# Alveolar bone regeneration in rats after grafting of anorganic bovine bone and thick synthetic bioceramic

Roberta D. **GARCIA**[1]
Roberto B. **GARCIA**[2]
Talita **TARTARI**[3]
Marco Antonio H. **DUARTE**[2]
Ivaldo G. de **MORAES**[2]
Clovis M. **BRAMANTE**[2]

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## ABSTRACT

**Introduction:** It remains uncertain whether the use of biomaterial in apical surgeries facilitates or induces bone regeneration. This study comparatively analyzed the effects of socket filling with anorganic bovine medullary bone and dense synthetic bioceramic on bone regeneration in 48 rats. **Methods:** Forty-eight Wistar albino rats were randomly divided into three groups which had the socket filled after dental extraction as follows: GI (n = 12) blood clot (control); GII (n = 18) anorganic bovine bone; GIII (n = 18) dense synthetic bioceramic. Specimens were harvested at 7, 15 and 30 days post-surgery. Quantitative microscopic analyses of inflammatory infiltration, fibroblastic density, angioblastic density, and bone neoformation were performed. Data were subjected to Kruskal–Wallis test ($\alpha < 0.05$) to detect differences between groups within the same time interval. **Results:** Although some differences were detected between experimental and control groups for inflammatory infiltrate and angioblastic density within 7 days, and bone formation in 15 days, the process of repair was similar for all groups within 30 days. **Conclusions:** There was no difference between the two types of material both of which did not delay the process of bone regeneration. Should they be used in apical surgery, they may act as osteoconductive and osteofilling material in large bone defects.

**Keywords:** Biocompatible material. Bone regeneration. Guided tissue regeneration. Material testing. Healing.

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[1]MSc in Endodontics, School of Dentistry — University of São Paulo/Bauru.
[2]Professor, Department of Endodontics, School of Dentistry — University of São Paulo/Bauru.
[3]PhD resident in Applied Dental Sciences, School of Dentistry — University of São Paulo (USP).

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Contact address: Clovis Monteiro Bramante
Av. Dr. Octávio Pinheiro Brisolla, 9-75 – Vila Universitária
CEP: 17012-901 – Bauru/SP — Brazil
E-mail: clobra@fob.usp.b

## Introduction

Apical surgery is indicated as a complementary procedure whenever conventional endodontic treatment fails. It has been demonstrated that the size of preoperative lesions is a significant predictor of this procedure outcomes.[1,2] In some cases, such as through-and-through osseous defects and in combined endodontic-periodontal lesions, there is large bone destruction which hinders apical healing by periodontal tissue regeneration.[3]

Osseous defects may be repaired in two ways: healing by periodontal tissue regeneration or by fibrous scar tissue. Natural regeneration of large bone defects is usually incomplete and occurs by fibrous tissue.[4]

A new treatment option has become available for such defects with the introduction of guided tissue regeneration (GTR) which consists in placing a mechanical barrier to prevent the proliferation of oral epithelium and gingival connective tissue into the defect and while giving preference to proliferation of cells with osteogenic potential that can refill the defect, thereby resulting in more predictable bone repair.[5,6,7]

The concept of GTR has led to the development of synthetic bone substitutes, such as membrane barriers and bone grafts that allow cellular re-growth in periodontal defects.[8,9] However, membranes are difficult to be applied in cases with no cervical cortical bone, and in through-and-through osseous defects. Additionally, if a non-resorbable membrane is used, a second surgery is required to remove the membrane.[3]

Lyophilized anorganic bovine bone is among the different types of material used for grafting. It is a xenograft obtained through deproteinization at high temperatures.[10] It has high contents of calcium and phosphorus, and not only is classified as biocompatible and osteoconductive, but it is also able to act as a scaffold, filling the bone cavity and guiding bone formation.[10-13]

Another biomaterial available is bioceramic, an alloplastic composed by hydroxyapatite (HA) and β-tricalcium phosphate (βTCP).[14] The biological response of the host tissue to this type of material may vary according to its porosity, shape and size of particles, as well as their compositional characteristics, such as the amount of HA and βTCP.[15-18] HA-βTCP ceramics have been reported as biocompatible, osteoconductive and osteoinductive. The latter is due to being capable of differentiating mesenchymal stem cells into osteoblasts and chondroblasts.[19]

It remains unclear whether biomaterial enhances alveolar and dentoalveolar healing after apical surgery. Thus, the aim of this study was to investigate the effects of socket filling with microgranular anorganic bovine medullary bone and microgranular dense synthetic HA-βTCP bioceramic on the process of bone repair in rats.

