Use of BioGran and Calcitite in Bone Defects: Histologic Study in Monkeys (*Cebus apella*)

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The present study compares the biologic behavior of BioGran and Calcitite as fillers for surgical cavities in the mandibles of 4 adult monkeys (*Cebus apella*). The surgical cavities were prepared through both mandibular cortices, with a diameter of 5 mm, in the angle region. Two cavities were prepared on the right side and 1 on the left and divided into 3 groups: R_1 sites were filled with bioglass (BioGran), R_2 sites were not filled, and L sites were filled with hydroxyapatite (Calcitite). After 180 days the animals were sacrificed and the specimens were removed for histologic processing. Results showed no bone formation in group R_2 (empty cavities). BioGran-treated sites showed bone formation and total repair of the bone defect, and the bioglass particles were almost totally resorbed and substituted by bone. The few remaining crystals were in intimate contact with newly formed bone. Calcitite did not allow bone formation, and granules inside the cavities were involved by connective tissue. Based upon those results, the authors concluded that bioglass resulted in total obliteration of the surgical cavity with bone and hydroxyapatite was present in a large amount and involved by connective tissue, without bone formation.
(Int J Oral Maxillofac Implants 1999;14:859–864)

Key words: bioglass, hydroxyapatite, new bone formation

Autogenous bone is generally considered the best material for filling bony defects. However, clinical situations, such as the size of the bony defect, absence of enough donor tissue, or the need for a second surgical intervention, may preclude its use. In the attempt to eliminate these difficulties, biomaterials have been developed. Among others, calcium phosphate materials, such as hydroxyapatite (HA), have a chemical structure similar to the mineral part of the bone. The most attractive characteristics of hydroxyapatite are the absence of local or systemic toxicity, absence of inflammatory or foreign body responses, and the bonding of those materials to bone,[1–4] although there may be woven connective fibrous tissue between the implanted material and bone.[5,6] Those materials also possess osteoconductive activity; that is, they provide a physical matrix suitable for deposition of new bone and can stimulate bone growth.[1,6–8] Hydroxyapatite can be classified as dense or porous, according to its physical characteristics. The dense form biodegrades more slowly than the porous form, and its resistance to compression is greater.

Another ceramic material is calcium phosphate containing glass or glass-ceramics. The bonding between these materials and bone results in a series of reactions that leads to the formation of a gel-like silicate-rich interlayer from which most of the calcium and phosphate ions have leached out.[9,10] After grafting of the material, a dissolution of the

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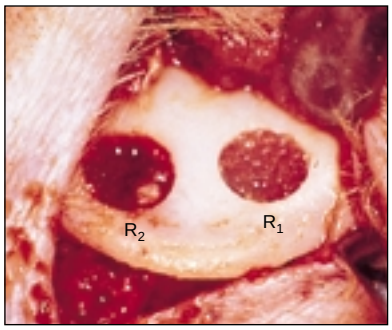

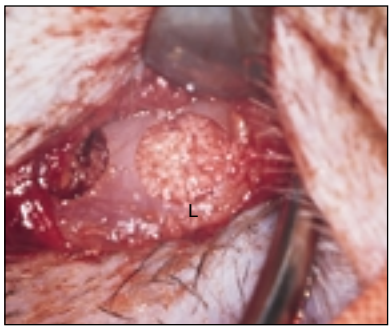

Fig 1a *(Left)* Cavity R_1 was filled with bioglass (BioGran) and Cavity R_2 was left unfilled.

Fig 1b *(Right)* Cavity L was filled with hydroxyapatite (Calcitite).

central part of the granules occurs because of the formation of a gel-like silicate. The lacunae formed by resorption of that gel will allow bone formation inside and around the granules, being the material incorporated to the newly formed bone.[3,11]

The forms of dense HA available provide different results when used to fill bone cavities.[6,10,12–14] The results reported with the use of bioglass are promising.[10,15–18] It is important to know the histologic, biologic behavior of those materials, so that they can be effectively and safely used. The purpose of this study was to evaluate and compare, histologically, the effectiveness of Calcitite (dense HA, Calcitek, San Diego, CA) and of BioGran (bioactive glass, Orthovita, Malvern, PA) as fillers for surgical bone defects in the jaws of *Cebus apella* monkeys.

