Published in final edited form as:

Oral Surg Oral Med Oral Pathol Oral Radiol. 2013 October ; 116(4): . doi:10.1016/j.oooo.2011.12.018.

The use of bovine screws to promote bone formation using a tibia model in dogs

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Abstract

The objective of this study was to evaluate the use of a unique resorbable bovine bone screw, to stimulate bone formation. Bovine bone screws were inserted in the tibia beagle dogs. Each animal received 8 screws, divided into Groups A (screws + no membranes), B (screws + titanium reinforced membranes) and C (bone defects treated with autogenous bone grafts). Animals were sacrificed at 2, 4 and 6 months. New bone was measured with a periodontal probe and reported an average of 7.4 mm in vertical bone gain for Group B, 3.6 mm for Group A and 1.7 mm for Group C. Submission to Kruskal-Wallis test showed statistical differences between groups ($p < 0.05$). Histological examination revealed an intimate contact between the newly formed bone and the resorbing bone screws. Conclusion: Bovine bone screws provide environment for new bone formation and thus may provide an alternative therapy for enhancing bone formation vertically, including for regenerative procedures as well as prior to implant therapy.

INTRODUCTION

Areas with poor quality and/or with limited quantity of bone present challenges for successful dental implant placement. Insufficient alveolar bone anatomy may require bone graft procedures to restore adequate bone volume prior to implant placement^{1,2}. Bone grafts are used frequently for socket preservation, sinus augmentation, and horizontal/vertical bone gain^{3,4,5}. Such bone grafts, including autogenous grafts from intraoral donor sites, allografts, xenografts, and alloplastic bone substitutes, are often used to increase bone prior to implant placement, with reports of positive integration of the implants with the newly formed bone^{4,6,7}. Most of the authors consider autogenous bone grafts the “gold” standard horizontal and vertical bone gain¹⁻⁷. In the studies here we focused on vertical bone gain, which has been a major limitation for regenerative procedures. Specifically, we hypothesized that new vertical bone growth would be established as the bone screws were undergoing resorption.

Bovine bone has been used as a substitute of autogenous grafts. As a xenogenic graft material, this type of commercial bone is found in both non demineralized and

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Conflict of Interest

The authors declare that there are no conflicts of interest in this study.

demineralized forms ^{8,9}. Mineral particles of bovine bone are well integrated during regeneration of bone with indication of osteoconductive properties and no immunological reactions ^{10–13}.

The purpose of the present research was to evaluate the use of a unique resorbable bovine bone screw, as a bone graft material, to stimulate vertical bone formation, using an animal model.

MATERIALS AND METHODS

Obtaining bovine bone screws

This project was developed in association with the Mechanical Engineering Department of the Federal University of Santa Catarina. Screws were produced under established law of the national sanitary agencies and according to international rules. The bovine bone* (cortical and inorganic bone) was approved by Brazilian Health Ministry. Screws samples for this research were modified from original model of Osteotite NT MicroMini 3.75 × 8.5 mm oral implants[†]. The implants were machined with dimensions of 4.0mm X 10.0mm.

Animal Selection

Eighteen young female beagle dogs (10kg, Canis Familiares), obtained from Central Vivarium at Federal University of Santa Catarina (UFSC) were used in this study. The animal procedures were approved by the Ethics Commission for Animal Use, register number 244/CEUA.

First Surgical Stage

The right tibia was used. An intramuscular antibiotic[‡] 40,000U per animal kilo, was administrated two hours prior to surgery. Aseptic procedures and tricotomy of surgical site were done before the initial horizontal incision. All tissues were separated and bone exposed. To insert the bovine bone screws, a sequence of surgical drills similar to that used for osseointegrated implants were used[§]. Primary stability of the screws was obtained manually, using a ratchet[§]. A number 8 drill was used for decorticalization of the adjacent areas in order to increase blood supply and stimulate cells in the local area. For each right tibia, four bone screws were inserted (figure 1) and established as Group A (4 bovine bone screws without membrane) and as Group B (4 bovine bone screws covered by expanded polytetrafluorethylene membranes titanium reinforced (e-PTFE), utilizing extra large size^{||}) and fixed with metallic screws[¶] to maintain the membrane in position. For group C (control group), an autogenous bone graft was used since it is considered by many clinicians to be the gold standard for horizontal and vertical bone gain ^{9–13}. These grafts were maintained in position using the same metallic screws as for Group B.