## Material and methods

This study was approved by the Ethics Committee on Animal Research of the School of Dentistry — University of São Paulo/Bauru (CEEPA 29/2005). Forty-eight male Wistar albino rats weighting 350 g each were randomly divided into three groups which had the socket filled after dental extraction as follows:

» GI (n = 12) blood clot (control group);

» GII (n = 18) microgranular anorganic lyophilized bovine medullary bone (0.25 to 1 mm, Gen-Ox, Baumer S. A., Mogi Mirim, SP, Brazil);

» GIII (n = 18) microgranular biphasic dense synthetic bioceramic (0.25 to 1 mm, GenPhos, Baumer S. A.) containing 70% HA and 30% β-TCP.

The surgical procedures were performed under intramuscular anesthesia with 10% ketamine (Dopalen, Vetbrands, Montes Claros, MG, Brazil) , and 2% xylazine (Anasedan, Vetbrands, Brazil) (0.05 ml/100 g body weight). Upper right incisors were extracted and alveolar bleeding was controlled with sterile gauze compression. Anorganic bone and bioceramic particles were manipulated after adding saline solution. They were carefully inserted into the socket of the experimental animals with a metallic micro-amalgam carrier after which the sutures were made. The control group received suture for blood clot contention, only. The lower incisor was worn with a diamond drill to avoid trauma on the gingival tissue of the sutured socket. The animals received a single intramuscular dose of 0.1 ml of antibiotics (Pentabiótico®, Brazil) associated with an inflammatory (Pencivet® Plus Super Forte, Intervet do Brasil Veterinaria Ltda, Cotia, SP, Brazil).

At the end of each experimental period (7, 15 and 30 days), four animals from the control group and six from the experimental groups were anesthetized and then killed.

The upper right maxillas containing the tooth socket were removed and immersed into 10% neutral buffered formalin solution for seven days. The specimens were demineralized in 4.3% EDTA, pH 7.2, for approximately

two months. Subsequently, they were embedded in paraffin and serially sectioned in the longitudinal plane (6 μm thick). All sections were stained with hematoxylin and eosin (HE) for histological analysis. Microscopic quantitative analyses were performed attributing scores between 0 and 3 according to the intensity of the following criteria: inflammatory infiltrate, fibroblast density, angioblastic density and bone neoformation.

The meaning of the scores were as follows:

0 = absent: no presence of the phenomenon evaluated;

1 = discreet: sparse presence or small extent of the phenomenon;

2 = moderate: general presence of the phenomenon;

3 = intense: abundant presence of the phenomenon.

## Statistical analysis

The data obtained were presented as medians, minimum and maximum scores values. Nonparametric Kruskal-Wallis statistical test ($\alpha < 0.05$) with Dunn's post-hoc was used to detect potential differences within the same group in different periods of time and among different groups within the same time interval.

## Results

The median, minimum and maximum score values for the presence of inflammatory infiltrate, fibroblast density, angioblastic density, and bone neoformation are summarized in Table 1.

Intergroup comparison revealed that there was statistical difference ($P < 0.05$) for the inflammatory infiltrate only in the 7-day period and between GI and GII, with a more significant inflammatory infiltrate for GII. As for fibroblast density, no statistical difference was found between groups at any time interval analyzed. Regarding angioblastic density, blood vessel proliferation was more pronounced in the 7-day period and in GII and GIII, with statistical difference ($P < 0.05$) between these groups and GI. With regard to bone neoformation, GI and GII showed a higher amount of bone neoformation up to 15 days, with a statistical difference between GI and GIII, only. In the 30-day period, however, no statistical differences were found between any groups for all criteria analyzed.

