Comparison of Biomaterial Implants in the Dental Socket: Histological Analysis in Dogs
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ABSTRACT

Background: Bone graft procedures have been used commonly in buco-maxillo-facial surgery. For this reason, many researchers have evaluated the bone substitutes.

Purpose: The present study evaluated soft and hard tissue reactions to two different hydroxyapatites HAs (synthetic HA and natural HA) and bioactive glass implanted into the sockets immediately after extraction.

Materials and Methods: First and third upper and lower premolars, on both sides, were extracted from six female dogs. The alveolar sockets were randomly assigned to four groups: Group 1 – control (unfilled), Group 2 – filled with synthetic hydroxyapatite, Group 3 – filled with bovine bone mineral (natural HA), and Group 4 – filled with bioactive glass. The animals were euthanized at 4 weeks (n = 2), 8 weeks (n = 2), and 28 weeks (n = 2) after extraction. The mandible and maxilla of each animal were removed for histological analysis to determine soft tissue reactions, newly formed bone, bone characteristics, and presence or absence of implanted materials.

Results: Most particles of synthetic hydroxyapatite had bone formation on their surface, although some particles showed a layer of fibrous connective tissue. The bovine bone mineral group exhibited particles partially replaced with bone formation. The bioactive glass group showed particles with a thin layer of calcified tissue, but was absent in some specimens, suggesting complete resorption.

Conclusion: All biomaterials had similar behavior. Bovine bone mineral, compared to synthetic hydroxyapatite and bioactive glass, showed a larger number of particles covered with osseous tissue. All biomaterials interfered with the socket repair process.

KEY WORDS: bioactive glass, biocompatibility, biomaterials, bone substitute, hydroxyapatite

INTRODUCTION

Several studies in the past have focused on determining an ideal biomaterial to be used as a bone substitute. Autogenous bone grafts (ABGs) are most widely used by surgeons for ridge augmentation and for reconstruction of osseous defects because these grafts contain viable cells such as bone marrow osteoprogenitor cells, collagenous and noncollagenous extracellular matrix, and growth/differentiating factors.1–3

Therefore, ABGs are still considered the “gold standard” therapy for bone repair because of its osteogenesis, osteoinduction, and osteoconduction properties. The major disadvantages of ABG are donor site morbidity, limitations on the quantity of grafted materials, and high cost.4–7 Other complications such as viral transmission and immunogenicity are of serious concern for allografts and xenografts. Therefore, there is a critical
need for the development of bone substitute materials that match the properties of bone without the drawbacks of autografts or allografts, that can be supplied at any time, in any amount, and at lower costs.8–11

Considerable attention has been directed toward the use of synthetic grafts including hydroxyapatite (HA), tricalcium phosphate, and bioactive glass (BG).12–18 HA bone substitutes have been developed synthetically, derived from corals or algae, or naturally derived from bone mineral.12,13,16,17,19–22 BGs are a class of biomaterials based on amorphous silicate compounds. When granules are implanted in the bone tissue, these granules fully react to form internal silica gel cores (Si-rich) with calcium phosphate-rich surfaces (Ca-P-rich). In this process, the internal silica gel core degrades, leaving an external calcium phosphate bulk. Inside the excavated granules, osteoprogenitor cells differentiate and form new bone tissue, which does not originate from the external surface of the granule or from preexisting bone.10,18,23–26

The purpose of this qualitative study was to evaluate tissue reaction to two different hydroxyapatites (synthetic and natural) and bioactive glass when implanted into fresh extraction sockets in dogs.

MATERIALS AND METHODS

Six 2-year-old male mongrel dogs in good general health were used (approximately 15-kg body weight) in this study. The animals were kept in individual cages with access to food and water ad libitum. Prior to the surgical procedures, all the animals were allowed to acclimatize to the facility environment for a period of 7 days. The surgical procedures were performed under general anesthesia with 0.05 mg/kg of subcutaneous preanesthesia sedation (atropine sulfate 0.5 mg, Ariston Inds. Química e Farms. Ltda, São Paulo, Brazil) followed by intravenous injection of a 3% sodium pentobarbital solution (0.5 mL/kg Tiopental™, Abbott Laboratório do Brasil Ltda, São Paulo, Brazil). Hydration was maintained by intravenous infusion with 0.9% NaCl solution during the whole surgical procedure.

Tooth Extraction

The extractions were performed on the right and left first and third mandibular and maxillary premolars. Thus, eight teeth were extracted per animal. As the third premolar teeth are biradicular, 12 alveolar extraction sites were available per animal for bone filling, a total of 72 extraction sites.

