Repair of defects in the alveolar ridge using rhBMP-2 in baboons

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Abstract

The objective of this research was to evaluate the regeneration of alveolar ridge width defects following surgical implantation of recombinant bone morphogenetic protein-2 (rhBMP-2) using two different carriers: a) Tricalcium Phosphate (TCP) / Hydroxyapatite (HA) / Absorbable collagen sponge (ACS) and b) α-BSM cement ($CaPO_4$) in the baboon model. Standardized alveolar ridge defects (15 X 8 X 5 mm) were made in 4 edentulous areas, in 4 baboons. Sites were balanced as to treatments and maxilla/mandible. Two titanium pins were placed at the mid apical and coronal levels to provide landmarks for defect measurements (width) and comparisons pre and post – treatment reentry. Impressions of the pre and post treatment ridges were also taken and models made to determine changes in clinical defect volume. Five treatments were performed: rhBMP-2/TCP/HA/ACS, TCP/HA/ACS alone, rhBMP-2/α-BSM($CaPO_4$), α-BSM($CaPO_4$) alone and unimplanted Control. A dose of 0.4-mg/ml rhBMP-2 was used in rhBMP-2 treated sites. Qualitative radiographic observations were recorded at pre implantation and before reentry. Block sections (mid-defects) were harvested at 12 -16 weeks, processed for light microscopy and stained with Mason's Trichrome. Three central histologic sections were evaluated for trabecular bone area, marrow space area and bone density using the Computerized Image Program. Statistical comparisons between treatments were made using ANOVA. Carriers by themselves demonstrated sufficient rigidity, resistance to compression and osteoconductive capacity to provide for modest ridge augmentation. Addition of rhBMP-2 resulted in almost double the increase in width and volume, and statistically significant more trabecular bone, less marrow space and higher density than the carriers alone. The rhBMP-2/α-BSM($CaPO_4$) construct demonstrated superior, but not statistically significant ($p \geq 0.05$) results over the rhBMP-2/TCP/HA/ACS implant Both TCP/HA/ACS and α-BSM($CaPO_4$) appear to be suitable carriers for rhBMP-2. The enhancement of both carrier systems with rhBMP-2 provided a viable alternative to second site grafting for the augmentation of alveolar ridge defects prior to implant placement. In addition, these treatments were the only ones that provided enough clinical ridge width for implant placement.

Keywords: rhBMP-2. Alveolar bone. Bone graft.

How to cite this article: Miranda DAO, Blumenthal NM, Francischone CE, Consolaro A. Repair of defects in the alveolar ridge using rhBMP-2 in baboons. Dental Press Implantol. 2012 Jan-Mar;6(1):54-66.

» The authors report no commercial, proprietary, or financial interest in the products or companies described in this article.

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Submitted: /9/21/2011
Revised and accepted: 10/31/2011

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Introduction

Dental implants are commonly used to support dental and maxillofacial reconstruction. Morphology and quality of alveolar bone are similarly important factors. The placement of the implant can become difficult due to aberrations in the alveolar ridge, resulting from functional requirements changed after tooth loss, surgical and accidental trauma, or due to pathological processes involving the alveolar bone. The dental implant surgery depends on the prosthetic planning. In many cases, the correct positioning of the implants needs the alveolar ridge augmentation. Several protocols can be used: Autogenous bone graft (in block or particulate, biomaterials, bone-guided regeneration, osteogenic distraction, and others. However, these protocols are significantly related to the morbidity of the patient because they have a limited biological potential, and/or are technically challenging. This leads to a continuous search for simpler and more effective procedures for alveolar ridge augmentation.

The rhBMP-2, Recombinant Bone Morphogenetic Protein 2, is a member of the superfamily of transforming growth factor β of multifunctional cytokines. It induces the formation and bone repair in adult vertebrates[1,2] and plays an important role in early embryonic development.[3] The rhBMP-2 in an absorbable collagen carrier sponge (ACS) has been shown to induce clinically relevant bone formation in different scenarios in maxillomandibular complex, including segment defects (resection),[4,5] cleft palate,[6] orthognathic defects,[7] alveolar ridge defects,[8,17] for increasing the maxillary sinus[18,19] and rebuilding the periodontal defects[20-23] in canine and primate models. Although rhBMP-2/ACS has been generally effective to the significant bone formation when used as a covering, it has been revealed less effective for the indications of onlay graft.[8,15] The ACS carrier undergoes compression of masticatory forces, thus reducing the graft volume with rhBMP-2/ACS, and consequently it does not produce the space on the bone to be formed. This failure of ACS transporter conducts the evaluation for transport systems with higher structural integrity and space providing biomaterials and devices to be used in combination with ACS.[11-14,20,24] For example, the combination of rhBMP-2/ACS to hydroxyapatite demonstrated to induce clinically relevant bone increase in the alveolar ridge defects; however, the quality of newly formed bone was compromised by residual biomaterial.[11] The objective of this study was to evaluate the alveolar ridge augmentation after grafting the rhBMP-2 with two carriers, in (*Papio anubis*) baboon model.