Post-Surgery Stage

All dogs were under veterinary care until the second surgical stage, no complications were observed and all dogs survived the surgical procedures.

*Baumer Brazilian Industries, Genius® Biomaterial division, Mogi Mirim, SP

†3i Implant Innovations, Inc. Riverside, FL

‡Pentabiotico®, Fort Dodge Saúde Animal Ltda, São Paulo, SP

§Nobel Biocare®, Zürich, Switzerland

||GORE-TEX®, Gore & Associates, Inc, Dover, DE

¶Neodent®, Curitiba, PR

Second Surgical Stage – Biopsies Removal

Samples were removed at three different time points of 2, 4 and 6 months after first surgery, with maintenance of animal at the Central Vivarium at the Federal University of Santa Catarina (UFSC). Biopsies were fixed in 10% formaldehyde, dehydrated in serial decreasing ethanol solutions and embedded in methyl metacrylate resin[#] for histological analysis^{**}.

Histological Analysis

Histological sections were prepared following the protocol which allows visualization of bone-implant interface stained by Toluidine Blue.

RESULTS

The design of a unique bovine bone screw is shown in Figure 1a. This model of bone screw was originally designed by Schiochett¹⁴ with modifications of Oliveira¹⁵ as shown here in Figure 1a and 1b, to use to evaluate the biocompatibility of a screw-like design vs. a smooth surface design, to increase surface area and also enhance stability upon insertion into bone. Figure 1c, demonstrates the insertion of the resorbable bone screws into the newly created bony defects and includes four bovine bone screws without membrane (Group A), four bone screws covered by membrane fixed with metallic screws (Group B) and autogenous bone graft fixed with metallic screws (control Group C).

Clinical outcomes 4 months post surgeries are shown in Figure 2a. Note new vertical bone formation around the resorbable bovine bone screws for groups A and B. Furthermore, upon probing the area surrounding the resorbing bovine bone screws or the bone graft using a periodontal probe, hard tissue formation was confirmed. The most robust response was noted for Group B (Fig. 2c) with significant bone gain noted in Group A vs. control as well, but not as great a gain as seen with Group B.

Figure 3 presents the results in a graphic form providing averages for vertical bone height around bone screws for all groups, Group A (without membrane), Group B (with membrane), Group C (control, bone graft) at all time points (2, 4 and 6 months). Displayed values are the average of six samples for each group. Kruskal-Wallis test was applied and a statistically significant difference was observed between all groups ($p < 0.05$). Results for group B were the most impressive revealing an average of vertical bone gain of 7.4mm. For this group, the newly formed bone around the screws was regular, filling the spaces between the screws with a significant increase in bone height at all time points. Moreover, this increase was 4 fold greater than that for control (Group C) and 2 fold greater than that for Group A at all time points. In contrast, while significant bone gain was noted in Group A (no membranes) vs Group C (control), the increase was about 2 fold greater than control. Histological analyses of sections were similar for Groups A, B and C. As seen in Figure 4, an interface was observed between the area of new bone formation (NB) and the bone screws (BS). Osteocytes and regular medullar spaces were observed within the newly formed bone at all time points and for all groups. For control samples, Group C, an intimate relation was noted between the bone graft and the tibia, with typical characteristics of bone, yet minimal gain in bone height was observed at the clinical level. This may be due to rapid resorption and/or loss of graft material. During the time periods measured in this study no necrotic tissue formation was observed in samples obtained from any of the groups.

[#]Technovit® 9100 Methyl Methacrylate, EMS, Hatfield, PA

^{**}EXAKT System, EXAKT Technologies, Inc. Oklahoma City, OK

DISCUSSION

Techniques that result in predictable and robust bone fill are desirable outcomes for regenerative therapies. The necessity for two surgical procedures when placing implants, increasing morbidity level during surgical procedures and difficulty in obtaining sufficient bone tissue are major challenges for the clinicians^{4,9,16}.