After disinfection of the oral cavity with 2% chlorhexidine solution, 2% lidocaine HCl with epinephrine (1:100,000) was administered by infiltration at the buccal and lingual aspects. A crevicular incision was made around the necks of the teeth, and full-thickness buccal and lingual mucoperiosteal flaps were raised to expose the crestal bone. Gingival incisions were extended mesially from canine and distally to the molar. In the biradicular teeth, a vertical interradicular section was performed with a dental bur under sterile irrigation to separate all teeth into two pieces and to avoid root and alveolar cortical bone fracture during extraction. Each root was carefully mobilized with a dental elevator and then was gently extracted with dental forceps (Figure 1A). All alveolar sites were checked visually after irrigation with 0.9% NaCl solution.

Next, the alveolar extraction sites were divided randomly and a split-mouth design was established in four groups: Group 1, control (unfilled) – 13 alveolar sockets; Group 2, filled with synthetic HA (OsteoGen™, Implant Dent Ltd., Holliswood, NY, USA) – 15 alveolar sockets; Group 3, filled with bovine bone mineral (natural HA; Bio-Oss™, Osteohealth Company, Shirley, NY, USA) –

![Figure 1](https://example.com/figure1.png)

**Figure 1** Photographs of surgical procedures. (A) Tooth extraction: after the root was separated with a dental bur, each piece was mobilized with a dental elevator and dental forceps before extraction. (B) Alveolar sockets filled with biomaterials (*).
16 alveolar sockets; and Group 4, filled with BG (Biogran™, Orthovita, Palm Beach Gardens, FL, USA) – 15 alveolar sockets (see Figure 1B). A total of 13 alveolar sockets were excluded from the study because of root and alveolar bone fracture during extraction. The connective tissue surfaces of the buccal and lingual flap margins were carefully joined together with nonresorbable hermetic sutures (3-0 silk sutures, Ethicon™, Johnson & Johnson S/A, São José dos Campos, Brazil).

**Postoperative Care**

After the surgical procedures, the animals were given an anti-inflammatory medication (betamethasone 2 mg, Celestone™, Schering-Plough S/A, Rio de Janeiro, Brazil) twice a day and with appropriate analgesic (paracetamol) for 3 days following surgery in order to reduce postoperative swelling and pain. Antibiotics were given through a single intramuscular injection of penicillin G benzathine (600,000 UI–40,000 UI/kg). The animals were checked daily and were fed with a soft-consistency diet. The sutures were removed under short general anesthesia 2 weeks after implantation, after which a normal diet was given.

The animals were euthanized 4 weeks ($n=2$), 8 weeks ($n=2$), and 28 weeks ($n=2$) postextraction through the induction of deep anesthesia followed by intravenous pentobarbital. The mandibles and maxillas were removed and then sectioned buccolingually into small tissue blocks isolating each extraction site using the remaining teeth as reference points. Specimens were fixed in 10% neutral buffered formalin for 10 days and were decalcified by Morse solution (1:1 of sodium citrate 20% and formic acid 50%). The specimens were embedded in paraffin, and histological serial sections were prepared. Six-micrometer-thick tissue sections were prepared as transverse (buccolingually) sections and were stained with hematoxylin and eosin. Tissue reactions, newly formed bone, bone characteristics, and presence or absence of implanted materials were qualitatively evaluated.

**RESULTS**

The animals did not present any complications following the immediate postoperative period until sacrifice, and no materials were rejected. The histological evaluation revealed the following results.

**Four Weeks**

**Group 1 – Control (Unfilled).** It was observed that the marginal soft tissue compartment harbored a well-organized fibrous connective tissue that was lined with keratinized epithelium. In addition, most of the extraction socket was filled with newly formed bone. This bone contained a large number of primary osteons and was contiguous with the old bone of the socket walls. In some areas, the woven bone was undergoing osteoclastic resorption. Osteoclasts were also observed in the surface of the old lamellar bone, indicating that the process of modeling/remodeling of the newly formed bone had begun (Figure 2A).

**Group 2 – Filled with Synthetic HA.** The specimens showed fibrous connective tissue in the cervical and middle portions of the socket. Presence of fibroblasts, blood vessels, and a few inflammatory cells was observed. Biomaterial particles were also seen to be involved by connective tissue (see Figure 2B).

**Group 3 – Filled with Bovine Bone Mineral – (Natural HA).** Many osteoblasts were observed on the surface of the apical portion of the woven bone. Connective fibrous tissue with collagen fibers and fibroblasts involving particles of biomaterials, which was partially resorbed, was located laterally to the woven bone (see Figure 2C).

**Group 4 – Filled with BG.** Extraction sockets exhibited newly formed bone with particles of biomaterials. Collagen fibers with variable thickness and a few inflammatory cells were observed among the osseous trabeculae. Additionally, granules of biomaterials showed fissures with infiltration cells (see Figure 2D).