Materials and Methods

Four young adult male monkeys were used, with weight ranging from 2 to 2.5 kg and age determined as described by Schultz.[19] Before surgery, the animals were maintained in the Primate Procreation Nucleus, Dental School at Araçatuba, UNESP, and received feedings of bananas, corn, rations, eggs, and varied fruits. After fasting for 15 hours, the animals were weighed and anesthetized with Tionembutal, aqueous solution, in the dosage of 30 mg/kg. At this time, penicillin G-procaine and penicillin G-potassic crystalline with streptomycin 300,000 IU veterinary were administered in a single intramuscular dose for each animal.

After trichotomy, the submandibular region of each animal was cleansed with Povidone-iodine surgical scrub. To prevent excessive bleeding, a solution of adrenaline 1:400,000 was infiltrated along the area to be incised. The incision was accomplished on the skin of the animal in the submandibular region, with divulsion of the subcutaneous tissue and platisma muscle. The masseter was incised, so that the mandibular angle was exposed. The procedure was done bilaterally. With a pneumatic handpiece and trephine drill (5-mm-diameter, Implant Innovations, Palm Beach Gardens, FL), under external and intense irrigation with physiologic saline solution, 2 cavities were prepared through both mandibular cortices on the right side, and 1 cavity was prepared on the left side. All preparations had a diameter of 5 mm.

At this time, the cavities were divided into 3 groups:

- Cavity R_1: filled with BioGran (Fig 1a);
- Cavity R_2 (control): left with no material for filling of the surgical bony defect (Fig 1a);
- Cavity L: filled with Calcitite (Fig 1b).

After bleeding was controlled, the cavities were filled with the corresponding material, with the exception of R_2, and the soft tissues were closed with 4-0 polyglactin-910. In the immediate postoperative period, 20 mg of diclofenac potassic were administered through an intramuscular injection.

The animals were maintained in individual cages during the entire experimental period and sacrificed after 180 days. For sacrifice, they were again anesthetized, and after thoracotomy, the right atrium was sectioned. A catheter was introduced into the left ventricle and 4 L of heparinized saline solution 0.9% were infused to wash the circulatory system. After that, 4 L of neutral 10% formaldehyde were infused. The jaws were dissected and the specimens with the surgical cavities were reduced by removing all muscle attachments.

After routine laboratory procedures were performed,[20] the specimens were embedded in paraffin and histologic serial sections were prepared. These were 6 µm thick and taken as transverse sections of the specimens. Tissue reaction, amount of newly formed bone, bone characteristics, and presence or absence of the implanted materials were evaluated.

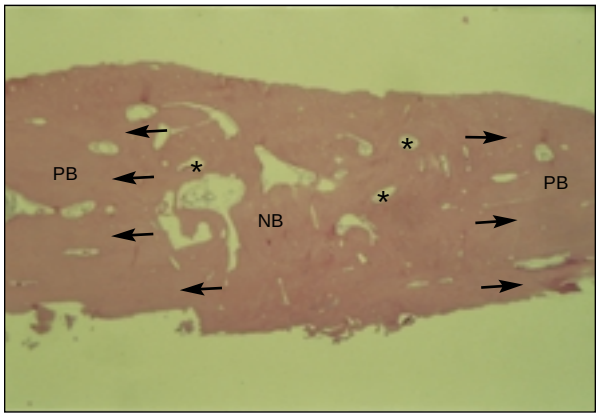

Fig 2 Photomicrograph of a cavity filled with BioGran (R_1). Note the presence of newly formed bone (NB), with an orientation that differs from that of the pre-existing bone (PB), and the edge of the cavity (*arrows*). There are few granules of BioGran (*asterisks*) bonded to new bone (hematoxylin-eosin, original magnification ×25).

