A Prospective, Randomized Controlled Preclinical Trial to Evaluate Different Formulations of Biphasic Calcium Phosphate in Combination With a Hydroxyapatite Collagen Membrane to Reconstruct Deficient Alveolar Ridges

Many patients and clinicians would prefer a synthetic particulate bone replacement graft, but most available alloplastic biomaterials have limited osteogenic potential. An alloplast with increased regenerative capacity would be advantageous for the treatment of localized alveolar ridge defects. This prospective, randomized controlled preclinical trial utilized 6 female foxhounds to analyze the osteogenic impact of different formulations of biphasic calcium phosphate (BCP) in combination with an hydroxyapatite-collagen membrane and their ability to reconstruct deficient alveolar ridges for future implant placement. The grafted sites were allowed to heal 3 months, and then trephine biopsies were obtained to perform light microscopic and histomorphometric analyses. All treated sites healed well with no early membrane exposure or adverse soft tissue responses during the healing period. The grafted sites exhibited greater radiopacity than the surrounding native bone with BCP particles seen as radiopaque granules. The graft particles appeared to be well-integrated and no areas of loose particles were observed. Histologic evaluation demonstrated BCP particles embedded in woven bone with dense connective tissue/marrow space. New bone growth was observed around the graft particles as well as within the structure of the graft particulate. There was intimate contact between the graft particles and newly formed bone, and graft particles were bridged by the newly formed bone in all biopsies from the tested groups. The present study results support the potential of these BCP graft particulates to stimulate new bone formation. Clinical studies are recommended to confirm these preclinical findings.

Key Words: canine, localized alveolar ridge defect, alloplast, biphasic calcium phosphate, hydroxyapatite collagen membrane

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INTRODUCTION

Alloplastic biomaterials offer an alternative to autogenous bone harvest morbidity and nonrealized fear of disease transmission with allograft. An alloplast with osteogenic potential will be a welcoming addition to a regenerative surgeon’s armamentarium for the treatment of localized alveolar ridge defects.

Biphasic calcium phosphate (BCP) composed of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) is a bone graft substitute that resembles the inorganic phase of human bone tissue. The insoluble HA retains its form and structure to maintain space, while the β-TCP will stimulate new bone formation by dissolving into calcium and phosphate ions.1,2 The alteration of the HA/β-TCP ratio has been demonstrated to positively influence the substitution rate as well as the bioactivity of these materials.1,2

One formulation of BCP (60% HA and 40% β-TCP) has been successfully used for alveolar ridge augmentation, maxillary sinus grafting, and the treatment of implant dehiscence and fenestrations.3–17 The percentage of new bone formation in human sinus augmentation procedures has ranged from 28.4% to 37.5% in 6 to 8 months.8,18 Thus, BCP has been shown to be safe, biocompatible, and bioresorbable, and can effectively serve as a scaffold for new bone formation.

A preclinical study was designed to further investigate the capacity of BCP to provide an osteoconductive matrix for ridge augmentation. The design of the study allowed for comparison of different formulations of HA/β-TCP grafting materials combined with a novel HA-collagen membrane for standardized large mandibular alveolar ridge defects.

MATERIALS AND METHODS

This prospective, randomized controlled preclinical trial utilized 6 female foxhounds to analyze the osteogenic impact of different formulations of BCP in combination with an HA collagen membrane and their ability to reconstruct deficient alveolar ridges for future implant placement.

Study animals and biomaterials

The study protocol was approved by the Institutional Animal Care and Use Committee at PARF in Massachusetts. Six female hounds (age 2–3 years, weight 20–24 kg) that have been bred exclusively for biomedical research purposes were obtained from a licensed vendor. They were acclimated for 2 weeks prior to the research commencement and were fed an appropriate diet with ad libitum access to water. The biomaterials utilized for this study were alloplastic BCPs in 3 different formulations together with a barrier membrane:

- Group A (Osteon I, Dentium Co, Ltd, South Korea): 70% HA and 30% β-TCP (0.5- to 1.0-mm particle size, porosity 77%, macro pore size 300–500 μm, crystallinity 93%, crystal size 0.059 μm); 7 sites.
- Group B (Osteon II, Dentium Co, Ltd.): 30% HA and 70% β-TCP (0.5- to 1.0-mm particle size, porosity 70%, macro pore size 250 μm, crystallinity 97%, crystal size 0.043 μm); 7 sites.
- Group C (Osteon II Collagen, Dentium Co, Ltd): a mixture of 30% HA and 70% β-TCP (0.5- to 1.0-mm particle size, porosity 70%, macro pore size 250 μm, crystallinity 97%, crystal size 0.043 μm) and collagen; 92% graft and 8% collagen by volume; 7 sites.
- Group D: negative control (no grafting); 3 sites.

