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

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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 of 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 killed 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 among groups ($P < .05$). Histologic examination revealed an intimate contact between the newly formed bone and the resorbing bone screws. We conclude that bovine bone screws provide an environment for new bone formation and thus may provide an alternative therapy for enhancing bone formation vertically, including for regenerative procedures as well as before implant therapy. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013; 116:e215-e220)

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 before implant placement.[1,2] Bone grafts are used frequently for socket preservation, sinus augmentation, and horizontal/vertical bone gain.[3-5] Such bone grafts, including autogenous grafts from intraoral donor sites, allografts, xenografts, and alloplastic bone substitutes, are often used to increase bone before 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 in horizontal and vertical bone gain.[1-7] 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 for autogenous grafts. As a xenogeneic graft material, this type of commercial bone is found in both nondemineralized and demineralized forms.[8,9] Mineral particles of bovine bone are well integrated during regeneration of bone with indication of osteoconductive properties and no immunologic 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.

MATERIAL 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 (Baumer Brazilian Industries, Genius Biomaterial Division, Mogi Mirim, SP, Brazil) (cortical and inorganic bone) was approved by the Brazilian Health Ministry. Screw samples for this research were modified from original model of Osteotite NT MicroMini 3.75 × 8.5-mm oral implants (3i Implant Innovations Inc., Riverside, FL). The implants were machined with dimensions of 4.0 × 10.0 mm.

Animal selection

Eighteen young female beagle dogs (10 kg, *Canis familiaris*), obtained from Central Vivarium at Federal University of Santa Catarina (UFSC) were used in this study. The animal procedures were approved by the

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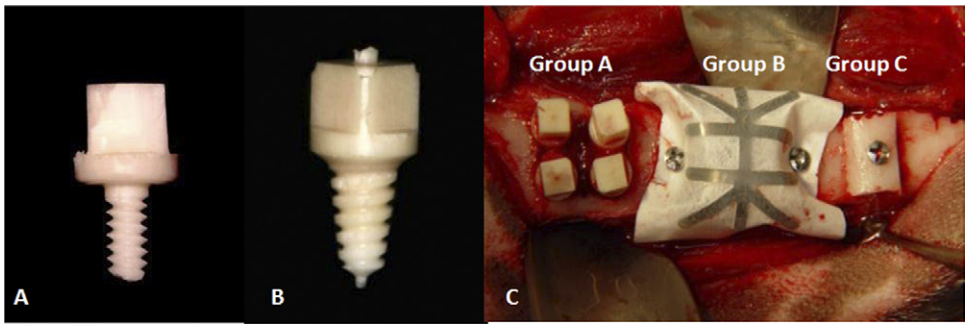

Fig. 1. Detection of resorbable bovine screws. **A,** Bovine bone screws first designed by Schiochett 2002. **B,** Bovine bone screw design used in this study with modifications (Oliveira, 2004). **C,** Bovine bone screws inserted in right tibia. Group A (4 bovine bone screws without membrane), group B (4 bovine bone screws protected by an expanded e-PTFE membrane, titanium reinforced, extra large size), and group C (control with bone graft).

Ethics Commission for Animal Use, register number 244/CEUA.

First surgical stage

The right tibia was used. An intramuscular antibiotic (Pentabiótico, Fort Dodge Saúde Animal Ltda., São Paulo, Brazil) 40,000 U per animal kilo, was administrated 2 hours before surgery. Aseptic procedures and tricotomy of the 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 (Nobel Biocare, Zürich, Switzerland). Primary stability of the screws was obtained manually, using a ratchet (Nobel Biocare). A number 8 drill was used for decorticalization of the adjacent areas to increase blood supply and stimulate cells in the local area. For each right tibia, 4 bone screws were inserted (Figure 1) and established as Group A (4 bovine bone screws without membrane). Group B consisted of 4 bovine bone screws covered by expanded polytetrafluoroethylene membrane, titanium reinforced (e-PTFE), using the extra large size (GORE-TEX, Gore & Associates Inc., Dover, DE), and fixed with metallic screws (Neodent, Curitiba, PR) to maintain the membrane in position. For group C (control group), an autogenous bone graft was used because 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.

Postsurgery stage

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

Second surgical stage—biopsy removal

Samples were removed at 3 different time points of 2, 4, and 6 months after 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 methacrylate resin (Technovit 9100 methyl methacrylate, EMS, Hatfield, PA) for histologic analysis (EXAKT System, EXAKT Technologies Inc., Oklahoma City, OK).

Histologic analysis

Histologic sections were prepared following the protocol that allows visualization of bone-implant interface stained by toluidine blue.

RESULTS

The design of a unique bovine bone screw is shown in Figure 1, *A*. This model of bone screw was originally designed by Schiochett[14] with modifications of Oliveira,[15] as shown in Figure 1, *A* and *B*, to use to evaluate the biocompatibility of a screwlike design versus a smooth surface design, to increase surface area, and also enhance stability on insertion into bone. Figure 1, *C*, demonstrates the insertion of the resorbable bone screws into the newly created bony defects and includes 4 bovine bone screws without membrane (Group A), 4 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 after surgeries are shown in Figure 2, *A*. Note new vertical bone formation around the resorbable bovine bone screws for Groups A and B. Furthermore, on 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