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Histologic Presentation of Alveolar Preservation with an Alloplastic Bone Graft - A Case Series

Lanka Mahesh[1], Sagrika Shukla[2*], Sumit Dubey[3], Gaurav Mathpal[4] and Anshi Jain[5]

[1]The Specialist Clinic Private Practice, New Delhi, India

[2]Department of Periodontology, SGT Dental College and Hospital, India

[3]Department of Prosthodontics, DJ Dental College and Hospital, India

[4]Senior Consultant, (Prosthodontics), Panacea Clinic R, New Delhi, India

[5]Department of Oral Pathology, ITS Dental College, India

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***Corresponding author:** Sagrika Shukla, Department of Periodontology, SGT Dental College and Hospital, SGTU, Gurgaon, Haryana, India

ABSTRACT

The aim of the study was to evaluate bone formation at 6 months in case of Alloplast (β-TCP) bone graft material. Socket grafting was done with Alloplast (β-TCP) and bone core was taken during osteotomy preparation for histological examination. For histological evaluation, tissues processing was done using standard protocol, wherein, tissue sections of 4-micron were fixed with Hematoxylin and Eosin stains. At 10X, thick bundles of collagen fibers were seen encircling newly formed mature bony trabeculae. Very mild diffuse inflammatory infiltrate was seen and large areas showing new bone formation with entrapped osteocytes within the osteocytic lacunae at higher magnification (40X) were also seen. β-TCP shows mature bone formation at 6 months and aids in regeneration.

Keywords: Guided bone Regeneration; Regeneration; Bone graft; Dental implants; Alloplasts

INTRODUCTION

Since the introduction of implants in the field of dentistry, bone grafting has become a vital component of the surgical procedure. As it is a well-known fact that autogenous bone is the gold standard, but its disadvantages such as rapid resorption, limited availability and additional surgical site make it a less suitable material as compared to other types of grafts. Because of which usage of other types of grafts such as xenografts, allografts and alloplasts has tremendously increased. However, any of these aforementioned grafts cannot match the osteoinductivity and osteoconductivity of autogenous bone. Ideally speaking, any bone graft used for regeneration, should be able to provide a 3-dimensional matrix support to precursor cells and ultimately one bone-sigma via ingrowth or on-growth [1]. Surprisingly, recent literature shows that β-tricalcium phosphate (β-TCP) is not only osteoconductive, but osteoinductive as well [2,3]. Unlike xenograft, β-TCP shows rapid resorption, according to Chung et al. [4], 55% of graft gets completely resorted within 12 months. β-TCP has also shown good bone formation, for the very same reason we histologically studied bone cores grafted with β-TCP in a case series as discussed.

CASE REPORT

Socket preservation was performed in 20 patients who needed tooth/teeth extraction due to poor periodontal status or decay and needed placement of implants according to general inclusion and exclusion criteria such as:

Inclusion criteria: -

- Patients aged between 20 -80 years.
- Decayed tooth/teeth ready to be extracted and socket grafted in any of the 3 quadrants.
- Compliant patients.

Exclusion criteria: -

- Uncontrolled systemic diseases/condition
- Presence of infection at the surgical site.
- Systemic antibiotic therapy in the preceding 3 months.
- Pregnant or lactating females.
- Habits such as smoking, tobacco chewing and or parafunctional.
- Other systemic disease/disorder/condition falling under absolute contraindication towards surgical procedure.

Once the patients participating in the study were enrolled, they signed a consent form and were treated by keeping Helsinki declaration into consideration [5]. The procedure was conducted under local anesthesia and after tooth extraction, socket grafting was done. 20 sockets were grafted with (β-TCP) Powerbone® and Sockets were covered with a Collagen plug (Synerheal, India) 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.

Histopathology

For the histopathological examination, trephine core biopsies of size 3.2 mm were obtained and fixed immediately for 24 hours to 48 hours in neutral buffered formalin solution, followed by decalcification of the specimens in a mild agent, 10% EDTA at a pH of 7.4. The tissues were then processed *via* standard protocol of dehydration, clearing and infiltration with paraffin wax. Paraffin wax was also used for block preparation, wherein, sections of 4-micron thickness were stained with Hematoxylin and Eosin. A research microscope namely, Olympus BX53 was used to view the slides thus obtained and Olympus EPL3 was used to capture the images digitally in low and high magnification.

