ORAL SURGERY

Comparison of different bone substitutes in the repair of rat calvaria critical size defects: questioning the need for alveolar ridge presentation

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Objective: This study aimed to evaluate the effectiveness of biomaterials in bone healing of critical bone defects created by piezoelectric surgery in rat calvaria. **Method and materials:** Histomorphologic analysis was performed to assess bone regeneration and tissue response. Fifty animals were randomized into five groups with one of the following treatments: Control group (n = 10), spontaneous blood clot formation with no bone fill; BO group (Bio-Oss, Geistlich Pharma; n = 10), defects were filled with bovine medullary bone substitute; BF group (Bonefill, Bionnovation; n = 10), defects were filled with bovine cortical bone substitute; hydroxyapatite group (n = 10), defects were filled with hydroxyapatite; calcium sulfate group (n = 10), defects were filled with calcium sulfate. Five animals from each group were euthanized at 30 and 45 days. The histomorphometry calculated the percentage of the new bone formation in the bone defect. **Results:** All data obtained were evaluated statistically considering $P < .05$ as statistically significant. The results demonstrated the potential of all biomaterials for enhancing bone regeneration. The findings showed no statistical differences between all the biomaterials at 30 and 45 days including the control group without bone grafting. **Conclusion:** In conclusion, the tested biomaterials presented an estimated capacity of osteoconduction, statistically nonsignificant between them. In addition, the selection of biomaterial should consider the specific clinical aspect, resorption rates, size of the particle, and desired bone healing responses. It is important to emphasize that in some cases, using no bone filler might provide comparable results with reduced cost and possible complications questioning the very frequent use of ridge presentation procedures. *(Quintessence Int 2024;55: 328–334; doi: 10.3290/j.qi.b4955867)*

Keywords: bone, bone substitutes, extraction, implants, wound healing

The field of implant dentistry has been improved by better utilization of guided bone regeneration (GBR). This technique uses barrier membranes to select osteogenic cells and promote bone regeneration in the jaws.[1,2] GBR also applies bone biomaterials to accelerate bone healing by providing a scaffold for new bone formation (NBF).[1,3] The careful management of the resorption rate of the biomaterials is essential to ensure that the scaffold is gradually replaced by bone.[4,5] Thus, it is crucial to know the unique behavior of each biomaterial in bone healing.

Autogenous bone grafts present osteoconductive, osteogenic, and osteoinductive properties; however, they increase morbidity and surgical time. Thus, xenogenous bovine bone substitutes are predicted biomaterials widely used in GBR.[6] These biomaterials are processed by heating and alkaline solutions to remove all organic components, leaving only the inorganic bone matrix.[7] The resulting xenograft is biocompatible, promoting the formation of new bone tissue by acting as a scaffold through its osteoconduction property.[8]

Xenogenous bovine bone substitutes have extensive purposes in implant dentistry.[6,9] Bovine bone substitutes are employed to augment the alveolar bone in cases of inadequate bone volume, providing relatively predictable bone regenera-