## Discussion

The dental socket is subject to a succession of biological phenomena inherent to the regeneration process, such as cell proliferation, synthesis of large amounts of fibers by fibroblasts, and, subsequently, tissue mineralization which simulates a periapical bone defect environment.[20]

The presence of any substance inside the dental socket, such as biocompatible material and blood clot, initially triggers an inflammatory reaction in the implanted area, a natural defense mechanism for reabsorption, thus enabling the formation of tissue granulation and, therefore, the repair process.[21] The anorganic bovine bone graft particles (GII) and bioceramic (GIII) triggered a moderate inflammatory process, confirming the organism reaction to an implanted foreign body.[20,21,22] In the 15-day period, the inflammatory infiltrate appeared

**Table 1.** Median (Med), minimum (Min) and maximum (Max) score values for the presence of inflammatory infiltrate, fibroblast density, angioblastic density and bone neoformation in the different groups assessed 7 (T07), 15 (T15), and 30 (T30) days after surgery.

| Groups | Inflammatory infiltrate | | | Fibroblast density | | | Angioblastic density | | | Bone neoformation | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T07 | T15 | T30 | T07 | T15 | T30 | T07 | T15 | T30 | T07 | T15 | T30 |
| | Med (Min - Max) | Med (Min - Max) | Med (Min - Max) | Med (Min - Max) | Med (Min - Max) | Med (Min - Max) | Med (Min - Max) | Med (Min - Max) | Med (Min - Max) | Med (Min - Max) | Med (Min - Max) | Med (Min - Max) |
| GI Control | $1.0^{B.a}$ (1.0 – 2.0) | $1.0^{A.a}$ (1.0 – 1.0) | $1.0^{A.a}$ (1.0 – 1.0) | $3.0^{A.a}$ (3.0 – 3.0) | $2.0^{A.b}$ (2.0 – 2.0) | $2.0^{A.b}$ (2.0 – 2.0) | $2.0^{B.b}$ (2.0 – 2.0) | $3.0^{A.a}$ (3.0 – 3.0) | $3.0^{A.a}$ (3.0 – 3.0) | $1.0^{A.b}$ (1.0 – 2.0) | $3.0^{A.a}$ (3.0 – 3.0) | $3.0^{A.a}$ (3.0 – 3.0) |
| GII - Anorganic bone | $2.0^{A.a}$ (2.0 – 3.0) | $1.0^{A.b}$ (1.0 – 1.0) | $1.0^{A.b}$ (1.0 – 1.0) | $3.0^{A.a}$ (2.0 – 3.0) | $2.0^{A.b}$ (2.0 – 2.0) | $2.0^{A.b}$ (2.0 – 2.0) | $3.0^{A.a}$ (3.0 – 3.0) | $3.0^{A.a}$ (3.0 – 3.0) | $3.0^{A.a}$ (3.0 – 3.0) | $1.0^{A.b}$ (1.0 – 1.0) | $2.0^{B.ab}$ (2.0 – 2.0) | $3.0^{A.a}$ (3.0 – 3.0) |
| GIII Bioceramic | $2.0^{AB.a}$ (2.0 – 2.0) | $1.0^{A.b}$ (1.0 – 1.0) | $1.0^{A.b}$ (1.0 – 1.0) | $3.0^{A.a}$ (3.0 – 3.0) | $2.0^{A.b}$ (2.0 – 2.0) | $2.0^{A.b}$ (2.0 – 2.0) | $3.0^{A.a}$ (3.0 – 3.0) | 3.0A.a (3.0 – 3.0) | $3.0^{A.a}$ (3.0 – 3.0) | $1.0^{A.b}$ (1.0 – 1.0) | $2.5^{AB.a}$ (2.0 – 3.0) | $3.0^{A.a}$ (3.0 – 3.0) |

Different capital letters in columns indicate statistically significant differences between groups within the same time interval (Kruskal-Wallis test, P-value < 0.05); different lowercase letters in rows for the same inflammatory phenomenon indicate statistically significant intragroup differences between the periods analyzed (Kruskal-Wallis test, P-value < 0.05).