On Histological examination at 10X, mineralized bony areas with well-formed bony cellular components such as, osteoid, osteoblasts, osteocytes and vascular connective tissue could be seen at the Eosin-and-Hematoxylin-stained sections (Figure 1). Abundant areas of mature bone formation with varying degrees of mineralization within a fibrocellular connective tissue stroma is seen. Lamellations were seen in mature bone, lined by bone lining cells and entrapped osteocytes with in the osteocytic lacune. Thick bundles of collagen fibers are seen encircling newly formed mature bony trabeculae. Inflammatory infiltrate was also seen which was very mild and diffused, suggestive of mild host-inflammatory reflecting graft biocompatibility. A higher magnification of 40X showed newly formed bone with entrapped osteocytes within the osteocytic lacunae (Figure 2). Very few areas show the Vascularity and areas of new blood vessel formation in the histology of the section.

At scanner view at 4x Under polarizing stain using picrosirus red, section showed the presence of bony trabeculae at various stages of mineralization present in fibrocellular connective tissue stroms. Collagen fibers in red indicating mature collagen fibers type I collagen (Figure 2A).

At low power and high power 10x and 40x polarizing stain using picrosirus red, section showed the presence of bony trabeculae at various stages of mineralization present in fibrocellualr connective tissue stroms. (Arrow). Bony trabeculae shows both yellow and red colour indicating both thin and thick bundle of collagen fibers of both type 1 and type 3 showing the process of mineralization arranged in lamellar pattern indicating mature bone formation (Figure 2B,2C).

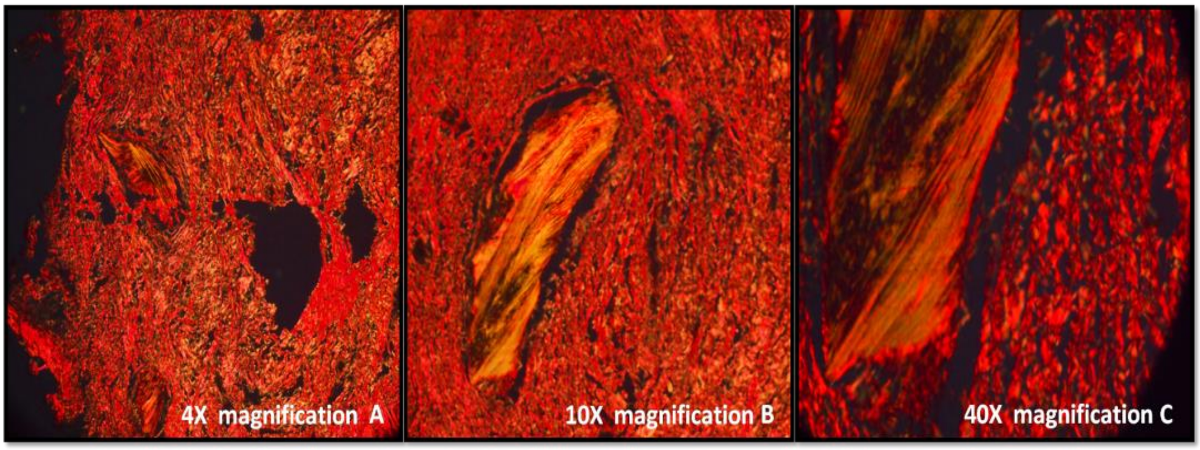

Figure 1: Bone histology at 10x and 40x.