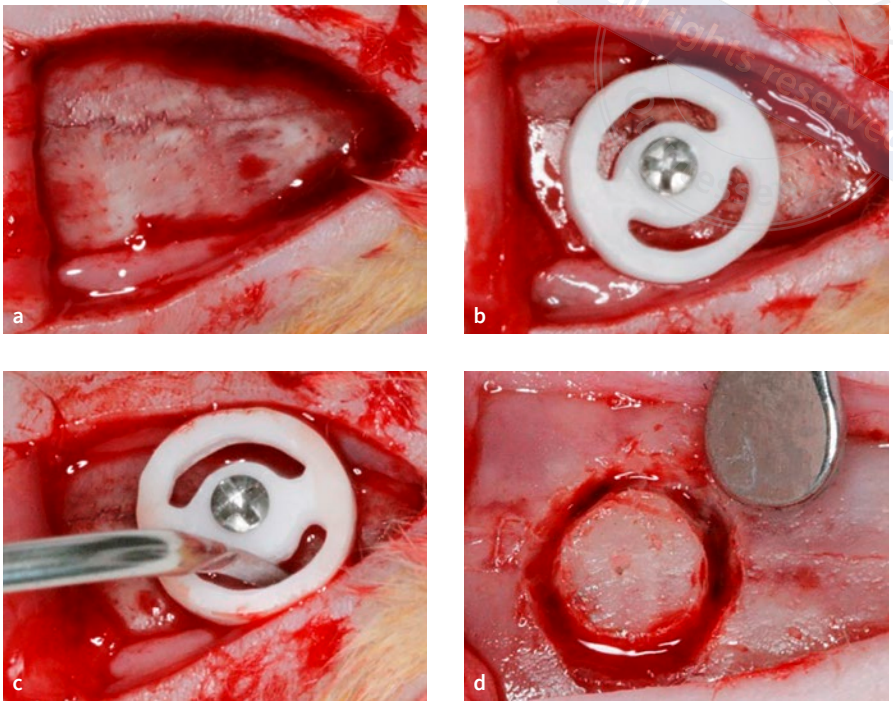

Fig 1a to d Creation of a critical size 5-mm bone defect in rat calvaria by piezoelectric surgery. A full-thickness flap with a U-shaped incision *(a)*. Surgical guide positioned containing an internal diameter of 5 mm *(b)*. Osteotomy by piezoelectric equipment with a power of 50 W and a frequency of 100 Hz *(c)*. Borders of the critical bone defect in rat calvaria *(d)*.

tion for implant placement.[6] Although xenograft bovine bone substitutes have shown promising performance due to their structural similarity to human bone, synthetic biomaterials can also be used as bone substitutes in GBR.[7,10,11]

Hydroxyapatite (HA) is a naturally occurring form of calcium phosphate.[12] It is a natural mineral component of the bone and tooth enamel, being a biocompatible material for GBR.[12] It provides an osteoconductive scaffold, promoting the deposition of calcium and phosphate ions, which gradually results in NBF.[13] This synthetic biomaterial, available in granular form, facilitates NBF to contribute to the stability of dental implants.[13]

Calcium sulfate (CS), available in alpha or beta hemihydrate forms, serves as a resorbable bone graft substitute.[14] This synthetic biomaterial displays biocompatibility, but is quickly reabsorbed during healing as it is substituted by newly formed bone.[14] CS can also be combined with other biomaterials, enhancing its osteoconduction.[15] Its ability to accelerate bone regeneration while minimizing morbidity makes CS a valuable biomaterial in GBR.[15]

Ridge preservation following tooth extraction has become a widely used procedure,[16] although in some cases its necessity can be questioned.

The present comparative study assessed negative controls with those four commonly employed biomaterials through histomorphometric analysis. The comparative evaluation of their regenerative properties was performed using a standardized model of bone healing of bone defects in rat calvaria.

Method and materials

Ethical committee

This research was approved by the Ethics Committee for Animal Experimentation, Faculty of Dentistry (process 1200-1203/2011), within the recent rules adopted by the Brazilian College of Animal Experimentation. Additionally, all steps of the experiment carefully respected the ARRIVE Guidelines 2.0.[17]

Sample size

In this study, a total of 50 male rats (*Rattus norvegicus* Albinus, Wistar), aged between 3 and 4 months and weighing approximately 250 to 300 g were used. The rats were kept in a controlled environment with a stable temperature of 22 ± 2°C and had access to food and water ad libitum. The sample size for this study was determined at n = 10 per group based on previous studies[18] and a sample size calculation to achieve a power of 0.8 and an alpha error of .05.