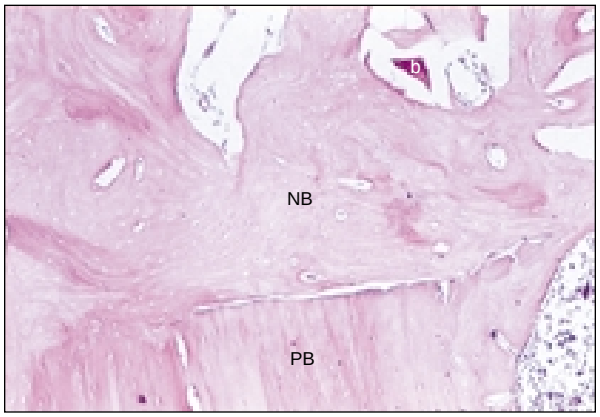

Fig 3 Note the presence of the Haversian system and different orientation of newly formed bone (NB) and pre-existing bone (PB). A granule of BioGran (b) is bonded to new bone (hematoxylin-eosin, original magnification ×40).

Results

The histologic evaluation revealed the following results:

- Cavities R_1 (BioGran) were completely repaired by newly formed bone (Figs 2 to 4) characterized by the organization of a Haversian system (Figs 3 and 4). Reversion lines limited newly formed bone and pre-existing bone (Figs 2 to 4). The newly formed bone demonstrated other features of the Haversian system, presenting a larger amount of osteocytes that showed a certain immaturity in relation to the surrounding bone, indicating a process of bone repair (Figs 3 and 4). A small amount of material could be seen in close contact with newly formed bone, and connective tissue was not observed between that material and the newly formed bone (Figs 2 to 4).
- Cavities R_2 (control) were not filled by bone. Fibrous tissue and muscle were present inside the cavities (Figs 5a and 5b). The bony edges of the cavity were remodeled (Figs 5a and 5b), with the presence of some osteoblastic activity. The connective tissue presents discrete inflammatory infiltrate (Fig 5a).
- Cavities L (Calcitite) were filled by fibrous connective tissue and muscle that involved the implanted material (Figs 6a to 6c). Some granules of the material were inside giant cells, characterizing macrophagic activity (Fig 6b). At the edge of the cavity, bone remodeling was seen, with deposition and resorption of bone (Figs 6a and 6b) exhibiting osteoblastic activity. Fibrous connective tissue was seen between granules and bone (Figs 6a to 6c), showing the absence of bonding of bone and materials. The connective tissue demonstrated mild inflammatory infiltrate characterized by the presence of mononuclear cells (Fig 6c).

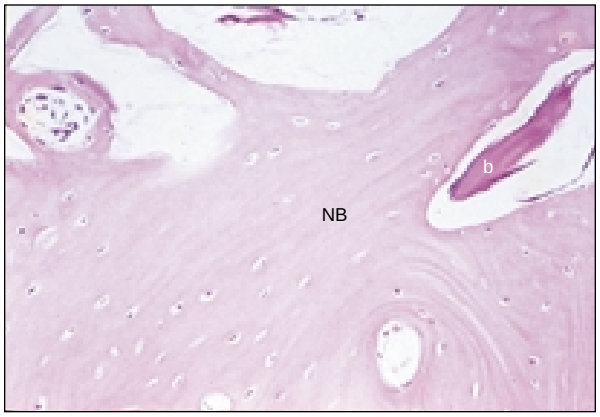

Fig 4 Newly formed bone (NB) with residual BioGran particles (b). There is no fibrous tissue between the material and bone (hematoxylin-eosin, original magnification ×400).

Discussion

Through the surgically created bone defects in the mandibular angle of monkeys, biocompatibility, resorption, osteogenic potential, and bonding of the introduced materials (BioGran and Calcitite) to bone tissue were evaluated. Manipulation of the materials differed from each other. The bioglass formed a cohesive mass when in contact with the blood of the animal, a feature that ensured its accommodation in the cavity with minimum loss of

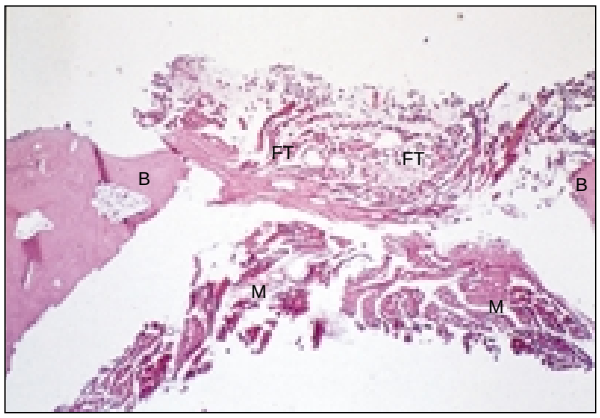

Fig 5a Photomicrograph of the cavity without filling (R_2). Note the presence of fibrous tissue (FT) and muscle (M) and bone remodeling (B) at the edge of the cavity (hematoxylin-eosin, original magnification ×25).