discreet such as in GI. This reduction is based on an initial aggressive reaction to a foreign body, which is later on attenuated due to the biocompatibility of the graft,[11,15,22,23] which may be attributed to the fact that both bone substitutes are composed of hydroxyapatite similar to the crystalline phase of natural bone.[24] It was also found that the presence of biomaterial inside the socket did not delay the healing process, thereby agreeing with the results of previous studies.[25,26] However, a study showed that even though there was a progressive increase in bone volume over time, there was a significant delay in the chronology of alveolar repair, when experimental groups were compared with controls, due to the presence of biomaterial.[20]

All groups showed intense fibroblastic proliferation within 7 days, which declined after 15 days and could be explained by the organization and maturation of the connective tissue.[27,28] This result differs from the findings of another study[22] in which only a few cells were observed in the central area of the granulation tissue within the first days. Nevertheless, the authors explained that this probably occurred due to the large amount of compacted material used, which may have retarded the penetration of connective tissue cells and blood vessels.

Angioblastic density proved intense in GII and GIII in all periods analyzed, although, in GI, this density was moderate after 7 days, increasing to intense after 15 days. It is natural to find an increase in blood vessels concomitant with new bone formation during the repair process, since osteoblast activity and mineralization process require adequate blood supply with transport of oxygen and nutrients.[21,29] Additionally, distribution of graft particle sizes can play an important role in the emergence of new blood vessels, particularly because if there is wide size variation, the smaller particles tend to obstruct the spaces among the large particles, thus reducing vascularization and also cell penetration.[16,22] Analysis of results reveals that although both types of grafting material are microgranular, with particle sizes ranging from 0.25 to 1 mm, this variation was not so great as to prevent the formation of blood vessels and cell migration.

After dental extraction, alveolar repair in rats is complete with the socket filled with well-organized trabecular bone lined by osteoblasts.[20] In the present study, the animals of all groups killed after 30 days had the socket completely filled with bone tissue (Fig 1), and the buccal and palatal bone ridge remodeled in accordance with a previous study.[30] The results of this study support the claims that anorganic bovine bone and bioceramic material are osteoconductive and osteofilling.[11,12,19,31] The osteoconductivity expressed by direct bone-to-particle contact[31] may be benefited by anorganic bone porosity and by macropores resulting from fast resorption of HA + β-TCP bioceramic which is initially dense.[18,32] The existence of pores can promote tissue growth and bone formation.[33,34] However, even though porosity reduces the material mechanical strength, the penetration of newly formed tissue in the pores provides more resistance to fracture.[33,35]

Porosity also favors the resorption process of material.[22,35] In fact, an optimal synthetic bone substitute should be reabsorbed and replaced by new bone, since the long-term presence of material can limit bone formation and affect healing.[11,35,36] Nevertheless, none of the GII and GIII specimens showed signs of grafting particle reabsorption within the studied periods, thereby agreeing with other authors who proposed that some Ca/P-derived material has slow resorption and require several months to complete the process.[31,37] A possible explanation for these findings is that HAs have slow *in vivo* resorption profiles,[34] and Gen-Ox, although porous, is processed at high temperatures which makes it a crystalline and, as a consequence, little degradable substance.[10] Even though the socket in the control group could be affected by bone remodeling much faster, the effects of particle biomaterial long-term implantation in the socket could result in the maintenance of its original dimensions.[20]

It should be emphasized that after 30 days there were no differences between the control and experimental groups in all inflammatory criteria analyzed, thereby proving the biocompatibility of implanted material and confirming that they did not delay the process of bone formation. The results of this study also suggest that, if used in apical surgery with large bone defects, this type of material can act as osteofilling and osteoconductive, helping in the healing process.

## Conclusions

The use of biomaterial in apical surgeries with large bone defects can be highlighted as a promising approach, since the material studied did not delay the process of bone regeneration and acted as osteoconductive and osteofilling material.