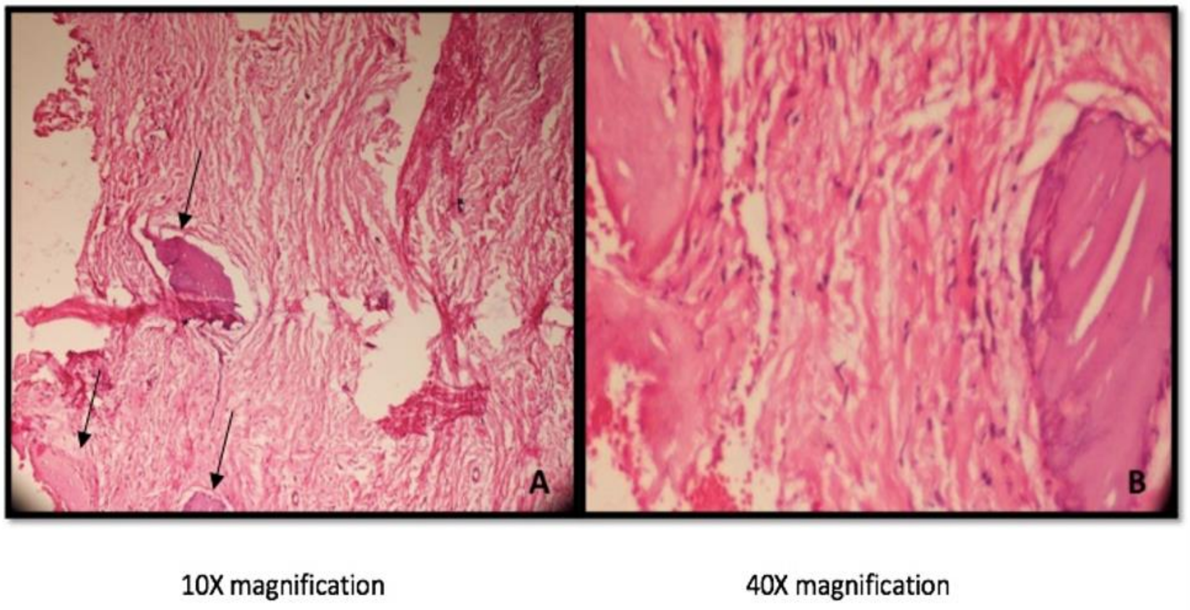

Figure 2: Bone formation at 4x, 10x and 40x magnification.

DISCUSSION

The science of ceramics is quite a fascinating one specially the ones used in medical/dental field. One such substance is tricalcium phosphate, which has been widely studies and still remains a topic for research. This substance occurs in subtypes or polymorphs, namely: α-Ca3(PO4)2, β-Ca3(PO4)2 and α′-Ca3(PO4)2 [6]. The α′-TCP polymorph exists above ~1430˚C and converts into α-TCP upon cooling. The main difference between the alpha and beta TCP is that β-TCP is biocompatible and stable at low temperatures (below 1125˚C) above whict it transforms to α-TCP, which is only stable at higher temperatures between 1120˚C to 1430˚C [7]. Hence, out of these two forms, former one is used in the bone grafts substitutes where regeneration is needed. Coming to β-TCP, clinically it is becoming the 'frequently used' bone graft substitute due its fast-resorbing property, which partially converts into hydroxyapatite *in vivo* [8]. The material acts as a scaffold, and like all the other majority of bone graft substitutes, material resorption facilitates bone formation at the site previously occupied by the bone graft, and by 26 weeks, most of bone graft material is being resorbed [9-11].

By now we have sufficient literature establishing that bone regeneration is influenced by the porous structure of calcium phosphate [12,13]. As aforementioned, recent literature indicates that β-TCP is osteoconductive but, osteoinductive as well, however it is interesting to note that not all types of β-TCP fall under this category. It is only after autoclaving β-TCP becomes osteoinductive as it triggers formation of apatite layer which can then grow right after implantation [14]. However, when it comes to the action of β-TCP on osteoblasts, the data is conflicting. In a study by Sun et a. [15], authors state that β-TCP has an inhibitory effect on osteoblasts due to increased synthesis of PGE2. According to Adams et al. [16], possible explanation could be the exchange of calcium and phosphate ions in the culture medium resulting in apoptosis of osteoblasts. And also depends upon the microporosity which is known to negatively modulates viability of human BMSCs osteoblastic differentiation *in vitro* [17]. Micropore (<50 µm in diameter) and macropore (>100 µm in diameter) are two different structures which have been commonly studied [18]. And it has been postulated that the surface area of biomaterials increases with the presence of micropores improving healing/regeneration, whereas, macropore structures demonstrated to enhance material resorption and boost osteoinduction [19], and this latter property has been making regeneration possible. In a study of femur defect model of rabbit, higher new bone formation was seen with the honeycomb β-TCP scaffolds with Interconnected Pore Structure (ihTCP) as compared to the Unidirectional Pore Structure (uhTCP) [20].

In a histological study by Ruiter et al. [21], authors observed formation of comparable bone volumes in the alveolar clefts between β-TCP and autogenous bone for alveolar clef repair, in fact the amount of bone formed in β-TCP was approximately 10% greater. In the current histsaological study, at 6 months, both thin and thick bundle of collagen fibers of both type 1 and type 3 can be seen showing the process of mineralization arranged in lamellar pattern indicating mature bone formation and regeneration. The only disadvantage of this synthetic graft is the inconsistent and unpredictable resorption property. Altermatt et al. [22], mentioned that on radiographic evaluation after 7 years of β-TCP placement, there was no evidence of biodegradation and this could attributed to the fact that some of the β-TCP graft gets converted to hydroxyapatite, which is slow to resorb and leaves unwanted fragments for years.

CONCLUSION

As aforementioned, much research has been done on β-TCP and it continues to remain the topic for studies, currently, there is vast literature available based on which it is safe to state that β-TCP irrespective of its controversial properties aids in bone formation and regeneration. Histology at 6 months showed mature bone formation, which within time will continue to mature further.

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