Cross-linked HA-collagen membrane (Genoss Co, Ltd, Suwon, South Korea).

General and local anesthesia

All surgical procedures were performed under general and local anesthesia in sterile conditions. Xylazine hydrochloride (2.2 mg/kg, intramuscularly) and tiletamine hydrochloride/zolazepam hydrochloride (10 mg/kg, intramuscularly) were administered initially, followed by inhalation of 1.5% to 2% isoflurane as a general anesthesia for the duration of the procedure. Local anesthesia (2% lidocaine with 1:100,000 epinephrine) was provided at the surgical sites.

Surgical extraction and defect creation

The bilateral mandibular first, second, third, and fourth premolars (P1–P4) were extracted, and bilateral standardized alveolar ridge defects (approximately 12 mm mesiodistally and 10 mm apicocoronally) were created by removing the buccal plate (leaving the lingual wall intact) (Figures 1 and 2). The flaps were then adapted for tension-
free wound closure with multiple interrupted sutures and allowed to heal for 2 months.

**Ridge augmentation procedure**

A healing period of 2 months followed the creation of chronic bony defects eliminating the possibility of spontaneous regeneration. Each site of the jaw was randomized to receive 3 different bone grafting materials (groups A, B, and C) in addition to a negative control site (group D, defect only). A crestal incision provided surgical access, and the mucoperiosteal flaps were reflected to expose the mental foraminae. The graft materials were condensed into the defects according to a randomized distribution pattern so that no two adjacent sites received the same material (Figure 3). HA-collagen membranes were used to cover all sites and were stabilized with stainless steel pins (Dentium) (Figure 4). The flaps were released by periosteal incision to allow for a tension-free primary wound closure, combining mattress and interrupted sutures. The animals received the standard postsurgical infection and pain control consisting of cefazolin sodium (20 mg/kg, intramuscularly) and buprenorphine HCl (0.02 mg/kg, intramuscularly). The sutures were removed after 14 days, and the animals received a soft diet during the entire healing period and treatment phase.

**Bone core biopsies**

The surgical sites were allowed to heal for 3 months, at which time radiographs and photographs were taken. Mucoperiosteal flaps were elevated and a 2.4-mm wide trephine bur (Dentium) was used to harvest a bone core from each site. The dental implants (3.4 mm wide, 8–10 mm in length, Dentium) were then placed into the trephined sites in submerged fashion and allowed to heal for 3 months.

**Light microscopy and histomorphometric analysis**

The bone cores were embedded following complete dehydration in ascending grades of ethanol (60%, 80%, 96%, and absolute ethanol) in a light-
curing one-component composite resin (Technovit 7200 VLC, Heraeus Kulzer, Wehrheim, Germany). Polymerized blocks were initially ground to bring the tissue components closer to the cutting surface. A 100-µm thick section attached to the second slide was sawed with a diamond blade and 50 to 100 g of pressure. The final thickness of 40 µm was achieved by grinding and final polishing with 1200-, 2400-, and 4000-grit sandpaper. Sections from each block were used for Sanderson’s rapid bone stain and acid fuchsin counterstain. Light microscopic overview images of the cores were taken digitally with a Leica M16 stereomicroscope (Leica Microsystems, Glattbrugg, Switzerland). Histomorphometric measurements were performed by using a software (Image-Access, Imagic, Glattbrugg, Switzerland) to calculate the percentages of mineralized bone, soft tissue components (connective tissue and/or bone marrow), and residual graft particles.

**Statistical analysis**

Means and standard deviations were calculated for all quantitative data. Due to the sample size, collected data of each group was confirmed with Shapiro-Wilk test of normality. Data were not significantly deviated from the normal distribution \((P > .05\) for all data). Therefore, they were compared by one-way analysis of variance (ANOVA) with post hoc Tukey test for pairwise comparisons. \(P < .05\) was considered to be statistically significant.

**RESULTS**

**Clinical and radiographic evaluations**

No early membrane exposure or adverse soft tissue responses were observed during the healing period. The grafted sites exhibited greater radiopacity than the surrounding native bone with BCP particles seen as radiopaque granules. The graft particles appeared to be integrated, and no areas of loose particles were observed.