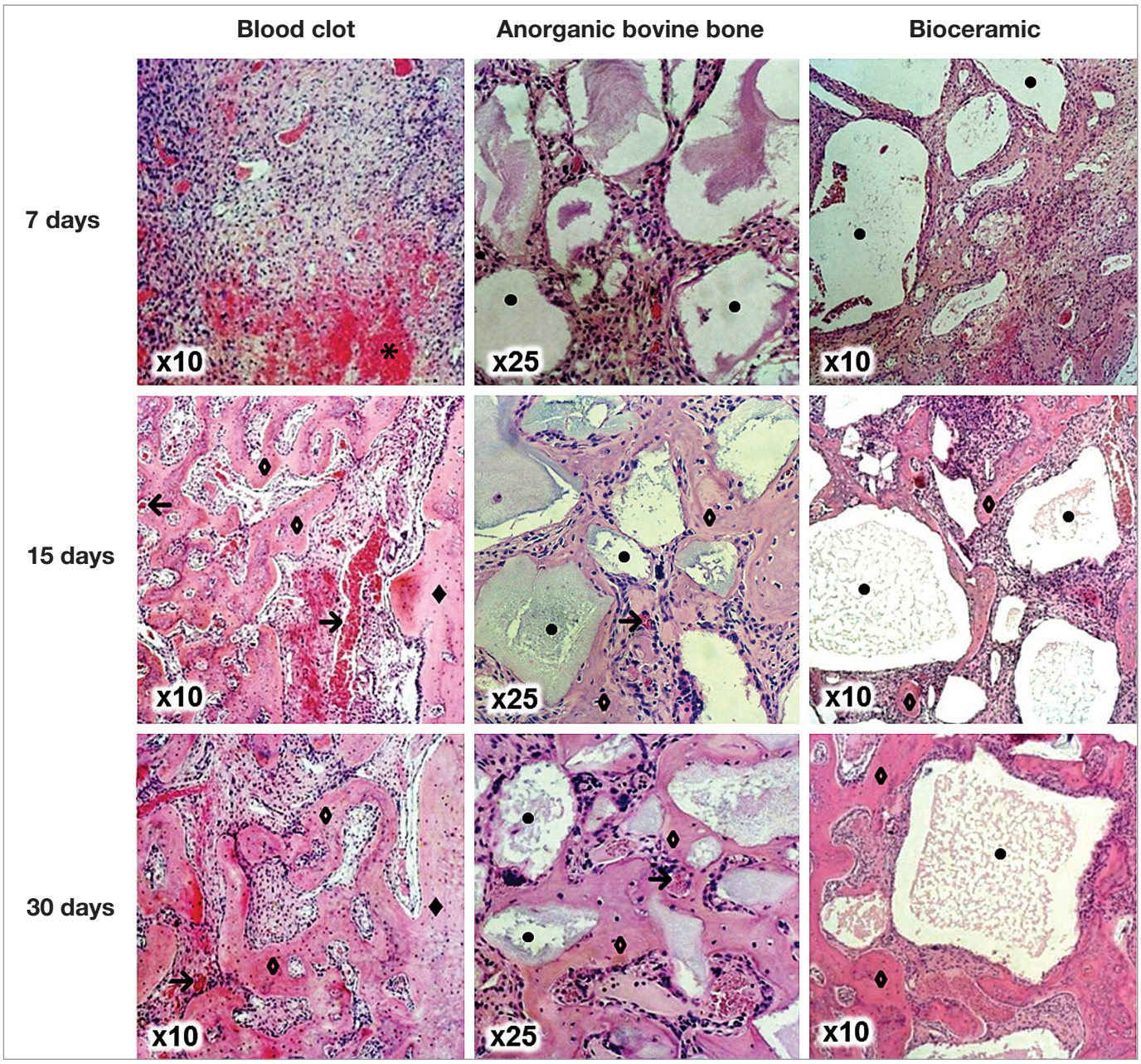


**Figure 1.** Blood clot (*), particles of implanted material (●), trabeculae of neoformed bone (◊), blood vessels (→), cortical bone (♦).

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