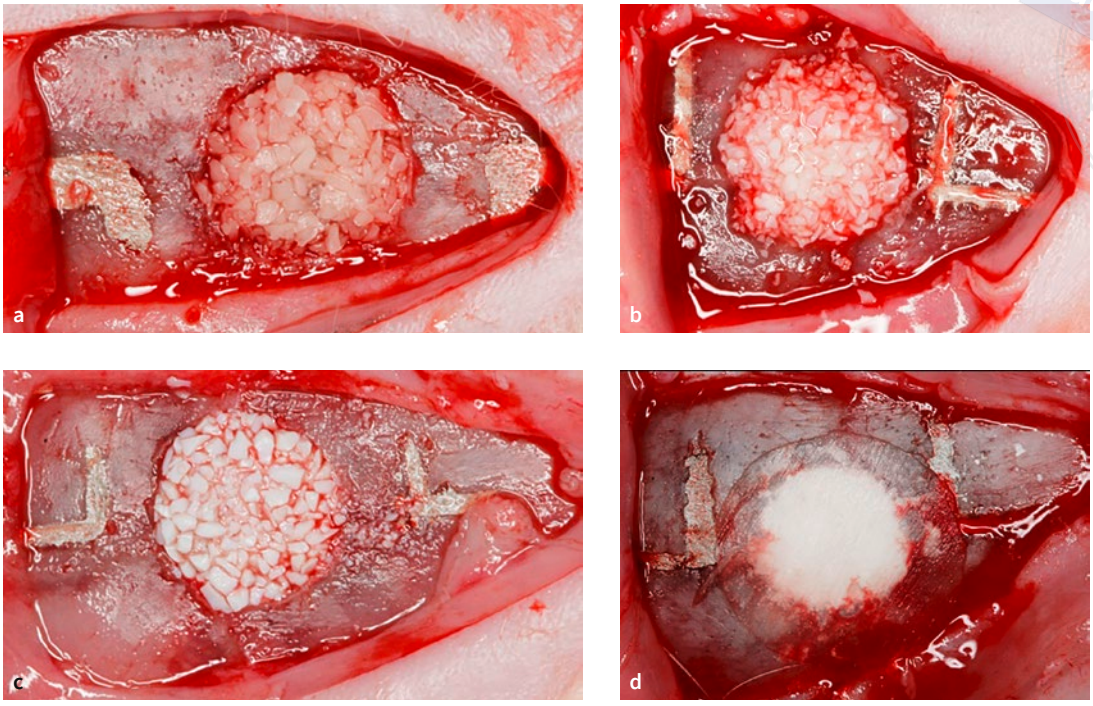

Fig 2a to d Bone defect filled with the four different biomaterials: BO *(a)*, BF *(b)*, HA *(c)*, CS *(d)*. Bone biomaterial substitutes were carefully inserted into the defects, and a collagen membrane of demineralized bovine cortical bone (GenDerm, Baumer) was placed over all defects.

Piezoelectric surgery for bone defect

All animals were anesthetized intramuscularly with 80 mg/kg of ketamine hydrochloride (Cetamin, Syntec do Brasil) and 60 mg/kg of xylazine hydrochloride (Xilazin, Syntec do Brasil). The steps of the piezoelectric surgery for bone defect creation are described in Fig 1. After trichotomy and antisepsis of the region, a surgical guide created especially for this purpose was screwed to the bone in the calvaria. The surgical guide had an internal diameter of 5 mm, creating a critical size defect of 5 mm in diameter involving the parietal bones, and sagittal suture in similar conditions for all animals. For osteotomy, the piezoelectric system with the SIN 300.0112 tip (VK Driller, Piezosonic Esacrom) was used coupled to the handpiece of the piezoelectric equipment with a power of 50 W and a frequency of 100 Hz (VK Driller, Piezosonic Esacrom) under abundant irrigation with sterile saline solution. Next, two L-shaped markings were created 2 mm from the defect margin and filled with amalgam; one in the posterior portion of the defect and the other in the anterior portion. These markings were used to locate the center of the defect during laboratory processing and as a reference to determine the original bone margin of the defect during histometric analysis.[18]

Study design

The randomization sequence of the experimental groups was performed using a computer-generated random number table. A blinded external member of the study labelled numerals from 1 to 50 on the rats' tails. The number order was then uploaded into Stata 9.0 (StataCorp). The animals were randomly assigned to five experimental groups, each consisting of ten animals. Each animal received one of the following actions (Fig 2):

- control group (CO; n = 10): spontaneous blood clot formation with no biomaterial
- BO group (n = 10): defects filled with inorganic bovine bone from bovine tibia (particles of 0.25 to 1.00 mm in diameter; Bio-Oss, Geistlich Pharma)
- BF group (n = 10): defects filled with anorganic bone matrix from bovine femur (particles of 0.6 to 1.5 mm in diameter; Bonefill, Bionnovation)
- HA group (n = 10): defects filled with HA (particles of 0.5 to 0.8 mm in diameter; Xydroxiapatite, Bionnovation)
- CS group (n = 10): defects filled with CS (Bionnovation).