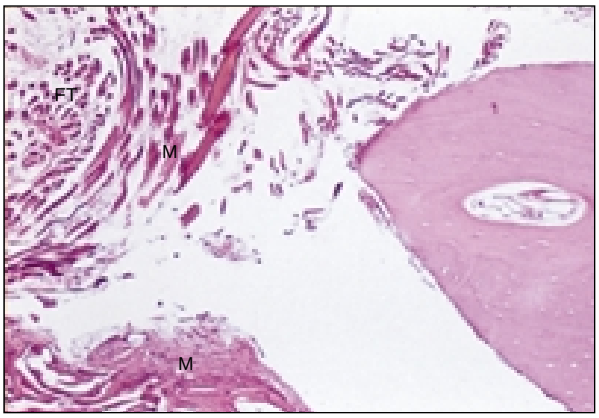

Fig 5b Fibrous tissue (FT) and muscle (M) in the cavity. Osteoblastic activity is taking place at the edge of the cavity (hematoxylin-eosin, original magnification ×40).

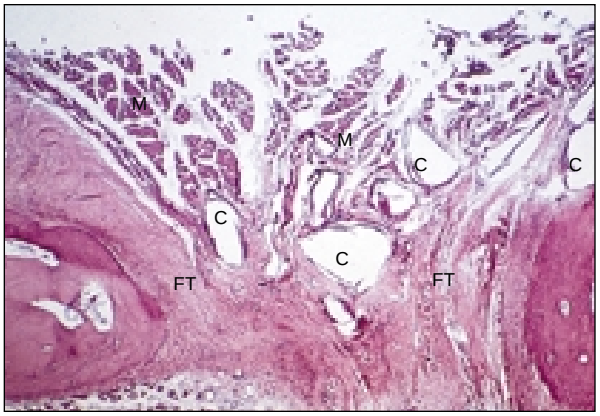

Fig 6a Photomicrograph of the cavity L filled with Calcitite (C). Fibrous tissue (FT) and muscle (M) surround the particles of Calcitite. Bone remodeling is apparent at the edge of the cavity (hematoxylin-eosin, original magnification ×25).

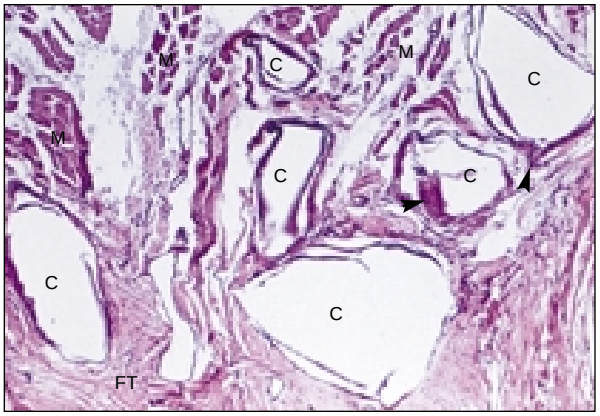

Fig 6b Calcitite granules (C) are surrounded by muscle (M) and fibrous tissue (FT). Note the presence of giant cells (*arrowheads*) (hematoxylin-eosin, original magnification ×400).

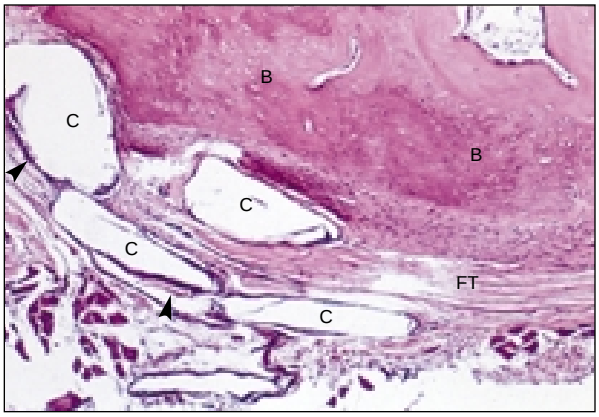

Fig 6c Note the giant cells around the Calcitite granules and the presence of fibrous tissue between Calcitite and bone (hematoxylin-eosin, original magnification ×100).

the material during implantation. It seemed to assist in controlling bleeding. The HA, even after hemostasis of the cavity, showed a tendency to move when in contact with blood. Consequently, because of the characteristics of the material, displacement of Calcitite granules may occur during stabilization of the clot and the initial healing period.