There was graft consolidation noted in all 3 groups, but some grafted sites were distinguishable from the surrounding host bone due to the visible presence of remaining graft particles (Figure 5). The negative control defects demonstrated minimal bone regeneration and concave ridge morphology. All 3 grafted sites (groups A through C) had a ridge contour that appeared to be stable and adequate to receive dental implants. There was no clear demarcation between the grafted area and the native bone.

Twenty-four bone biopsies were obtained from the 24 treated sites, and 24 implants were placed into the sites with adequate primary stability (Figure 6).

**Histologic evaluation**

For groups A through C, remaining graft particles were easily distinguishable from the native bone and connective tissue due to differences in staining and morphology (Figures 7a through 9c). The BCP particles were embedded in woven bone and in dense connective tissue/marrow space. New bone growth was observed around the graft particles as well as within the cavities of the bone graft material (Figure 9b). There was intimate contact between the graft particles and newly formed bone, and graft particles were bridged by the newly formed bone in all biopsies from the tested groups (Figures 7b, 8b, and 9b). The use of backscattered scanning electron microscopy images of the ground sections allowed contact, which allowed graft materials to be distinguished from the new bone due to their dense, whiter appearance (Figures 7c, 8c, and 9c).

The presence of a rim of osteoblasts that were in the active process of depositing osteoid matrix can be seen around some particles. Osteoblasts lined the layers of osteoid matrix deposited around the graft particles (Figure 8b). Bone remodeling was observed in all 3 groups by the presence of osteoclast-like multinucleated giant cells lining resorbed graft particles. The ratio of bone formation or resorption could not be clearly identified from these histologic specimens.

Histomorphometric analysis revealed a high quantity of new bone formation in all groups (Figure 10). The mean percentage of vital bone was 41.0\% ± 10.0\% for group A, 46.6\% ± 5.5\% for group B, 49.3\% ± 10.9\% for group C, and 54.1\% ± 8.8\% for group D. The mean percentage of remaining graft particle was 23.9\% ± 2.1\% for group A, 10.4\% ± 2.0\% for group B, and 12.9\% ± 9.6\% for group C. No statistical difference was noted in terms of vital bone, but there was a statistically significant difference in remaining graft particles for all 3 groups (\(P = .02\), ANOVA). Group B had lower amount of remaining graft particles compared to groups A and C, although only difference between
group A and group B turned out to be statistically significant ($P = .022$, post hoc Tukey test). Thus, all defects ultimately healed with similar amount of bone, but the graft resorption seemed to be slower for group A due to high concentration of HA (70% in group A vs 30% in groups B and C).

**FIGURES 7-9.**

**Figure 7a.** Overview ground section of a group A specimen in light microscopic (top) and backscatter scanning electron microscopy views (below). Note the high density of the HA graft particles (G) embedded in newly formed bone (NB). Also, note minimal resorption of graft particles. **Figure 7b.** Light microscopic view of a group A specimen demonstrating an intimate contact between graft particles (G) and surrounding newly formed bone (NB). Graft particles were bridged by the newly formed bone. **Figure 7c.** Backscatter scanning electron microscopy corresponding to the area shown in Figure 7b. Note the intimate integration of the graft particles (G) into newly formed bone (NB). **Figure 8a.** Overview ground section of a group B specimen in light microscopic (top) and backscatter scanning electron microscopy views (below). Note the intimate integration of the graft particles (G) into newly formed bone (NB). **Figure 8b.** Light microscopic view of a group B specimen at higher magnification. Note the presence of osteoblasts lining an osteoid, indicating ongoing bone formation. NB indicates newly formed bone; G, graft particles. **Figure 8c.** Backscatter scanning electron microscopy corresponding to the area shown in Figure 8b. NB indicates newly formed bone; G, graft particles. **Figure 9a.** Overview ground section of a group C specimen demonstrating new bone (NB) formation in light microscopic (top) and backscatter scanning electron microscopy views (below). G indicates graft particles. **Figure 9b.** Light microscopic view of the specimen shown in Figure 9a at higher magnification. Note the intimate contact between graft particles (G) and surrounding new bone (NB). New bone growth was observed around the graft particles as well as within the cavities of the bone graft material. **Figure 9c.** Backscatter scanning electron microscopy corresponding to the area shown in Figure 9b. NB indicates newly formed bone; G, graft particles.