Bone biomaterial substitutes were hydrated with saline solution before implantation and were carefully inserted into the defects, without excessive condensation. A collagen membrane of demineralized bovine cortical bone (GenDerm, Baumer) was

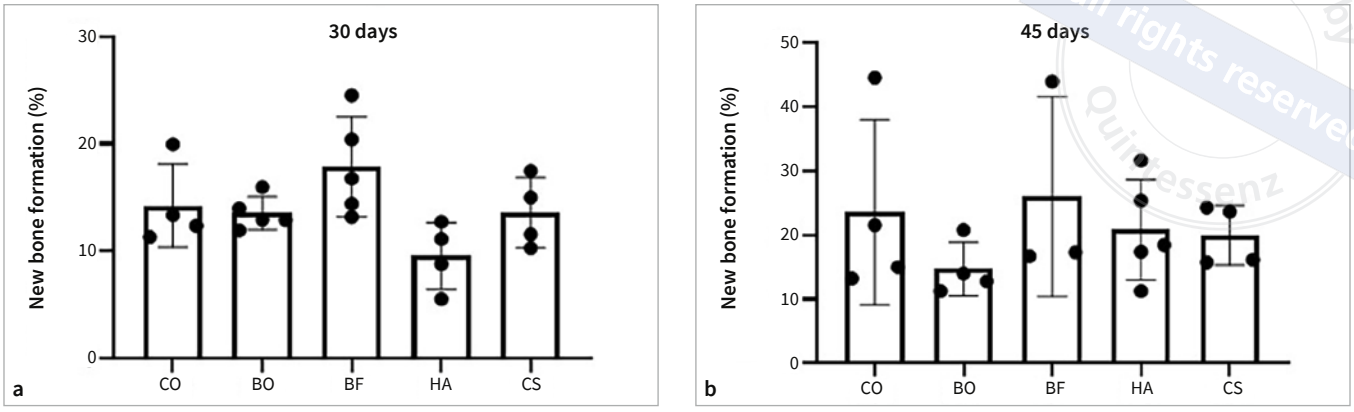

Fig 3a and b Scatter plot graphs with columns representing the histomorphometry analysis for all groups at 30 days *(a)* and 45 days *(b)*. There were no statistically significant differences between all groups ($P > .05$; Kruskal–Wallis test, Dunn post-hoc test).

then placed over all defects. Soft tissue sutures were performed to stabilize the collagen membrane. All evaluations were conducted following calibration and blinding.

Five animals from each group were euthanized at 30 and 45 days after surgery. The animals' calvaria including the bone defect areas were removed and preserved in a 4% formaldehyde solution for 48 hours and then rinsed in running water for 24 hours. The demineralization process was done using a solution of 16% ethylenediaminetetraacetic acid (EDTA). The sections were then washed and embedded in paraffin blocks. From the center of the bone defect, six consecutive sections with a thickness of 5 µm were created. These sections were stained using the hematoxylin and eosin (h&e) technique for histomorphometric analysis.[18]

Histomorphometry

A computer image evaluation software, ImageLab 2000 (Diracon Bio Informática), was used to perform histomorphometry analysis. The analysis was conducted by a single examiner who was calibrated and blinded to the periods and treatments (ML). The analysis followed previously established methods.[18]

Histologic sections were selected from the central area of each specimen's surgical defect in a sagittal direction. A digital camera coupled with an optical microscope was used to capture each section. In each image, the analyzed area was delimited, corresponding to the region of the calvaria where the defect was created. This area was determined by identifying the external and internal surfaces of the original calvaria on the right and left margins of the surgical defect. The surfaces were related to drawn lines following their respective curvatures. To identify the margins of the surgical defect, 2 mm were measured from the right and left extremities of the specimen towards the center of the defect, considering the total length of the histologic specimen.