Biocompatibility of the bioactive glass (BioGran) used in this experiment was shown by the few granules of the remaining material in the defect that were bonding to bone tissue without the presence of inflammatory cells (Figs 2 to 4), as reported by Schepers et al.[10,21] The presence of a few granules of BioGran inside newly formed bone demonstrates that resorption or dissolution of the material occurred during the period of analysis, and the few remaining granules of the material were bonded to bone with the presence of osteocytes, as demonstrated by Schepers et al[10,16] and Furusawa and Mizunuma.[15] The material aspect inside the newly formed bone suggests that the process (described in the literature) of formation of chambers in the granules, as a result of removal of the gel-like silicate, allowing bone formation around the inside of bioactive glass granules, has occurred.[10,16,21,22] This demonstrates that the material was a good scaffold for bone formation.

The HA (Calcitite) showed granules involved by giant cells (Fig 6b), which can characterize a foreign body reaction. Another possibility is that a process of slow resorption of the material was occurring. Therefore, after 180 days there were a large number of granules involved by inflammatory cells (Figs 6a to 6c). These results are similar to those of Schepers et al,[10] who observed that, after a period of 12 months, there was disintegration of the particles of Calcitite involved by connective tissue cells. However, those results differed from those seen by Misiek et al,[13] who described the presence of a giant cell foreign body reaction only in the initial period of their investigation, while in the longer term (180 days), resolution of the inflammatory process was described. Butler et al[14] observed similar results 90 days after implantation in rats. The behavior of Calcitite in bone defects was also evaluated by Bye et al[12] and Pettis et al.[6] These authors affirmed that continuous bony formation took place, probably the result of the characteristics of the bony defects, because the literature suggests that more available bone walls will result in improved new bone formation.

Furthermore, in transfixing the defects in this experiment, because of their location (mandibular angle), there was more probability of displacement of the material as a result of the presence of the masseter and medial pterygoid muscles, favoring the ingrowth of connective tissue in the bone defect. The lack of resorption of the materials also complicates the process of bone substitution. It is known that the synthetic form of HA is generally nonresorbable, and the dense form has less tendency to resorb than the porous form because of the surface area.[23] The dense form permits peripheral formation, while the porous allows diffuse tissue ingrowth.[1] This renders the materials weaker than the dense crystals, limiting their use as permanent implants.[1] However, Klein et al,[24] in their evaluation of dense microporous and macroporous HA, did not find histologic differences in response to those materials.

Some granules of the material were involved by giant cells, which raises the question of the osteoconductive capacity of the material as described by some authors,[1,6–8,22] because 180 days should be enough time for bone repair to take place. Some articles have described bonding between HA and bone.[1,6–8] However, in this experiment, most Calcitite granules were involved by fibrous connective tissue (Fig 6), and even those granules close to bone presented involvement by connective tissue. It could be proposed that Calcitite could be used as a bone filling material, but that in this investigation it did not present osteoconductive characteristics, although it did not allow penetration of muscle as occurred in the control sites.

Conclusions

According to the methodology used in this investigation, it can be concluded that bone healing did not occur in the surgical transfixing of bone defects 5 mm in diameter in the mandibular angle of monkeys. BioGran allowed new bone formation, with total repair of the defect. The material was largely resorbed and replaced by bone, and the remaining particles were in close contact with bone. Calcitite did not facilitate repair of the bone defect by newly formed bone, and the granules inside the cavity were involved by fibrous connective tissue.

Acknowledgments

The authors thank Dr Carlos Benatti, who contributed to histologic analysis; Dr Idelmo Rangel Garcia Jr for assistance during the surgical experimental procedures; and Dr José Américo de Oliveira, chairman of the Primate Procreation Nucleus of the Dental School of Araçatuba, UNESP.

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