Morfologic osteoconduction evaluation of Gen-Phos and Gen-Mix in rat calvaria

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Abstract

Introduction: the use of bone replacements in Dentistry has increased as a result of satisfactory and predictable clinical results, lower postoperative morbidity and affordable prices. The most common material used is still the inorganic bovine bone with osteoconductive properties. In the 1980's began the search for a synthetic material with osteoconductive results equal or superior to inorganic bovine bone. **Objective:** To compare histological and histometric property of osteoconductive bone compound (GenMix, Baumer) with phosphate beta-tricalcium (GenPhos, Baumer) implanted in critical size defects in rat calvaria. Results: It was observed 32.5% of new bone formation in the group of compound bone and 45.9% in the phosphate beta-tricalcium group. **Conclusions:** Along the time of microscopic observation of this work, one can state that the materials studied are not absorbable and that phosphate beta-tricalcium is more osteoconductive compared to the compound bone..

Keywords: Biocompatible materials. Bone regeneration. Bone transplant.

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Introduction

The use of biomaterials in Dentistry has been on the rise. The development of materials with adequate osteoconductive properties and diminished surgical trauma encourages the use of these materials. So far the best clinical and histological results are achieved with autogenous bone.[1,2] However, autogenous bone graft has the disadvantages of increased morbidity and not being readily available.[3-7]

In this context, the search for a material with properties similar or superior to those of autogenous bone is the subject of much research in Implant Dentistry. As of 1982 researchers have agreed that ideally a biomaterial should be biocompatible, radiopaque, osteogenic, readily available, hydrophilic and allow guided tissue regeneration, among other features.[8,9,10]

In the 1980's the search for synthetic materials led to the introduction of calcium phosphate ceramics, which is particularly biocompatible and shows no adverse reactions. More recently, it has been associated with beta-tricalcium phosphate (β-TCP).[11] Commercially, these features can be found in BoneCeramic (Straumann, Basel, Switzerland) with a ratio of 6:4 β-TCP / hydroxyapatite, and GenPhos (Baumer SA, Mogi Mirim, Brazil), available in particles (0.5 mm to 0.75 mm) at a ratio of 7:3 hydroxyapatite / β-TCP. Theoretically, this is an osteoconductive material which acts as a scaffold for new bone formation due to the presence of hydroxyapatite, while degrading TCP, thereby enabling replacement by new bone. It is common knowledge that hydroxyapatite features very wide applicability in the medical field as well as in dental care for the filling of cavities.[12,13] It should be recalled that in terms of origin it can be either natural or synthetic, whereas its biological behavior is directly related to its physicochemical characteristics.

On the other hand, composite bone (organic and inorganic portions) (GenMix, Baumer SA, Mogi Mirim, Brazil) was developed with the purpose of reducing the amount of remaining material and inducing new bone formation, since inorganic bone (natural hydroxyapatite) features as its main disadvantage the presence of particles in the recipient site for longer than 24 months.[5,14,15]

This study aimed to assess composite bone graft (GenMix, Baumer SA) and β-Tricalcium Phosphate (GenPhos, Baumer SA) as osteoconductive materials in rat calvarial critical size defects performed by microscopic and histometric evaluation.

Material and methods

This study was approved by the Ethics Committee of the School of Dentistry of Bauru (USP) (Procotol 033/2009). The 18 Wistar rats (*Rattus norvegicus albinus*) used in this study were supplied by the laboratory of animal facility of the São Paulo State University (UNESP) in the Brazilian city of Araçatuba and were randomly divided into three equal groups which comprised the study sample. The rats were kept in individual cages and fed *ad libitum* throughout the experimental period except on the surgery day.

After fasting for 12 hours, the animals were intramuscularly anesthetized with a mixture of 2% xylazine and 5% ketamine hydrochloride in a 1:1 ratio at a dose of 0.2 ml per 100 g body weight.

After anesthesia, the region of the calvaria was shaved, the animal was placed in prone position, antisepsis with topic PVP was performed in the area (Fig 1) and sterile drapes were placed.

Next, surgical access was performed by means of an incision in the midline of the occipital protuberance as far as the eyes. The entire flap was folded back and retractors used to expose the parietal bone from both sides. Osteotomy was performed in the median region between the parietal bones as far as the inner cortex (Fig 1) using a

trephine drill (7 mm) and with the aid of a low speed motor. Once osteotomized the parietal bone was removed and the dura mater kept intact (Fig 1).

