Histologic Findings in Sinus Augmentation with Autogenous Bone Chips Versus a Bovine Bone Substitute

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Purpose: *The aim of this study was to compare a bovine bone substitute (Bio-Oss) to autogenous bone with respect to its value as a material for sinus augmentation.* ***Materials and Methods:*** *In 10 beagle dogs 12 months of age, the 3 maxillary premolars were extracted on both sides. Six weeks later, 2 cavities of predefined size were produced in the region of the nasal cavity. The antral window was 25 mm long and had a vertical extension of 7 mm. Two Frialit-2 implants (3×8 mm) were placed in each bone defect (n = 20). Every implant was primarily stable because of fixation in native bone. In each maxilla, 1 bone defect was filled with autogenous bone harvested from the mandible and 1 was filled with Bio-Oss (material selected at random). The animals were sacrificed at 90 and 180 days, and histologic specimens were examined and the results subjected to statistical analysis by the Wilcoxon test for paired observations.* ***Results:*** *No healing problems were observed. Histologically, after 90 days the volume of the augmentation showed a reduction of 14.6 ± 4.4% within the Bio-Oss group and 3.8 ± 2.5% in the group with autogenous bone. Bone-implant contact of 52.16 ± 13.15% in the Bio-Oss group and 60.21 ± 11.46% in the autogenous bone group was observed. At 180 days, the Bio-Oss group showed bony ingrowth of the substitute, whereas in the autogenous group a differentiation from original bone could no longer be made. The volume reduction was 16.5 ± 8.67% in the Bio-Oss group and 39.8 ± 16.14% in the autogenous group. Bone-implant contact of 63.43 ± 19.56% in the Bio-Oss group and 42.22 ± 12.80% in the autogenous bone group was measured.* ***Discussion and Conclusion:*** *The results indicated that because of the nonresorptive properties of the bone substitute Bio-Oss, regeneration of the defects is achievable. It was demonstrated that the bone substitute seemed to behave as a permanent implant. The volume of the area augmented by autogenous bone decreased over the observation period.* (Int J Oral Maxillofac Implants 2003;18:53–58)

Key words: *bone resorption, bone substitutes, dental implants, maxillary sinus, osseointegration*

In the resorbed edentulous maxilla, the amount of bone available for implant placement is often insufficient. For patients who have a normal maxillomandibular relationship of the jaws according to height, onlay grafts may be unfavorable in attempting to provide an esthetic and functionally satisfying prosthetic rehabilitation. Therefore, in these situations sinus floor elevation using autogenous bone or bone substitutes is usually the method of choice.[1–10] Sinus floor elevation and implant placement can be accomplished simultaneously, assuming initial implant stability is obtained. If primary stability of the implants cannot be achieved, initially a 2-step approach is suitable.

The procedure of sinus floor elevation was initially proposed by Tatum and published by Boyne and James.[1] They described a 2-stage procedure with a healing phase of 4 to 6 months to allow biologic integration of the graft. The surgical technique reported by Boyne and James[1] used the preparation of a bony window in the lateral sinus wall. The created bone plate remained fixed to the inner sinus mucosa, which

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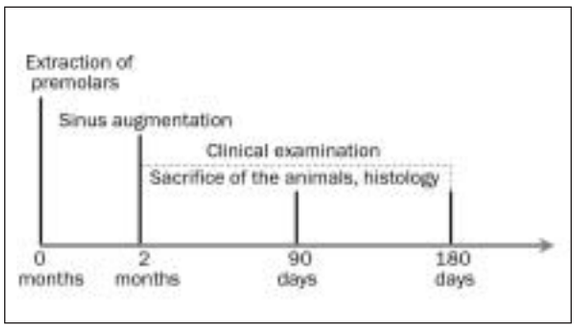


Fig 1 Flow chart of the experimental design.