The areas of NBF that occupied the remnants of the implanted bone biomaterial substitutes, BO, BF, HA, and CS, were delineated within the limits of the total area. The NBF areas of the respective specimens were evaluated three times by the same examiner on different days. The three measurements obtained were statistically analyzed, and the significance level was set at 5% using the Kappa test. The mean values were used for the statistical analysis.

For the reason of the magnification used, it was not viable to capture the entire defect in only one image. Therefore, digital images were created with a combination of three images using Adobe Photoshop (Adobe).

Statistical analysis

The Shapiro–Wilk test was employed to test the data distribution for normality. Based on the lack of normal and homoscedastic distribution of the data, statistical comparisons were performed using the Kruskal–Wallis test and Dunn post-hoc test, using GraphPad Prism version 8.0 (GraphPad Software). All data were plotted as mean ± standard deviation (SD), and $P < .05$ was considered statistically significant.

Results

Histomorphometry at 30 days showed no statistically significant difference between groups ($P > .05$). The NBF at 30 days was higher (not statically significant) in the BF group, with 17.9 ± 4.9%. The CO presented 14.3 ± 3.8% of NBF. The BO, HA, and CS groups

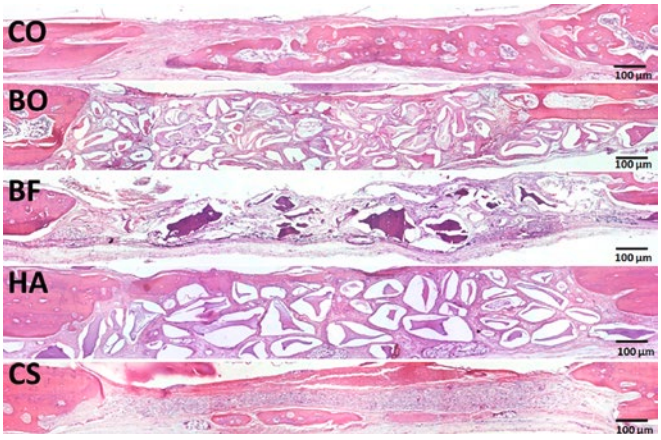

Fig 4 Histologic panoramic aspect of the bone healing of bone defects at 45 days from the CO, BO, BF, HA, and CS groups, respectively. Histologic images show the existence of newly formed bone inside the edges of the bone defects in the CO group. Moreover, a thin layer of newly formed bone is noted around the surface of the remaining bone grafts in the BO, BF, HA, and CS groups (h&e stain, original magnification 50 ×, scale bars 100 µm).

presented 13.6 ± 1.5%, 9.58 ± 3.1%, and 13.6 ± 3.2% of NBF, respectively (Fig 3a).

Additionally, the NBF at 45 days showed no statistically significant difference between groups ($P > .05$). The greatest NBF was in the BF group, with 26.0 ± 15.6%. The CO, BO, HA, and CS groups presented 23.7 ± 2.1%, 14.7 ± 4.2%, 20.8 ± 7.8%, and 20.0 ± 4.6% of NBF at 45 days, respectively (Fig 3b).

A representative histologic section of each biomaterial group is presented in Fig 4. A narrow band of NBF tissue within the edges of the surgical wound was observed in the CO group. The patterns of new bone in the BO, BF, HA, and CS groups were analogous with NBF surrounding the surface of the different biomaterials. All groups also were filled with thin layer of dense connective tissue. No signals of an intense or undesired inflammation were noted in the bone defects, thus all tested biomaterials confirmed their biocompatibility as well as their osteoconductive properties.

Discussion

GBR using biomaterials has significantly improved the effectiveness and safety of bone regeneration procedures in dentistry.[19] Biomaterials such as BO, BF, HA, and CS have proven to be versatile and reliable options in GBR. The results of the present study indicate that there were no statistically significant differences between the materials evaluated in terms of bone formation at 30 and 45 days, including the control group without bone grafting. This suggests that all tested biomaterials might be reliable for promoting NBF through histomorphometry analysis. It also suggests that the use of no filler, in certain cases, might be enough to create new bone with no additional statistically significant added value of the filler. It is essential, thus, to consider the specific features during the bone healing of each biomaterial for GBR and to match them with the specific needs of the patient and the site.