In Group I the critical size bone defect was filled with particulate composite bone (GenMix, Baumer SA), homogenized in the blood of the animal and covered with a cortical bovine bone membrane (GenDerm, Baumer SA) trimmed into a circle to ensure a better fit (Fig 1) and subsequently a simple suture of the skin tissue was carried out with 5-0 nylon.

Likewise, Group II was filled with β-TCP particles (GenPhos, Baumer SA) homogenized in blood and covered with the same membrane used in Group I. In Group III, the control group, the critical defect was filled with blood clot and lined with a cortical bovine bone membrane (GenDerm, Baumer SA).

After 60 days the animals were euthanized by anesthetic overdose, and subsequently the parietal bone was removed with a 3 mm margin and fixed in buffered neutral formalin at 10%. The pieces were then demineralized and processed by semi-serial coronal sections (10 sections per piece) and stained with hematoxylin-eosin, which allowed microscopic and histometric analysis by Merz grid.[15,16]

Merz grid consists of a camera lens or photographic objective with 10 horizontal and 10 equidistant points on each line totaling 100 points. The technique of histometry consists in counting manually the structures on each point along the length of the blade. Subsequently, one obtains the sum of these measurements on the blade and in all sections of each piece, which ultimately yield the percentage of each structure in the piece.[15,16]

Microscopic analysis results

The control group showed that at the site of the critical bone defect new fibrous connective tissue was formed with collagen bundles parallel to the skull surface (Fig 2). This tissue was formed instead of new bone as the neighboring

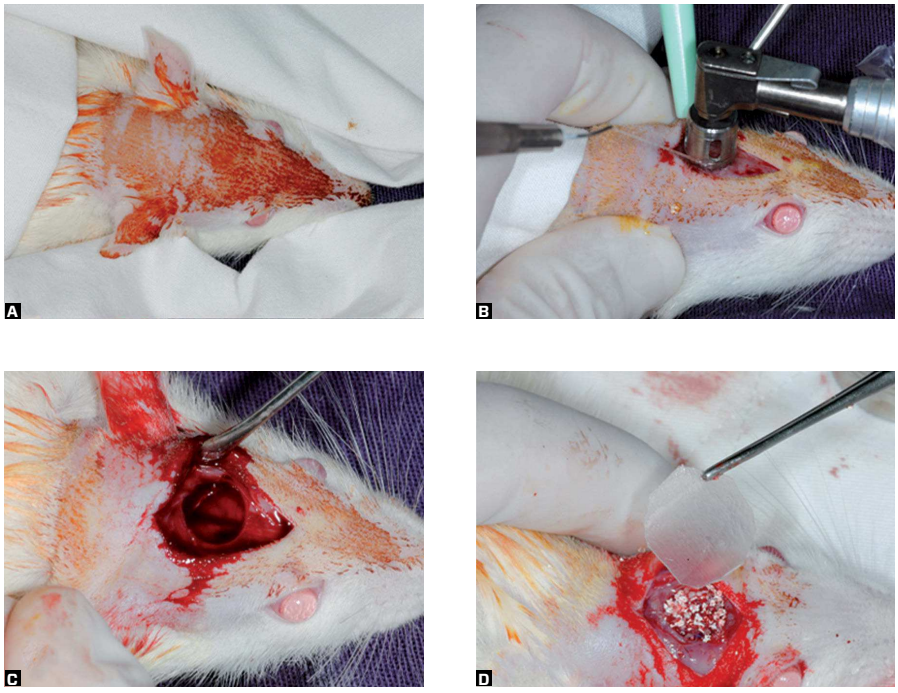

Figure 1 - **A**) Trichotomy and antisepsis with PVPI; **B**) Drilling with 8 mm trephine; **C**) Bone defect of critical size; **D**) Filling of cavity with biomaterial and placement of GenDerm membrane (Baumer SA).

bone cells were unable to move to the large surgically induced defect. A small formation of new bone was noted only on the margins of the defect (Fig 3). In the periods studied no inflammatory infiltrate was observed (Fig 4).