**Eight Weeks**

**Group 1 – Control (Unfilled).** All specimens showed a newly formed hard tissue, primarily composed of woven bone, separating the marginal mucosa from the extraction socket (Figure 3A).

**Group 2 – Filled with Synthetic HA.** Particles of biomaterials present were close to the socket wall and were involved by newly formed bone. Several osteoblasts were observed on the trabeculate surface. In the central part of the socket, particles of biomaterials were involved by a thin calcified tissue that was involved by connective
tissue. The particles did not induce inflammatory response (see Figure 3B).

**Group 3 – Filled with Bovine Bone Mineral – Natural HA.** Extraction sockets showed particles of biomaterials with different sizes in the cervical portion of the socket. In addition, the newly formed bone had several osteocyte involved particles of biomaterials. The newly formed bone exhibited reversion lines opposite to the concentric woven bone (see Figure 3C).

**Group 4 – Filled with BG.** Newly formed bone exhibited several osteocytes. Particles of biomaterials showed resorption process with fissures and cracks; the central area was infiltrated by connective cells. A slight inflammatory reaction and the presence of dense fibrous connective tissue were observed (see Figure 3D).

**Twenty-Eight Weeks**

**Group 1 – Control (Unfilled).** All extraction sockets were completely filled by compact bone tissue with layers of lamellar bone that was deposited on top of the previously formed woven bone. Concomitantly, collagen fibers from the mucosal lining had become inserted in the new “cortical” bone and, hence, a periosteum-like structure had been established (Figure 4A).

**Group 2 – Filled with Synthetic HA.** Some particles of biomaterials were involved by connective tissue, although the majority was involved by bone tissue (see Figure 4B).

**Group 3 – Filled with Bovine Bone Mineral – Natural HA.** Particles of biomaterials were involved by bone tissue. The direction of woven bone in some extraction sockets was random and was concentric in others. In some specimens, particles of biomaterials were involved by connective fibrous tissue. The histological aspects in this group were more heterogeneous than in other groups (see Figure 4C).

**Group 4 – Filled with BG.** Some extraction sockets did not have biomaterials and showed compact bone, suggesting total resorption. Other specimens, however, exhibited particles of biomaterials involved by a thin calcified tissue. Fissures and cracks were observed in the

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**Figure 2** Photomicrographs at 4 weeks: (A) Group 1 (control site): The majority of the extraction socket was filled with newly formed bone (arrows). (B) Group 2 (synthetic hydroxyapatite site): Specimens showed particles of biomaterials (*) involved by connective tissue. (C) Group 3 (bovine bone mineral site): Particles of biomaterials (*) were involved by connective fibrous tissue. (D) Group 4 (bioactive glass site): Extraction sockets exhibited newly formed bone with particles of biomaterials (*). Granules of biomaterials showed fissures (arrows) with cell infiltration (arrowheads). Hematoxylin and eosin stain; original magnification ×60.
particles, and the central portion showed connective cells. This group showed some variation among specimens (see Figure 4D).

**DISCUSSION**

The aim of this study was to evaluate tissue reaction to two different HAs (synthetic and natural) and BG when implanted into fresh extraction sockets in dogs.

Many experiments have been conducted in dog models to study the bone filling of periodontal defects or extraction sites.9–13,18,19 Dental socket morphology is similar in humans and dogs, and the healing process has been studied extensively, with some interference of normal healing events being easily detected microscopically. Thus, dental sockets have been considered adequate for the study of the biological behavior of biomaterials in the oral cavity.9,27

The control group specimens showed a better healing response than the experimental groups, and through deposition and bone resorption, the extraction socket was filled by lamellar bone after 4 weeks. After 8 weeks, all extraction alveolar sockets were filled by lamellar bone, and after 28 weeks, extraction alveolar sockets showed compact bone in the control group. This result is in accord with other studies using this model.11,27 However, Gauthier and colleagues9 showed similar results between the test and control groups, although in this study, extraction sockets were debrided with a dental curette to remove the periodontal ligament, which consequently interfered with the normal sequence of alveolar healing. Normal healing requires the presence of a periodontal ligament because the periodontal ligament cells proliferate and migrate into the center of the extraction socket where they differentiate into osteoblasts that have become involved in the formation of new bone.27 In the present study, the alveolar socket was simply irrigated with 0.9% of NaCl solution and no manipulations were made to the periodontal ligament.