**DISCUSSION**

This study presents clinical, radiographic, histologic, and histomorphometric comparison of the osteogenic potential of 3 BCP bone substitutes to regenerate large mandibular alveolar ridge defects. Group A BCP (70% HA and 30% β-TCP) formulation
has been reported to be effective in sinus augmentation,\textsuperscript{12,19} while group B BCP (30\% HA and 70\% \(\beta\text{-TCP}\)) formulation has been shown to be effective in alveolar ridge augmentation.\textsuperscript{16,17} After a healing period of 3 months, all 21 grafted areas (groups A through C) responded with stable ridge dimensions to ensure successful dental implant placement.

A number of contemporary investigations have supported the use of BCP for various maxillofacial indications.\textsuperscript{3–17} They need to be biocompatible and biodegradable in order to support the formation of new bone tissues. Porosity and pore interconnectivity have an effect on the BCP rate of biodegradation as well as new bone formation, vascularization, and graft stability. The degradation rate of \(\beta\text{-TCP}\) is thought to be 3 to 12 times faster than HA, thus higher composition of \(\beta\text{-TCP}\) in BCP may allow faster resorption of graft material while promoting faster new bone formation.\textsuperscript{20} Therefore, an optimum balance between the stable phase of HA and the soluble phase of \(\beta\text{-TCP}\) can enhance new bone formation.

Most histologic samples revealed regenerated bone with remaining graft particles that did not seem to interfere with the ridge restoration process. It was possible to correlate the histologic findings with the clinical outcome. All grafted sites showed evidence of gradual resorption of graft materials and the substitution by newly formed bone. Osteoblastic seams forming new bone directly on the BCP surface can be seen, although it is still unclear about the mechanism by which BCP is resorbed.\textsuperscript{7} Superficial disintegration or fragmentation of BCP particles into small grains has been previously reported,\textsuperscript{7} while osteoclasts were responsible for material resorption in another human study.\textsuperscript{9,21} The high percentage of vital bone in regenerated sites indicated that bone cells might still be actively replacing BCP, and additional time may increase the amount of vital bone formation while reducing the residual graft amount.\textsuperscript{21} The outcome of the implant placement at the grafted sites from this study will be presented in another report.

In Jensen et al.,\textsuperscript{22} 3 BCP bone substitute materials with HA/\(\beta\text{-TCP}\) ratios of 20/80, 60/40, and 80/20 were compared to particulate autogenous bone in a large defect in the mandible of minipigs; there were no significant differences with regard to the amount of bone formation, quality of newly generated bone, or graft degradation rate between the BCP 20/80 and the autograft at any time points. Autograft and BCP 20/80 demonstrated a high resorption rate, while BCP 80/20 and BCP 60/40 had a low resorption rate.\textsuperscript{22} A study comparing new bone formation and the graft degradation of 2 different formulations of HA/\(\beta\text{-TCP}\) graft implanted in canine mandibles reported that 15/85 HA/\(\beta\text{-TCP}\) resulted in earlier and more bone formation than 85/15 HA/\(\beta\text{-TCP}\).\textsuperscript{2} In our study, all 3 groups presented with similar amount of vital bone formation, but the amount of remaining bone graft was significantly less in group B compared with group A (\(P = .022\), post hoc Tukey test). Thus, higher concentration of \(\beta\text{-TCP}\) in BCP was favorable in terms of having less amount of residual amount of graft after a healing period of 3 months.

Numerous combinations of bone substitutes and cell occlusive barrier membranes have been reported, but this is the first report of the combination of BCP and an HA collagen membrane to treat large alveolar ridge defects. The HA was incorporated into the collagen membrane to provide space maintenance and to possibly benefit the mineralization process.\textsuperscript{23} Collagen membrane was designed to allow exposed HA particles to induce bone formation, while the collagen portion was designed to resorb in 6 to 8 weeks. Although the membrane was useful for graft retention and form, we cannot conclude whether it played an active role in bone formation. The HA collagen membrane without underlying graft was not rigid enough to maintain the space to allow complete bone fill of the defect.

\section*{FIGURE 10.} Histomorphometric analysis revealed a high quantity of new bone formation in all groups.
CONCLUSION

HA/β-TCP combinations were biocompatible and osteoconductive and clinically and histologically successful in forming bone. Clinical and histologic evidence supported the suitability of all 3 formulations to treat large alveolar ridge defects to allow subsequent dental implant placement after a 3-month healing period.

ABBREVIATIONS

BCP: biphasic calcium phosphate
HA: hydroxyapatite
β-TCP: β-tricalcium phosphate

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REFERENCES