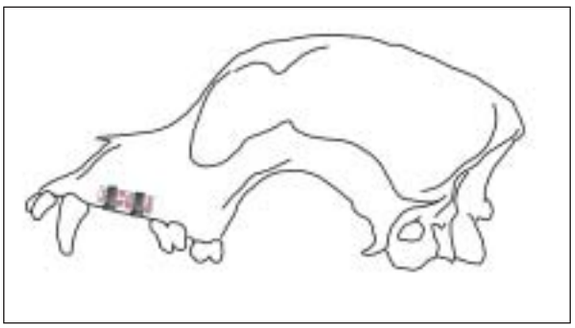


Fig 2 Drawing of sinus augmentation procedure.

functions like a hinge when subsequently the caudal mucosal layer is mobilized cranially. With this procedure, a cavity is created, which then can be filled by autogenous bone, bone substitutes, or a mixture of both.

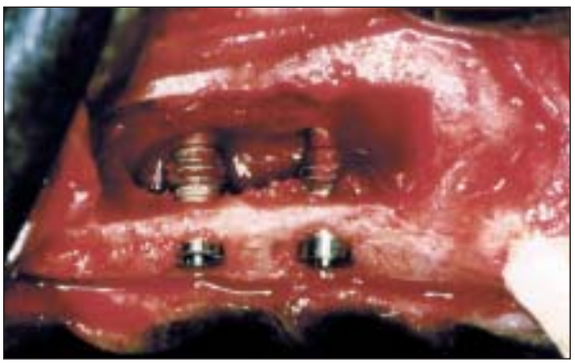
As an augmentation material, or so-called gold standard, autogenous bone is considered ideal because of the fact that remodeling takes place without any immunologic resistance.[3,4,11] Donor sites for these grafts are generally the iliac crest for bilateral approaches and the oral cavity for unilateral augmentations.[9] Patients may consider the second surgical intervention in the donor area uncomfortable and may prefer the use of bone substitutes for the procedure.[9,12–22] Since there are a large number of bone substitutes available, the properties of these materials related to biocompatibility and function in comparison to the gold standard must be considered by surgeons in making recommendations and assisting in the patient's treatment-planning process.

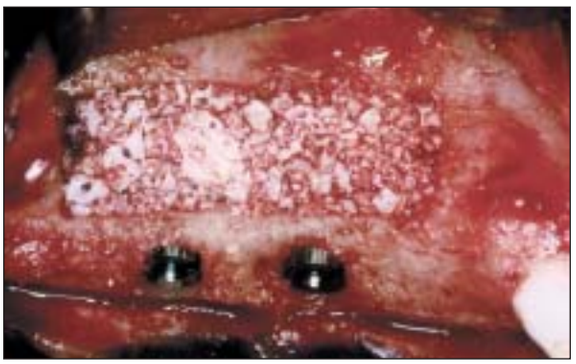
MATERIALS AND METHODS

This research project was approved by the Ethical Committee for Animal Research, Department of Justice, Copenhagen, Denmark, before the project commenced. Ten 12-month-old beagle dogs were included in the proposed study (Fig 1). For all surgical interventions, the animals were anesthetized by intravenous injection of Immobilon (Pherrovet, Malmö, Sweden). In the first session, the 3 premolars in the maxilla were removed bilaterally. The teeth were divided to minimize bone trauma, and before closure of the wound, bony spicules were removed. The wound was sutured with resorbable sutures (Vicryl 4.0, Ethicon, Norderstedt, Germany). Anesthesia was terminated by injection of Revivon (Pherrovet). The animals were allowed 6 weeks healing of the bone and soft tissues before the second surgery. In this study, the healing period to secure bone defect regeneration was based not on scientific evidence but in accordance with some previous publications.[11,15]

For the second surgery, the animals were anesthetized in the same way with Immobilon. A full-thickness mucoperiosteal flap was created in each edentulous jaw area. With a Lindemann bur, a standardized 25×7-mm window was created in the lateral sinus wall on each side of the maxilla. The sinus membrane was carefully removed from the basal bone and elevated to create a bony cavity. With a Lindemann bur, monocortical bone was harvested from the lateral aspect of the mandible and was kept in a sterile saline solution after being milled in a bone mill to particles of 0.7 mm^3. Two titanium Frialit-2 implants (3.0×8 mm, Friadent, Mannheim, Germany) were placed in each defect. All implants had primary stability in native bone and perforated the sinus cavity apically (Fig 2). One side of the maxilla was randomly assigned for treatment with autogenous bone, and in the other side Bio-Oss (Geistlich, Wolhusen, Switzerland) was used (Fig 3). The defect was completely filled using the selected material. Finally, the mucoperiosteal flap was readapted and sutured with resorbable sutures (Vicryl 4.0, Ethicon). Anesthesia was terminated by injection of Revivon (Pherrovet).