Because synthetic HAs are not able to induce bone formation when implanted in subcutaneous and muscle tissues, these biomaterials are considered to be osteoconductive and do not produce chronic or acute

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**Figure 3** Photomicrographs at 8 weeks: (A) Group 1 (control site): Specimens showed a newly formed hard tissue, mainly composed of woven bone. (B) Group 2 (synthetic hydroxyapatite site): Particles of biomaterials (*) close to the socket wall were involved by newly formed bone; in the central part of the socket, biomaterials were involved by thin calcified tissue, which was involved by connective tissue. (C) Group 3 (bovine bone mineral site): Specimens showed particles of biomaterials (*) involved by newly formed bone and connective fibrous tissue. (D) Group 4 (bioactive glass site): Particles of biomaterials (*) showed resorption process with fissures and cracks (arrows); the central area was infiltrated by connective cells (arrowheads). Hematoxylin and eosin stain; original magnification ×60.
inflammatory response. The results, however, are controversial, with some studies showing particles of biomaterials predominantly involved by connective fibrous tissue, and in other studies, newly formed bone is observed on the particle’s surface. The reason for the disparate results may be explained by the different sites of implantation, variation in the animal species, and periods of analysis. Within 4 weeks, synthetic HA had particles involved by fibrous connective tissue; after 8 weeks, particles were found at the extraction socket margin involved by newly formed bone; and in the central socket portion, particles were involved by a thin calcified tissue. After 28 weeks, biomaterial particles were involved by bone tissue and sometimes were involved by fibrous connective tissue. These results are in agreement with other studies with similar synthetic HA implanted in different sites, confirming its osteoconductive properties. Considering the analysis time (4, 8, and 28 weeks), no inflammatory response was observed with this biomaterial, although a foreign body reaction was presented. These results are similar to other studies. Particles were not completely resorbed within 28 weeks but were partially resorbed in some specimens. Other studies showed similar results.

Alveolar extraction sockets implanted with bovine bone mineral (natural HA) for 4 weeks exhibited particles involved with fibrous connective tissue and, within 8 weeks, these particles had variable sizes in the connective tissue and the presence of newly formed bone. Finally, within 28 weeks, granules in some specimens were involved by connective tissue and by newly formed bone in others. Bovine bone mineral was not able to induce newly formed bone when implanted in muscle tissue and in maxillary defects in rats, although the particles were involved by fibrous connective tissue. Several studies in animals with natural HA (bovine bone mineral) implanted in different sites show particles involved by fibrous connective tissue and by newly formed bone. Studies in humans showed similar results, as bovine bone mineral implanted in periodontal defects and in extraction sockets exhibited particles involved sometimes by connective fibrous tissue and at other times by newly formed bone. Biocompatibility of this biomaterial has been shown in animal studies.
implanted in different sites such as the skull, maxillary sinus, tibia, mandible, alveolar sockets, and peri-implant defects. According to animal and human studies, bovine bone mineral particles do not induce an inflammatory response and are partially resorbed. These results are in agreement with this research. Although the bovine bone mineral did not induce inflammatory response in the extraction socket, it did show some particles involved by bone tissue, which was partially resorbed, while in other specimens, the particles were involved by connective fibrous tissue.

Several studies with BGs show that biomaterial particles are well tolerated by tissues and do not induce an inflammatory response. In this research, the results are similar, as no inflammatory response was observed surrounding the particles. BG, when mixed with blood before implantation, produced a cohesive mass, which improved the biomaterial retention. When implanted in different sites such as the mandible, tibia, femur, radius, alveolar ridge, maxillary sinus, periodontal defects, and peri-implant defects, BG exhibited particles involved by newly formed bone and no connective tissue. Fissures and cracks in particles and cellular infiltration could be observed. Therefore, BG particles are sometimes not completely resorbed and in some cases are only partially resorbed. Schepers and colleagues analyzed BG and observed bone growth around particles, while other ones were totally resorbed and replaced by bone tissue. Cancian and colleagues observed a small amount of biomaterial in contact with newly formed bone in mandible bone defects, with most of the particles being resorbed. In this study (sockets), BG exhibited particles involved by thick calcified tissue and showed fissures and cracks, with cellular infiltration observed in the central portion, suggesting central dissolution and silica gel release. After 28 weeks, some specimens showed compact bone and particle absence, suggesting resorption. These results are similar to those from other studies.

The qualitative analysis showed that bovine bone mineral had the highest number of particles involved by bone tissue. Synthetic HA had similar results of bovine bone mineral (natural HA) and showed particles involved with newly formed bone, while other particles were involved by fibrous connective tissue. BG had granules involved by a thin calcified tissue. In some specimens, particles were not observed. None of the tested biomaterial was completely resorbed, but showed different rates of resorption, probably because of differences in the chemical composition and physical forms.

In conclusion, the materials analyzed showed similar histological characteristics, with BG having the highest reabsorption rate. Bovine bone mineral had the most particles involved by bone tissue, followed by synthetic HA and BG. All biomaterials retarded the extraction socket healing. Further studies are necessary to define an ideal alloplastic material for long-term experimental investigations of periodontal defects and dental implant osseointegration after bone filling.

REFERENCES