In Group II, most of the critical size surgical defect was occupied by the material's particles (Fig 5). Connective tissue was found amid these particles, which showed predominantly mononuclear inflammatory infiltrate and macrophages with granuloma formation of the foreign body type, directly related to most of the material's surface. The inflammatory multinucleated giant cells and macrophages that comprise these granulomas — as well as the connective tissues — were morphometrically affected by the demineralization process used. It is noteworthy that a portion of the surfaces of the particles of the material which is not in contact with the foreign body granuloma, is in contact with the newly formed bone as shown in Figures 5-8. No infiltration of neutrophils was found in the specimens examined, indicating the absence of bacterial contamination. Newly formed bone is predominantly associated with the particles located more internally in the surgical defect, i.e., the inner layers facing the brain, probably influenced by a greater stability of the particles.

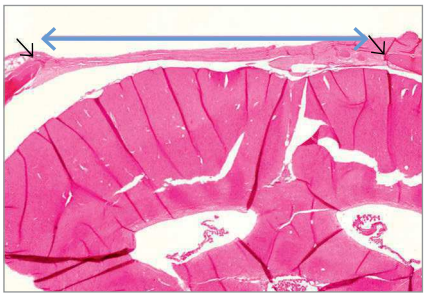

Figure 2 - Photomicrograph of control group showing critical size defects with no bone fill. Black arrows: Margin of bone defect. Blue arrow: Extension of bone defect.

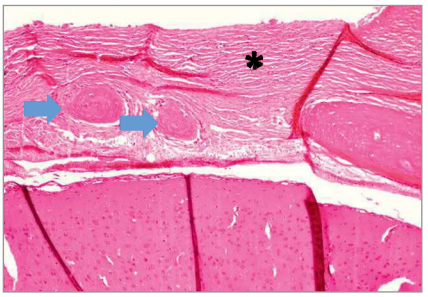

Figure 3 - Photomicrograph showing maximized fibrous connective tissue (*) and outline of bone formation near margin of defect (arrows).

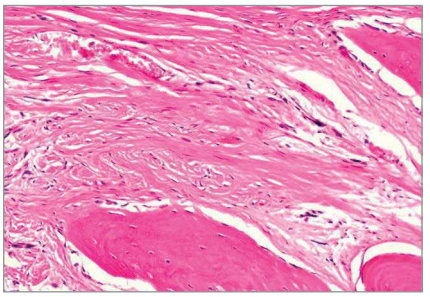

Figure 4 - Photomicrograph showing detail of connective tissue and no inflammatory infiltrate.

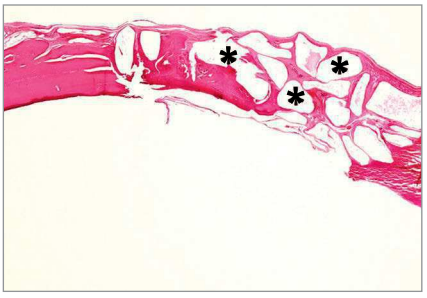

Figure 5 - Photomicrograph showing bone defect filled with GenPhos (*). Space occupied by GenPhos particles.

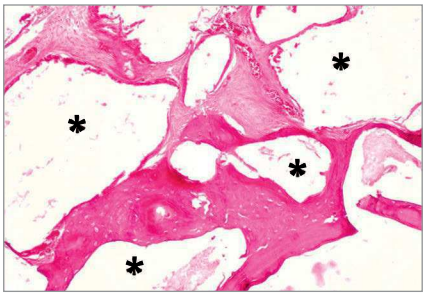

Figure 6 - Zoomed area shows GenPhos (*) particles in contact with connective tissue and with newly formed bone.

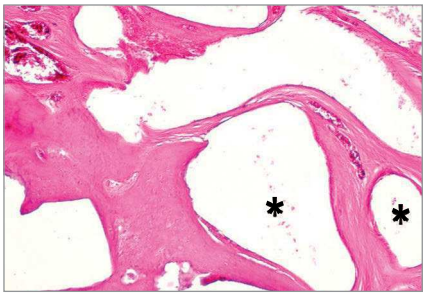

Figure 7 - Zoomed area shows GenPhos (*) particles in contact with connective tissue.