The rationale for designing this unique bovine bone screw was to promote bone growth in a vertical direction, a current limitation of existing regenerative materials. In this regard, the results obtained here are encouraging. Bovine grafts are known to be osteoconductive and thus it is reasonable to assume that the bovine screws provided a source for cells within the local region to migrate into the healing site with subsequent ability to promote bone formation, even without membranes (Group A). The results here support the use of membranes to prevent collapse (e.g., function as space maintainer) and therefore provide additional bone during remodeling as shown by previous studies^{13,17–26}.

Importantly even without the use of these supportive membranes, i.e., Group A, the results are also encouraging, with greater bone formation noted in this group vs. the control group. Although the new bone was irregular, versus the well-formed bone in group B, the bone exhibited characteristics of mature bone and should be able to stand implant placement.

In group B (screw plus membrane) robust bone formation was observed around bovine bone screws and thus bone gain could be measured accurately. Existing data have shown that a thin layer of connective tissue is often formed underneath membranes as we reported here^{12,13,18–26}.

Histological evaluation confirmed new bone formation to include components such as osteocytes and osteons, showing the effectiveness of the use of the bone screws to provide an environment conducive for bone formation.

Our findings of vertical gain of bone in Group A and B, while speculative, suggest that this osteoconductive material provided sufficient support for the migration of cells to the site to promote osteogenesis in a vertical direction not seen with current regenerative therapies.

In conclusion, bovine bone screws may be considered a unique model for promoting an increase in bone height at sites of periodontal bone defects and for preparing sites for implant placement. The association of titanium reinforced membranes ensures the architecture of new bone formation as well as enabling an environment suitable for cells in the region to function in a capacity allowing for osteogenesis to occur. While these results are encouraging we recognize that there were some limitations in the present study including the need for future studies to include comparisons of this model with additional controls such as autogenous bone grafts covered by titanium mesh membranes, bovine bone screws covered by resorbable membranes and autogenous bone covered by resorbable membranes. Moreover, the quality of the new bone needs further analysis to include determining whether of not implants are able to integrate with the newly formed bone. Within these limitations, the results here provide evidence that bovine bone screws are attractive candidates for use in regenerative therapies targeted at periodontal regeneration and implant rehabilitation.

Acknowledgments

We acknowledge the facilities of Laboratory of Surgical Technique at the Department of Endodontics of Federal University of Santa Catarina and the laboratory of University of Bauru for technical assistance with Exakt System.

Sources of Funding Statement

This study has been self-supported by the authors.

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Figure 1.

Detection of Resorbable Bovine Screws. Fig 1a showing bovine bone screws first design by Schiochett, 2002. Fig 1b bovine bone screws design used in this study with modifications (Oliveira, 2004). Fig 1c with bovine bone screws inserted in right tibia. Group A (4 bovine bone screws without membrane), Group B (4 bovine bone screws protected by an expanded polytetrafluorethylene membrane titanium reinforced (e-PTFE, extra large size§) and Group C (control with bone graft).

Figure 2.

Results 4 months post-implantation of screws. Fig 2a all groups exposed after 4 months. Fig 2b in higher magnification photograph (10X) of Group A, four months after surgery. Note the irregular pattern of bone formation around the screws. The screws head were not covered completely by new bone. Fig 2c higher magnification photograph (10X) showing Group B 4 months after surgery. Regular bone formation was observed, showing an impressive gain in bone volume around the bone screws.

Figure 3.

Graphic representation of vertical bone height around bone screws. Group A (without membrane) and Group B (with membrane) compared with Group C (control) at all time points (2, 4 and 6 months). Displayed values are the average of six samples for each group. Kruskal-Wallis test was applied and a statistically significant difference was observed between all groups ($p < 0,05$).

Figure 4. Histological Analysis. Photomicrography of slides stained by Toluidine Blue. New bone (NB), bone screw (BS), bone graft (BG), technique artifacts (*) (10X). Histological sample of Groups A, B and C at 2 months (Fig 4a), 4 months (Fig 4b) and 6 months (Fig 4c) post surgery respectively. New bone (NB) with typical osteons was noted. Bone screws (BS), were in close apposition to newly formed bone in Group B. For Group C an interface between autogenous bone graft and tibia was noted.