Material and methods

Four baboon adults in good health were used in this study in the Biological Resources Laboratory, University of Illinois — USA (Illinois Health Science Center). The guidelines for the care of animal in the research were strictly followed, as well as the guidelines established and approved by Animal Research Committee, University of Illinois Health Science Center. Screening procedures included all physical examinations and laboratory tests or radiographic evaluations required. Bacteriology and virology tests were conducted to establish the absence of infectious disease agents that may represent a risk to other non-human primates or human researchers.

The Tricalcium phosphate/absorbable collagen sponge/hydroxyapatite (TCP/HA/ACS, Wyeth Research, Cambridge, MA) contain TCP/HA (15/85 ratio) in a collagen sponge of bovine tendon. Then, TCP/HA/ACS was cut into 1-inch square and wetted with 0.65 ml of buffer (Wyeth Research, Cambridge, MA, 30 mm L-glutamic acid, 2.5% glycine, 0.5% sucrose and 0.01 polysorbate 80%, pH 4.5). The TCP/HA/ACS vehicle was subsequently cut into 5 x 5 x 1.5 mm cubes (Fig. 1B) and layered into the defect site to restore the alveolar ridge. For calcium phosphate

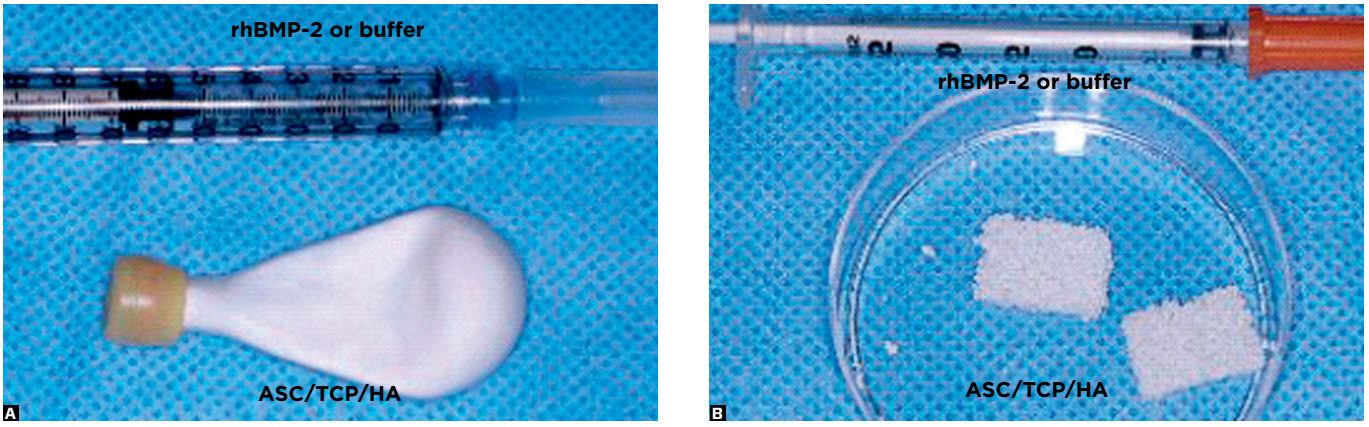

Figure 1 - Materials used for the graft. In A we may see the rh-BMP-2 (0.04 mg/ml), Buffer (30 ml glutamic acid, 2.5% glycine, 0.5% sucrose and pH 4.5. In figure B, we may also see the rhBMP-2 or Buffer and the HA/TCP/ACS carrier.

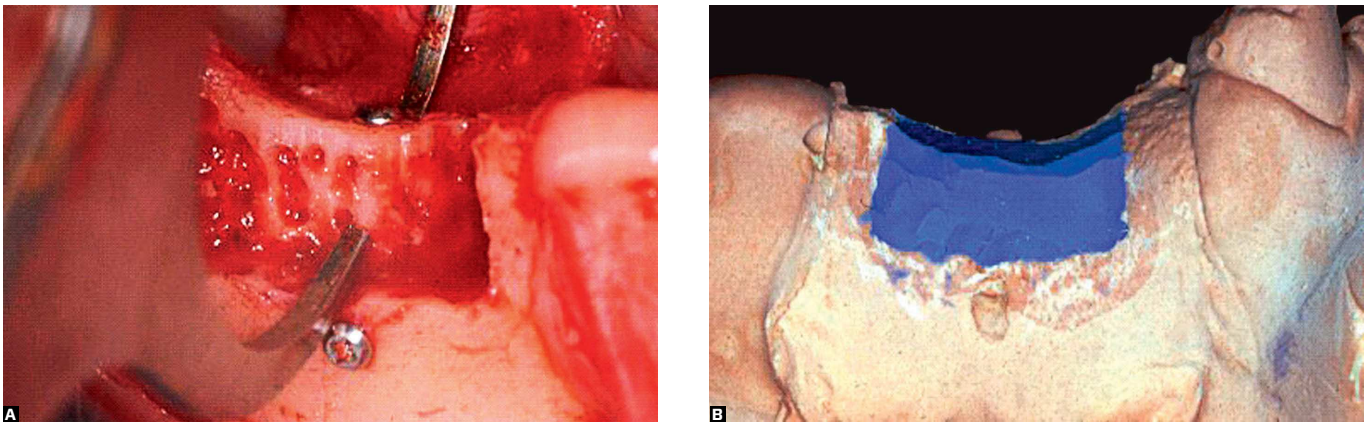

Figure 2 - Clinical measurements used to evaluate the increased thickness (**A**) and alveolar ridge volume (**B**).