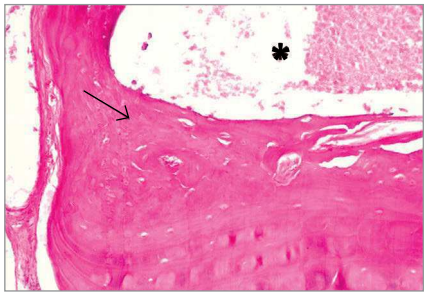

Figure 8 - Zoomed area shows GenPhos (*) particle in contact with newly formed bone (arrow).

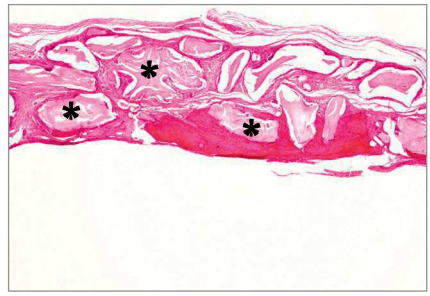

Figure 9 - Photomicrograph showing bone defect filled with GenMix. (*) Space occupied by GenMix particles.

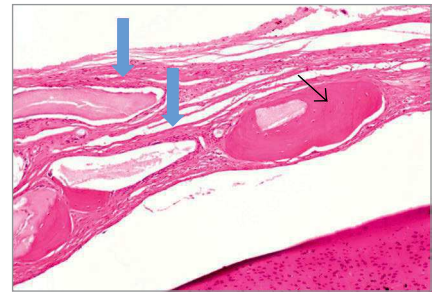

Figure 10 - Zoomed area shows GenMix particle surrounded by connective tissue (blue arrows) and by newly formed bone (black arrow).

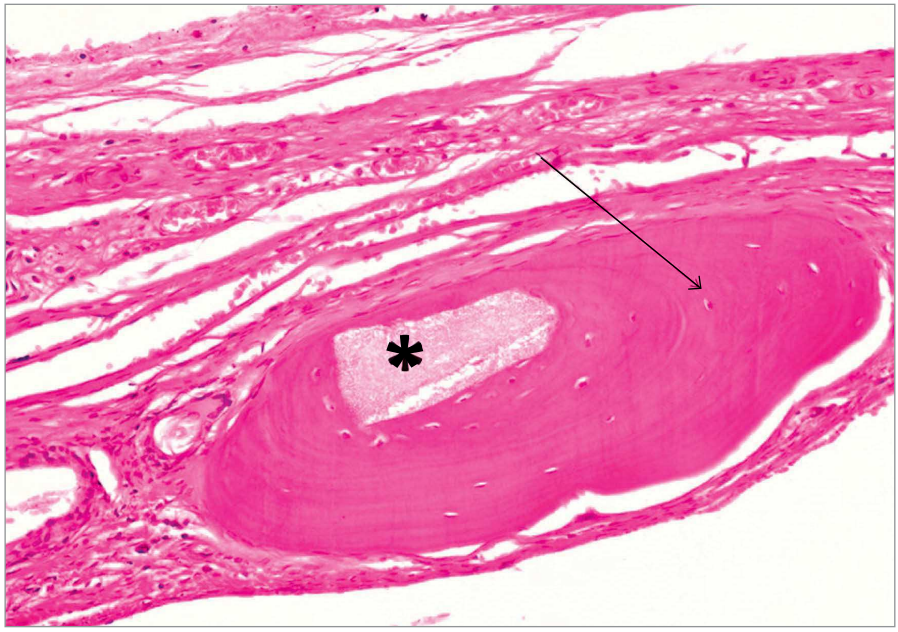

Figure 11 - The detail shows GenMix (*) particle surrounded by newly formed bone (arrow).

Table 1 - Connective tissue.

	Connective tissue	Newly formed bone tissue	Remaining material
Control	78.5%	21.5%	
Group 1	38.5%	32.5%	29%
Group 2	10%	45.9%	44.1%

Likewise, in Group I, most of the surgical defect was occupied by the material and its particles implanted at the site. Many particles of the material displayed part of their surface in direct relation to the newly formed bone and bone cell colonization. The other portion of the particle surfaces — which was also the largest — interfaced with the foreign body granulomas comprised of macrophages and inflammatory multinucleated giant cells. The morphological interaction with osteoblast cells can be clearly perceived (Figs 9-11) since the tissue components were very well stained and different from one another, which did not occur in Group II, probably due to technical problems. New bone formation around the particles — which was also found in this group — is more closely linked to those located more internally and closer to the brain. There is no infiltration of neutrophils in the specimens examined indicating the absence of bacterial contamination.

