Mahesh L, Grover HS. Effect of addition of Platelet Rich Plasma to Calcium Phosphosilicate Putty on healing at 9 months in periodontal intrabony defects. J Contemp Dent Prac. 2016;17(3):1-5. doi: 10.5005/jp-journals-10024-1832.

11. Andre M. Imaging methods in periodontology. Periodontol 2000. 2004; 34:34-38. doi: 10.1046/j.0906-6713.2003.003423.x.

12. Eggli PS, Muller W, Schenk RK. Porous hydroxyapatite and tricalcium phosphate cylinders with two different pore size ranges implanted in the cancellous bone of rabbits. A comparative histomorphometric and histological study of bony ingrowth and implant substitution. Clin Orthop Relat Res. 1988;232:127-138.

13. Shimazaki K, Mooney V. comparative study of porous hydroxyapatite and tricalcium phosphate as bone substitutes. J Orthop Res. 1985;3(3):301-310. doi: 10.1002/jor.1100030306.

14. Chung H, Kim S, Chung SH. Clinical Outcome of Beta-Tricalcium Phosphate Use for Bone Defects after Operative Treatment of Benign Tumors Clin Orthopedic Surg. 2019; 11(2):233-236. doi: 10.4055/cios.2019.11.2.233.

15. Kokubo T, Takadama H. How useful is SBF in predicting in-vivo bone bioactivity? Biomaterial. 2006;27(15);2907-2915. doi.org/10.1016/j.biomateruals.2006.01.017

16. Ohtsuki C, Kokubo T, Neo M, Kotani S, Yamamuro T, Nakakura T et al. Bone bonding mechanism of sintered β-3CaP-$P_2O_5$. Phosphorus Res Bull. 1991;1:191-196.

17. Neo M, Kotani S, Fujita Y, Nakakura T, Yamamuro T, Bando Y et al. Difference in ceramin-bone interface between surface-active ceramics and resorbable ceramics- a study by scanning and transmission electron microscopy. J biomed Mater Res. 1992; 26(2):255-267. doi: 10.1002/jbm.820260210.

18. Kitsugi T, Yamamuro T, Nakakura T, Kotani S, Kokubo T, Takeuchi H. Four calcium phosphate ceramics as bone substitutes for non-weight-bearing. Biomaterial. 1993;14(3): 216-224. DOI: 10.1016/0142-9612(93)90026-x

19. Bohner M, Baroud G, Bernstein A, Döbelin N, Galea L, Hesse B et al. Characterization and distribution of mechanically competent mineralized tissue in micropores of β-tricalcium phosphate bone substitutes. Mat Today. 2017;20(3)106-115. DOI.org/10.1016/j.mattod.2017.02.002

20. Le Huec JC, Clément D, Brouillaud B, Barthe N, Dupuy B, Foliguet B et al. Evolution of the local calcium content around irradiated β-tricalcium phosphate ceramic implants: in vivo study in the rabbit. Biomaterrial. 1998; 19(7-9)733-738.
DOI: 10.1016/s0142-9612(97)00189-0.

21. Obata A, Fujimoto T, Kasuga T. Enhancement of Bone-Like Apatite Forming Abilities of Calcium Phosphate Ceramics in SBF by Autoclaving. Ceram Soc Jpn 2006; 114:63-66.
https://doi.org/10.4028/www.scientific.net/KEM.309-311.247.

22. Altermatt S, Schwöbel M, Pochon JP. Operative Treatment of Solitary Bone Cysts with Tricalcium Phosphate Ceramic. A 1 to 7 Year Follow-Up. Eur J Pediatr Surg. 1992; 2(3):180-182. DOI: 10.1055/s-2008-1063435

23. Wang Z, Sakakibara T, Sudo A, Kasai Y. Porosity of β-TCP affects the results of lumbar posterolateral fusion. J Spinal Disord Tech. 2013;26(2):E40-45. DOI:10.2139/ssrn.3600188

24. Hulbert SF, Young FA, Mathews RS, Klawitt JJ, Talbert CD, Sterling FH. Potential of ceramic materials as permanently implantable skeletal prosthesis. J Biomed Mater Res. 1970;4(3)433-456.
DOI: 10.1002/jbm.820040309.

25. Mahesh L, Calvo-Guirado JL, Shukla S, Jain A. Histological study to compare the appropriate particle size of Beta TCP for socket preservation. Int J Sci Res. 2023;12(2)1-4. DOI:10.36106/ijsr/3503764