(αBSM — Etex Corp., Cambridge, MA), 0.8 mL of buffer (30 mM glutamic acid, 2.5 glycine, 0.5% saccharose, 0.01% Tween 80, pH 4.5) was removed and injected into a mixture vial ("mixing bulb"), containing 1.0 g of αBSM (Fig. 1A). The material was gently handled until the contents were well mixed. The consistent and malleable mixture (putty) αBSM was grafted and adapted to restore the crest contour.

The rhBMP-2 (Wyeth Research, Cambridge, MA) was reconstituted and diluted with buffer to obtain a final concentration of 0.4 mg/mL. For rhBMP-2/TCP/HA/ACS, 0.65 mL of 0.4 mg/mL the rhBMP-2 solution was uniformly distributed over the entire surface of 1-inch square inch TCP/HA/ACS. The rhBMP-2 embedded in the sponge was placed in the alveolar bone defect (Fig. 3B). In the compound of rhBMP-2/αBSM, 0.8 mL of rhBMP-2 was removed and injected in the mixture into the vial containing 1.0 g of αBSM. Both carrier with the recombinant protein were placed in the defect (Fig. 3A) to restore the all the missing alveolar bone contour.

Mucoperiosteal flaps were performed under general anesthesia (xylazine 3-5 mg/kg and ketamine 35 mg; IM) and routine dental infiltration anesthesia. Extraction of the third premolars and first molars were performed to create edentulous alveolar ridge. Flaps were coapted and sutured with no tension to ensure primary wound closure.

For the preparation of alveolus defect, the incisions were initiated in the alveolar mucosa and taken toward the mesial and distal surface of adjacent teeth following anesthesia routines described above. Vertical incisions were performed to ensure the mobility of the flap by allowing sufficient primary closure without tension. Standardized ridge defects class III, 25 at least two per quadrant of the mandible, were produced in the four edentulous areas in each animal. The defects had dimensions of 5 mm vestibulolingually, 8 mm apico-occlusally and 15 mm mesiodistally. High-speed burrs were used with sterile saline irrigation and chisels. Lingual wall remains intact. Flaps were repositioned to cover defects, ensuring primary and sutured closure. Animals were placed on a pasty diet.

Six to eight weeks after the induction of the defect, flaps were created following the surgical protocol described above. Sites designated before were measured (width and volume) after spontaneous repair. Adherent soft tissues were debrided, and the cortical walls were perforated with burrs under continuous irrigation to expose the marrow spaces before the graft. The remaining defects to be implanted were also measured with rhBMP-2 and vehicles to provide pretreatment observations. Measurements of all defects were implanted with appropriate graft materials. Treatments were alternated between left and right maxilla and mandible, following a schedule randomly. Defects received grafts (test and control). The flaps were coapted, sutured and the animals returned to their cages. Radiographic examinations were performed before the grafting, and within 4, 8, 12 and 16 weeks post-implantation to monitor the repair and eventual occurrence of adverse events. Clinical bone and reentry biopsies were taken within 16 weeks.

Long-acting opiates (buprenorphine HCl, 0.01 to 0.02 mg/kg; IM, twice every 48 hours) was given for pain. A broad-spectrum antibiotics (enrofloxacin, 5 mg/kg IM once a day) was used for 7 days for infection. The diet consisted of monkey diet after a 3-week initial repair period during which bananas and water pre-softened feed were served. Seeds and nuts were retained. The animals were lodged individually without the presence of branches or sticks to avoid possible traumatic lesion in the treated sites. The sutures that were not reabsorbed were removed under sedation, approximately 14 days post-surgery. Experimental sites regarding the gingival health, maintenance of the suture line, edema and evidence of tissue necrosis or infection were observed daily until the suture removal, and at least twice a week thereafter. All observations were recorded in the animal's chart. Investigators were informed of any complications or undesirable reactions to the repair process.

Pre-operative conditions of the oral tissues were noted in the animal's chart, and photographs taken. Photographs were taken before and immediately after the implantation and after wound closure.

Two stainless steel pins (3i, Palm Beach Gardens, FL) were placed in the middle-apical and coronal aspect of the defect to provide reference points for estimating the width of the pre- and post-treatment alveolar ridge. The vestibulolingual width between two marked points was measured with a Vernier caliper and recorded (Fig. 2). Determinations of defect volume were made using protocol from Silverstein et al.[26] Molding of the defects were obtained in the initial surgery and surgical reentry procedure (Fig. 4A, B).