That these findings differ from the control group is quite evident, since morphologically new bone formation was found in the critical size surgical defects in both materials.

Histometric analysis results

By superimposing the Merz grid, histometry of the histological sections was performed taking into account in each group the presence of connective tissue, newly formed bone tissue and remaining material. Percentage means of the results are shown in Table 1.

By using the t-test to compare the amount of newly formed bone, connective tissue and remaining material between the groups, a statistically significant difference was found in the remaining material of Groups I and II (p=0.007), and in the amount of connective tissue between Groups I and II (p=0.002). There was no statistically significant difference in the amount of new bone formation between Groups I and II (p=0.066). When Groups I and II were compared with the control group, statistically significant differences were found in terms of connective tissue and in bone formation in Group II. New bone formation in Group I compared with the control group was not statistically significant.

Discussion

Control group results revealed that the size of the bone defect used in this study is indeed a critical size defect as it exhibits new collagenized connective tissue formation and no bone formation in its interior.

As described in the results, Groups I and II were similar with respect to cellular phenomena and new bone formation occurred amid a large amount of remaining material, which clearly showed the osteoconductive potential of GenMix and GenPhos, thereby corroborating the literature.[17-23] Histometric evaluation, however, disclosed a more effective osteoconductiveness in Group II, perhaps due to (1) inorganic characteristics of ceramics, (2) Resorption of β-TCP, and (3) presence of a greater amount of biomaterial after 60 days in the bone defect area (44.1% in Group II and 29% in Group I).

This outcome reflects an interesting clinical applicability. It is a well known fact that in lifting the maxillary sinus with autogenous bone or organic bone substitutes graft material is "resorbed," thus reducing the height of newly formed bone. The presence of remaining material for a longer period than the autogenous bone or organic bone substitute can reduce this "resorption," allowing the placement of greater length implants in the region of the sinus graft, which might theoretically increase the success rate of the implants.[24,25]

The stability of bone resorption in maxillary sinus lifting occurs after 2 or 3 years when a 2:1 ratio of autogenous bone to inorganic xenogenic bone is used.[24]

Other authors have compared the resorption of bone graft in maxillary sinus lifting with deproteinized xenogenic bone, xenogenic inorganic bone and porous hydroxyapatite and reported a higher resorption with xenogenic inorganic bone, concluding by suggesting that the latter be mixed with synthetic materials to reduce the resorption of newly formed bone.[26]

Another study evaluated the success rate of implants in the posterior maxilla with and without maxillary sinus lifting using β-TCP. In this study, the authors reported the failure of one implant in each group prior to the prosthetic phase (more than 99% success) and concluded that β-TCP can be used for implant placement.[27]

Histomorphometrically, in comparing TCP with autogenous bone in maxillary sinus lifting, nearly 32% consisted of new bone formation with no statistical difference between the groups. Furthermore, the amount of connective tissue was similar and bone marrow formation was higher in the autogenous bone group.[28]

Cellular phenomena during bone formation with synthetic materials are not well defined. Some authors have reported that the biomaterial particles appeared surrounded by connective tissue,[4,18,29] as shown in Figures 6 and 10. Others have reported finding bone on the surface of the particles (Figs 8 and 11).[19-23] However, this difference in the description of cellular phenomena is due to the type of animal used in the experiment and the assessment time. A study in dogs revealed the presence of particles surrounded by connective tissue within 4 weeks, and 28 weeks later the presence of particles surrounded by newly formed bone as well as the presence of foreign body type granulomas in earlier periods.[30] These findings corroborate the results of this study in rats and provide a similar description of the changes in cell population.

It is therefore obvious from the study results that these materials can be used for reconstructing bone defects for subsequent rehabilitation with osseointegrated implants.

Conclusions

This study has led to the conclusion that the tested materials in the experiment feature osteoconductive properties and can be used clinically for filling cavities. A significant difference was found between the use of these materials and the control group, which was filled with blood clots.

Further investigation is warranted to measure the amount of remaining material left by these materials, the amount of new bone formation and bone resorption in the long term.

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