BF showed promising results due to its biocompatibility and favorable tissue response.[7] Although the BF group exhibited the highest amount of NBF compared to the other biomaterials, the difference was not statistically significant. This can be attributed to the larger particle size of BF, which provides a greater surface area for the NBF. BF graft is often chosen for its ability to balance resorption and bone regeneration, making it suitable for a range of bone defects.[7,20]

Research has shown that CS stimulates the body's natural healing mechanisms and promotes bone regeneration.[13] However, due to its smaller particle size, CS may require additional support for long-term bone stability.[15] It is commonly used for bone defects that involve fast resolution; however, its rapid resorption can limit its long-term structural support compared to other biomaterials.[15] CS has a faster resorption rate compared to BO,[21] making it not suitable for all types of regeneration.

HA is a calcium phosphate ceramic that closely resembles the mineral composition of natural bone.[22] It exhibits slow resorption and bone formation.[23] HA has shown capability to maintain its structure.[24] In the present study, BO showed the lowest, though not statistically different, amount of NBF among the biomaterials at 45 days. BO has a slower resorption rate than CS but faster than HA, making it suitable for cases where gradual replacement with native bone is desired.[25] The smaller particle size of BO occupies a larger space in the bone defect, thus probably leaving less space for NBF.

BO is a widely used xenogenous biomaterial derived from bovine medullar bone, known for its biocompatibility and tolerance without immunogenicity.[11] Its structure with porous surface is a distinguishing feature, enabling angiogenesis and osteoconduction.[11] This property allows the bone cells to migrate and proliferate within its structure.[10] BO is gradually resorbable,[4] and has been used successfully in procedures such as alveolar bone preservation, sinus augmentation, and ridge augmentation.[11] Nevertheless, some studies, including the present results, revealed no additional benefits for the use of BO.[7,26]

BF is another xenogenous biomaterial gaining popularity due to its adaptability in GBR procedures.[7] It is derived from the bovine xenogenous graft collected from the cortically bovine femurs, and offers osteoconductive properties due to its porosity.[27] Its adjustable particle size allows different GBR applications.[8]

The utilization of piezoelectric surgical equipment was another notable aspect in the present study. This technique had been shown to be a more accurate and faster method for creating defects in the calvaria, as opposed to traditional methods.[28] The use of piezoelectric surgical devices has grown in acceptance in various fields of health clinical practice, including oral and maxillofacial surgery.[28] The piezoelectric device with the surgical guide used in the present study, enabled a precise osteotomy without causing harm to blood vessels and nerves. Arguably, the piezoelectric surgery used here preserved the posterior adequate blood supply for the biomaterials which might attribute to the lack of difference in NBF between the groups and time periods.

Different biomaterials have distinctive properties that can be designed to meet the specific needs of the specific patients and sites. BO and BF offer suitable biocompatibility and controlled resorption, making them appropriate for long-term applications.[8,29] HA closely mimics natural bone and promotes ample bone integration, while CS is favored for situations of small bone defect.[25,29] The selection of biomaterial and surgical approach should be carefully considered based on factors such as the patient's overall health, the location and size of the bone defect, and the desired clinical outcome. It is also important to emphasize that in some cases, using no bone filler might provide comparable results with reduced cost and possible complications.

Conclusion

Histomorphology analysis of GBR using BO, BF, HA, and CS grafts demonstrated their potential for enhancing bone regeneration, with no statistical difference between them in both evaluated periods of 30 and 45 postoperative days including the control group without bone grafting. The selection of biomaterial should be designed to the specific clinical scenario, considering resorption rates, and desired bone healing responses. Using no bone filler should also be considered as it might provide comparable results with reduced cost and possible complications.

Acknowledgment

The authors are grateful for funding from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES), in the scope of CAPES/Print - Funding code: 001 (Process: 88887.194785/2018-00). The authors thank the Faculty of Dentistry of Araçatuba – UNESP for providing the necessary infrastructure to conduct the study. The authors thank Bionnovation biomedical for providing the biomaterials.

Disclosure

The authors have no conflicts of interest regarding the publication of this paper.

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First submission: 26 Jan 2024
Acceptance: 4 Feb 2024
Online publication: 8 Feb 2024