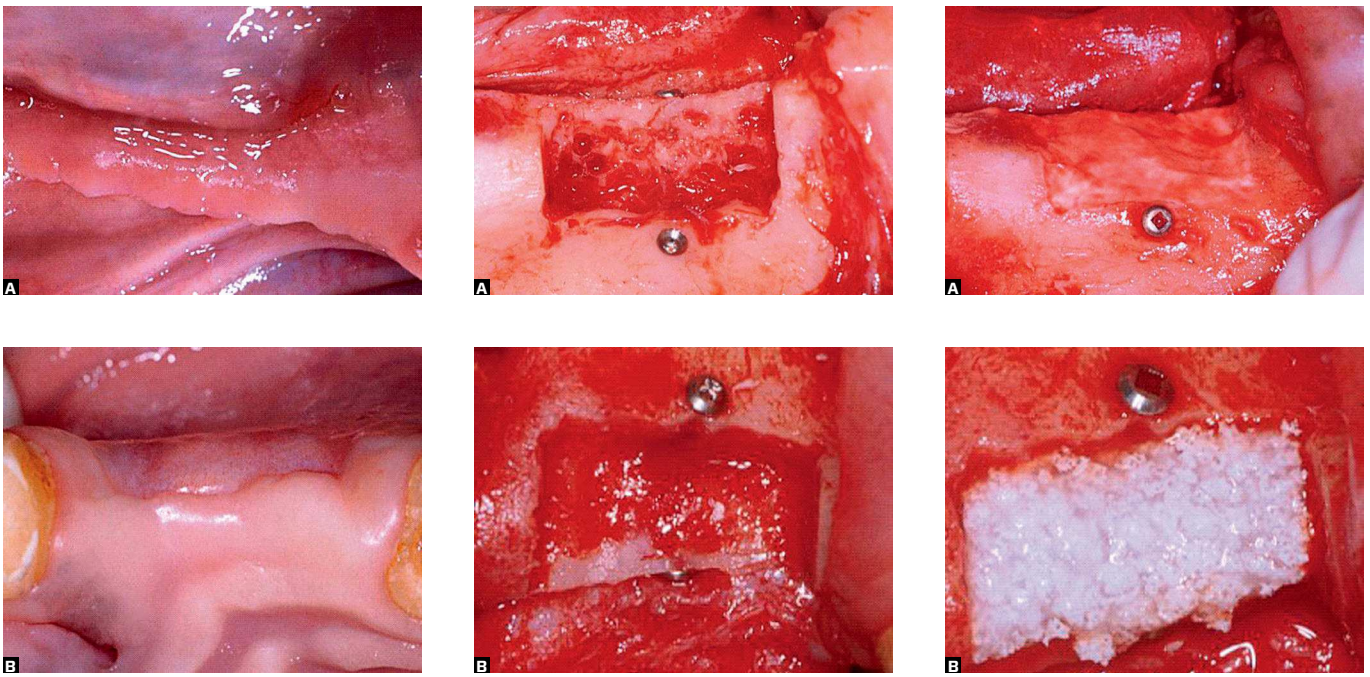

Figure 3 - Alveolar ridge defects receiving rahBMP-2 or Buffer in alpha BSM (1a) vehicle and TCP/HA/ACS (2b).

Sections in blocks (5 x 5 x 10 mm) (Fig. 4C, D) in the center of the repaired defect were collected within 16 weeks post-surgery observed through clinical measures and moldings. Resulting defects were covered with adjacent flaps and post-operative protocol described above was followed. The animals were returned to their colony, when the injuries were considered fully repaired. The tissue blocks were fixed in 10% neutral buffered formalin for 8-10 weeks, decalcified with 10% EDTA,[27] and subsequently processed for histology. 28

Three stained core sections of each defect site were used for histometric analysis. Using light microscopy and Image Tool UTHSCSA for Windows version 2.0, two blinded independent evaluators recorded the trabecular bone, the marrow space and bone density. All measurements were repeated twice.

Summary of statistical analyzes (mean ± SD) for each clinical and histological parameters from four animals for each treatment protocol (rhBMP-2/TCP/HA/ACS, TCP/HA/ACS, rhBMP-2/αBSM, αBSM and sham (control surgery) are provided in Tables 1-4. Differences between the treatment protocols were evaluated by one-way ANOVA. Tukey post-test was used to compare all pair combinations of mean for comparisons among treatments and a pair of t-test sample to compare (Fig. 3A, B) pre- and post-treatment values. All measurements were compared by ANOVA to determine whether the site suffer interference from the animal. All interaction terms of the graft site per animal were not significant ($p > 0.05$), then the region of individual treatment was used as analysis unit.

Results

The primary wound closure was performed in all defects. All sites remained closed without signs of infection. Repair process was within normal limits without any external reaction or complications. The animals were returned to their colony in good health with little impairment of masticatory function at the end of the study.

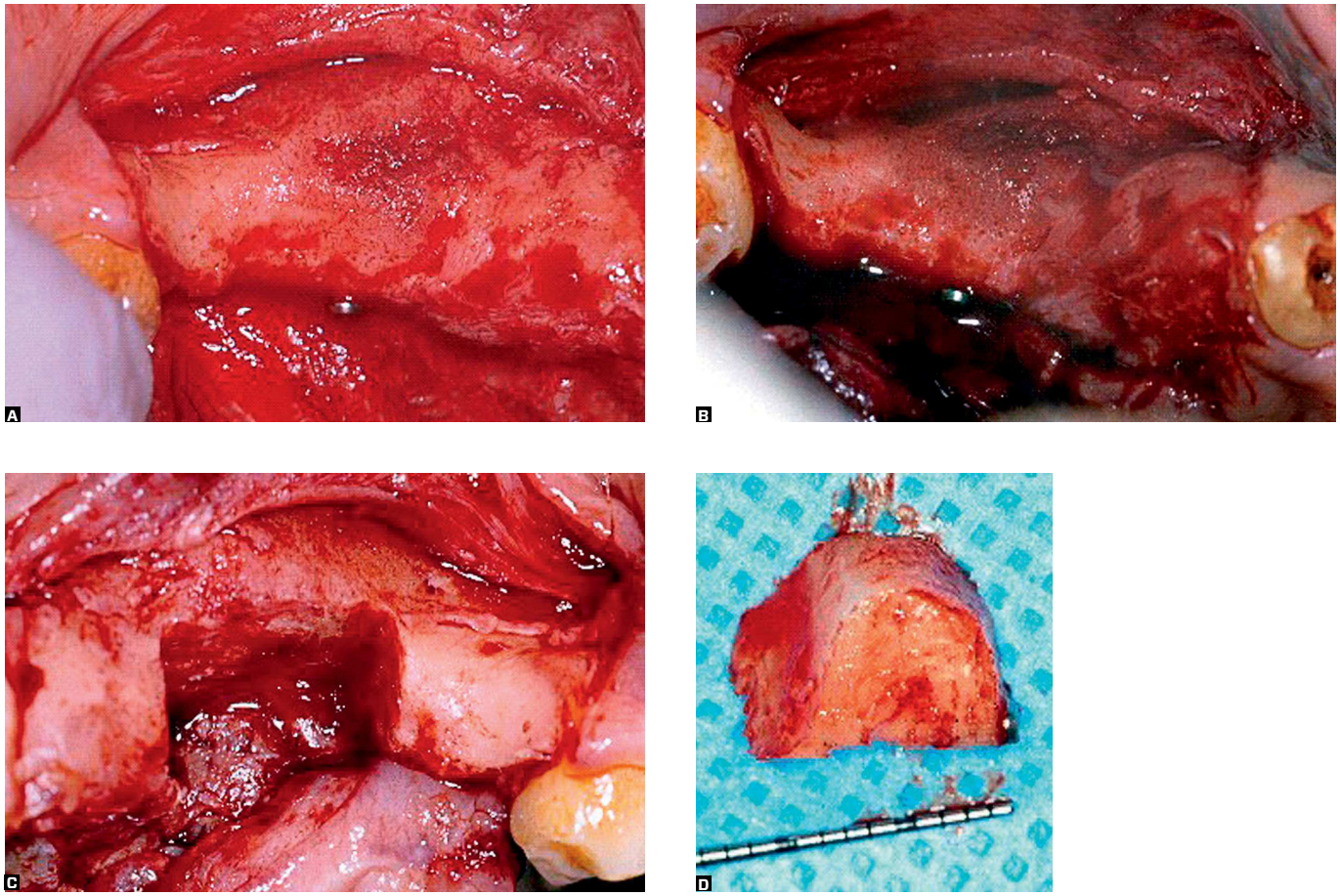

Figure 4 - Defects in maxilla (**A**) and mandible (**B**) receiving rhBMP-2 treatment with alpha BSM and HA/TCP/ACS carriers. **C** and **D** show the section in block removed for histological analysis..

Defects treated with rhBMP-2/TCP/HA/ACS and rhBMP-2/αBSM maintained the tissue contour produced in augmentation procedure during 16-week healing interval. The hardness of the ridge was comparable to the adjacent alveolar structures within 16 weeks. Sites receiving hBMP-2 showed mild resistance to probing.

The radiographic observations suggested higher radiopacity in defect sites implanted with some vehicle with or without rhBMP-2. There was no radiographic evidence of residual support material at sites receiving αBSM or TCP/HA/ACS within 16 weeks. The rhBMP-2 treated sites showed evidence of increased bone density and cortical restoration within approximately 4 weeks post-implantation.

Mean increase in width of the alveolar ridge and volume for different treatment protocols is shown in Table 1 and 2. Bone morphology and dimensions of surgically induced defects did not change significantly throughout the 8-week healing interval for control surgery (Sham).

A slight insignificant increase (0.84 ± 0.04 mm) in alveolar width was observed ($p<0.05$). All other

treatment protocols showed statistically significant improvement compared to the pretreatment (P M 0.05). Defects receiving TCP/HA/ACS and αBSM showed modest improvement in mean alveolar bone width of 2.740.5 mm and 3.340.7 mm, respectively, and in alveolar bone volume mean of 56.7% and 71.0%. Sites where rhBMP-2/TCP/HA/ACS showed an increased ridge width of 5.841.4 mm and an increased ridge volume of 94%. Observations corresponding to rhBMP-2/αBSM were 6.941.0 mm and 97%. When all the treatments were compared, and rhBMP-2/TCP/HA/ACS rhBMP-2/αBSM demonstrated statistically significant improvements compared to controls (Fig. 5) (PM0.05). There were no significant differences between rhBMP-2/TCP/HA/ACS and rhBMP-2/αBSM (p > 0.05).

The histological observations provided evidence that there was formation of new trabecular and cortical bone in all defects. TCP/HA/ACS and αBSM wastes were observed in some biopsy specimens. Specimens from sites receiving TCP/HA/ACS and αBSM exhibited moderate amounts of new trabecular bone. From moderate to large, the amount of osteoblasts and capillaries were observed in the marrow space of the newly formed bone. Histological analysis confirmed the clinical observations on the sites and received

Table 1 - Alveolar thickness measures.

Treatment		Measures (mm)	
		Pre-treatment	Post-treatment
	N	x ± s.d.	x ± s.d.
(1) Sham surgery	8	3.9 ± 0.5	4.7 ± 0.4
(2) TCP/HA/ACS	7	4.0 ± 0.6	6.7 ± 1.0
(3) rhBMP-2/TCP/HA/ACS	8	3.9 ± 0.4	9.8 ± 1.4
(4) αBSM	8	4.0 ± 0.5	7.1 ± 0.6
(5) rhBMP-2/αBSM	8	4.1 ± 0.4	11.0 ± 1.2

Table 2 - Changes in alveolar ridge volume.

Treatment		Measures (mm^3)			
		Pre-treatment	Post-treatment	Change	Filling of the defect
	N	X ± s.d.	X ± s.d.	X ± s.d.	%
(1) Sham surgery	8	368.2 ± 6.5	205.1 ± 12.4	163.0 ± 5.9	44.2
(2) TCP/HA/ACS	7	370.7 ± 6.7	160.6 ± 7.8	210.2 ± 9.0	56.7
(3) rhBMP-2/TCP/HA/ACS	8	367.6 ± 7.6	26.8 ± 9.0	345.7 ± 15.9	94.1
(4) αBSM	8	364.5 ± 5.3	105.2 ± 7.2	259.3 ± 7.9	71
(5) rhBMP-2/αBSM	8	366.6 ± 7.6	10.0 ± 6.9	356.6 ± 10.1	97.3

rhBMP-2/TCP/HA/ACS and rhBMP-2/αBSM. Trabecular bone thickness and density per area unit generally increased compared to the sites receiving vehicle without rhBMP-2. Spaces between the newly formed trabeculae also appear smaller. Defect sites receiving rhBMP-2/TCP/HA/ACS and rhBMP-2/αBSM exhibited dense trabecular bone (46.7% and 52.2%), lower marrow spaces (26.3% and 25.1%) and higher total bone density (82.5% and 87.1%) compared to the controls (Tab. 3). When rhBMP-2/TCP/HA/ACS and rhBMP-2/αBSM protocols were compared (Fig. 6), there were no statistically significant differences between the protocols (Table 4).

Discussion

The objective of this study was to evaluate the increased alveolar ridge after the grafting with rhBMP-2/αBSM, αBSM, rhBMP-2/TCP/HA/ACS, TCP/HA/ACS, and a control (sham surgery) in primates. The results provide clinical and histological evidence of the effectiveness of rhBMP-2 using these carriers. Defects receiving these grafts produced a clinically relevant increase of the alveolar bone. The sites receiving rhBMP-2 showed higher repair achieving the original alveolar ridge contour. Furthermore, there were no adverse reactions related to surgical or biomaterial procedures used.

Table 3 - Histological measures in post-operative alveolar ridge to the trabecular bone, marrow space and bone density (16 weeks).

Types of treatment	N	Measures		
		Trabecular bone %	Medullary Space %	Bone density %
(1) Sham surgery				
(2) TCP/HA/ACS	7	24	54.1	40.2
(3) rhBMP-2/TCP/HA/ACS	8	46.6	26.3	82.5
(4) αBSM	8	30.5	43.2	55
(5) rhBMP-2/αBSM	8	52.2	25.1	87.1

Table 4 - Significance level measures of trabecular bone, marrow bone and bone density.

Measures	Types of treatment			
	TCP/HA/ACS	rhBMP-2/TCP/HA/ACS	αBSM	rhBMP-2/αBSM
Trabecular bone		TCP/HA/ACS* αBSM*		TCP/HA/ACS* αBSM*
Medullary Space	αBSM*	TCP/HA/ACS αBSM*		TCP/HA/ACS* αBSM*
Bone density		TCP/HA/ACS* αBSM*	TCP/HA/ACS*	

Several previous studies evaluating BMP technologies[4,5,6,9,14,16,19,29] and other regenerative protocols[30,31,32] for craniofacial reconstruction have used non-human primates. Parts of the anatomy of alveolar ridge in baboon species (*Papio anubis*), physiology and bone remodeling are similar to those of humans,[33] making the adult baboon appropriate to study procedures for increasing the alveolar ridge. However, some extrapolations should be kept in mind when using primate models, as here. Although the animals were lodged individually without tree branches or other objects, they have a tendency to rub the injured area in their cages. This may impair the wound stability and, finally, the repair. Since animals may not be prevented from chewing on the operated areas, these sites may be exposed to trauma and compression forces almost immediately after the surgery. In addition, effective oral hygiene measures are difficult to achieve during the critical initial phase of repair. The impact caused by food and plaque accumulations showed to adversely affect the repair and its long-term stability. Thus, it can be assumed that the results obtained in this study represent the repair in conditions which can be better controlled in humans.[34]

It is essential that a biomaterial for onlay grafting procedures may be implanted without breaking the grafted material or leaving implantation site. Such difficulties of these handlings were found with other carriers for rhBMP-2. 20 The handling of αBSM and TCP/HA/ACS was uncritical. The αBSM was easily mixed with rhBMP-2 or buffer solution in an vial, allowing to obtain a malleable consistency for application in the ridge defect and it can be molded to achieve appropriate contours. TCP/HA/ACS carrier was easily absorbed by the rhBMP-2 solution or buffer for easy implantation and could be cut for the desired contour. At the sites, both materials appeared rigidity and resistance to compression forces. These observations corroborate the continued assessment of biomaterials αBSM and TCP/HA/ACS as vehicle to rhBMP-2.

This study provided additional evidence on the importance in creating space so that rhBMP-2 induces bone formation. Furthermore, it is very important the selection of carriers (biomaterials) working as vehicles, may be compatible with bone formation. The slow-resorbing of biomaterials may impair bone formation. In a long-term perspective, they can affect the mechanical properties of the bone and the primary and secondary osseointegration.[24] A previous study assessed the alveolar ridge augmentation with rhBMP-2/ACS, 21 in which HA was added to the ACS carrier to increase the rigidity, in order to reduce the compression of the space and the potential for repair. However, HA material remained present within 12 weeks after the surgery without evidence of osteoclastic resorption. HA particles appeared to partially prevent the bone formation. Similar observations were made when rhBMP-2 was combined with a bovine bone or DL-polylactic acid.[20] Both biomaterials remained at the site 8 weeks after the surgery preventing apparently rhBMP-2 induced bone formation in supraalveolar periodontal defects. In addition, DL-polylactic acid biomaterial caused a significant giant cell reaction, reaching to impair the formation and maintenance of bone. Inflammatory reactions were also observed

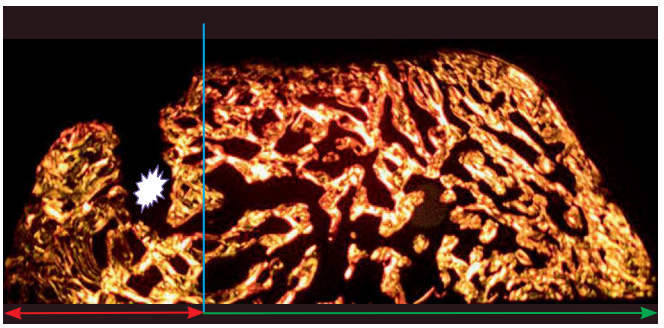

Figure 5 - Histological section under polymerized light where we may note the bone formed with the rhBMP-a protein (green arrow), native bone (blue arrow) and site where the screw was for measurements.

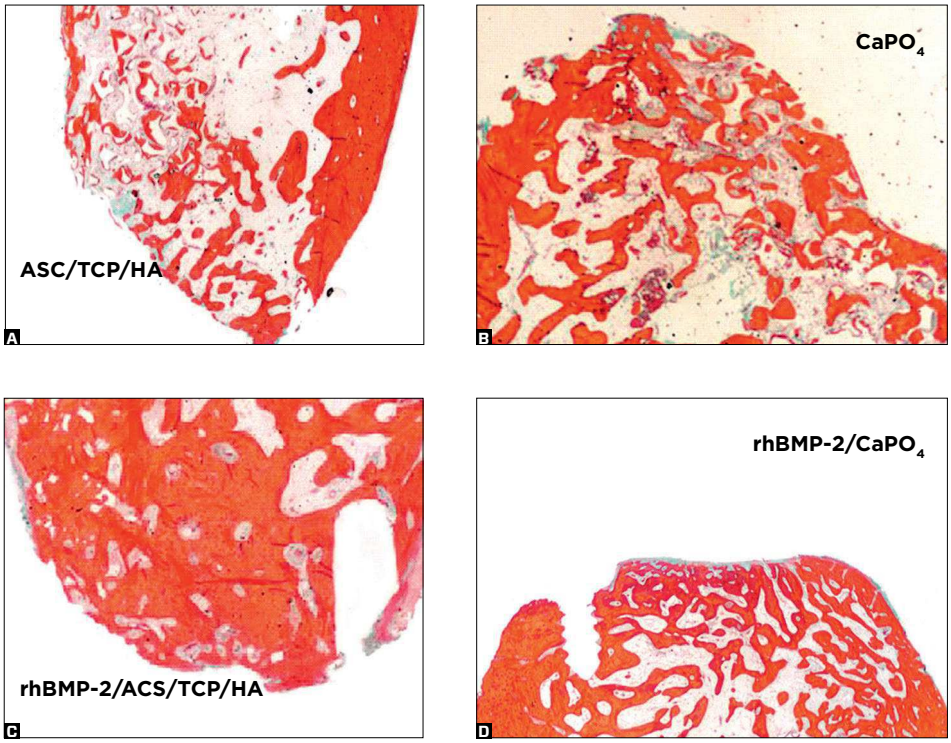

Figure 6 - **A** and **B** show the ridge augmentation with the carriers without the recombinant protein. In Figures **C** and **D**, carriers with rhBMP-2, showing higher trabecular bone and lower marrow space (H.E.; 20X).

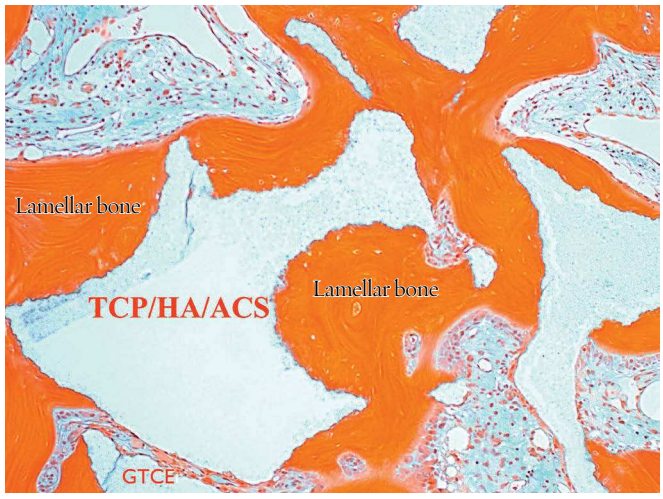

Figure 7 - Histological section of the biomaterial receiving rhBMP-2. Note the alveolar bone formation in almost all of the TCP/HA/ACS and the most apical part of a giant cell infiltrate (GTCE, 30X).

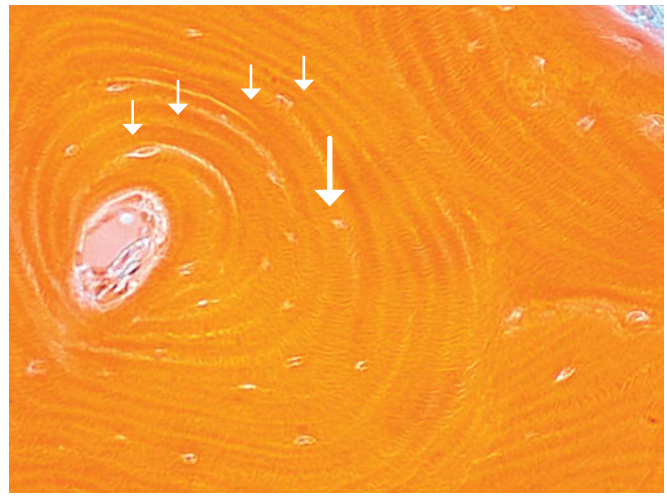

Figure 8 - Note Havers system consists of Havers canal and concentric bone lamellae (blue arrow). Observe osteoplast (red arrow) (40X).

after the OP-1 implantation (rhBMP-7) in a collagen carrier to the increased maxillary sinus. This particular construction was related to an intense inflammatory reaction apparently with the partially remaining collagen carrier with 14 weeks post-implantation. 29 In this study, particles of carries (TCP/HA/ACS or αBSM) remained at 16th week post-implantation and biomaterials do not seem to interfere with the bone formation or induce adverse inflammatory reactions. Apparently, with vehicles offering suitable space (Fig. 7), rhBMP-2 could induce clinically relevant bone formation for the alveolar ridge augmentation, but also for other indications in craniofacial complex in which compression forces can exist (Fig. 8).

The clinical success of growth and differentiation factors to bone repair seems partly to be dependent on the specific characteristics of carriers. Appropriate concentration of factors should be properly located, deliberated and sustained at the site to be repaired so that the cascade of events occurs for desirable repair process. The vehicle should be clinically and mechanically manageable, biologically acceptable, and support the defect stability by maintaining space. Moreover, the carrier should increase, or at least does not interfere or block the cascade of repair events. Critical questions remain related to the release of coordination factor regarding the repair response to maximize the results supplying a single dose (bolus) or continuously and constantly (pulse). Undoubtedly, the future therapeutic efforts will be refined with these concerns in mind.[32] The results of this study support the use of rhBMP-2/αBSM or rhBMP-2/TCP/HA/ACS to improve the augmentation of alveolar ridge defects. Although this technology has a refinement promise, the carrier systems can provide the key to further enhance the reparative potential.

Acknowledgment

The authors would like to thank the resources of the State University of Feira de Santana for their support in data analysis, and biological resources laboratory at the University of Illinois at Chicago for their veterinarian support.

The authors report no conflict of interest with the products presented in this work.

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