The animals were maintained at the research animal facility of Aarhus University (Paskehojgard, Aarhus) after surgery. All animals were treated with prophylactic antibiotics (Penicillin G), which commenced during surgery and continued for 3 days after surgery. The animals also received analgesic treatment (Temgesic, Boehringer, Mannheim, Germany) for 3 days postoperatively to avoid pain associated with the surgical procedure. All animals were kept on a liquid diet for the duration of the study. Once per week, the animals received a professional oral hygiene treatment of tooth brushing and local application of chlorhexidine gel. Five animals were

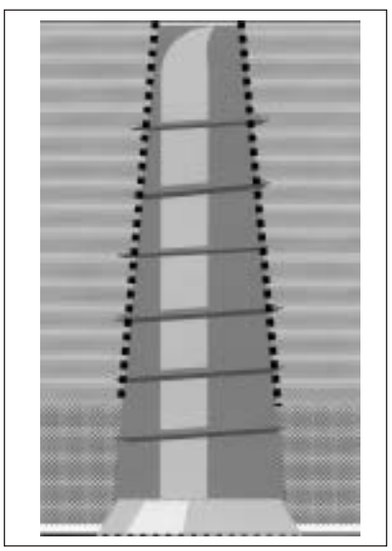




Figs 3a and 3b A site augmented with Bio-Oss. *(Left)* After elevation of the sinus floor and implant placement; *(right)* after augmentation with Bio-Oss.

Fig 4 *(Left)* Illustration of the region of interest for the measurement of implant-bone contact.

Fig 5 *(Right)* Histologic specimen used for measurement of implant-bone contact (red arrows indicate zones without contact; magnification ×1.25).



sacrificed after 90 days of observation, and 5 animals were sacrificed after 180 days by an overdose of pentobarbital, and they were intravenously perfused with 10% neutral buffered formalin injected through the carotid arteries. The maxillae were removed in total, and the specimens were fixed by immersion in formalin solution, dehydrated in alcohol, and embedded in acrylic resin for histologic examination by means of undecalcified sections using the technique described by Donath and Breuner.[23]

These histologic specimens were subjected to qualitative histologic analysis with respect to amount of bone regeneration and amount of bone-to-implant contact of the titanium implants (Fig 4). Undecalcified sections 100 µm thick were produced in a mesiodistal direction parallel to the long axes of the implants. They were stained with Sudan black, toluidine blue, basic fuchsin, and light green. Three sections per block, 750 µm apart and representing the midportion of the specimen, were subjected to histometric analysis using the Quantimet image analysis system (Leitz, Cambridge, United Kingdom) (Fig 5). The cross-sectional area of the defect was measured and expressed as a percentage of the cross-sectional area of the original defect. The distance between the coronal border of the dental implants and the coronal border of the regenerated bone was also measured, as well as the implant-bone contact rate, as described by Matsui and coworkers.[24] The mean for each specimen was then measured (Fig 6) and the data were subjected to statistical analysis by the WIlcoxon test for paired observations.

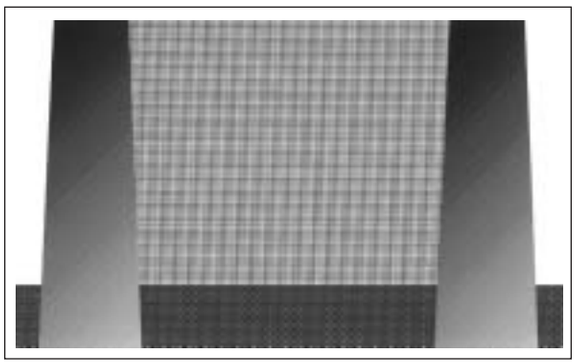


Fig 6 Illustration of the region of interest for measurement of the volume of the augmentation.

RESULTS

Clinical observation after surgery in the 2 groups showed no significant differences in healing patterns.

Results at 90 Days

In 1 animal in which Bio-Oss was used, the caudal contact zone was already infiltrated by newly formed woven bone filling the gap. All Frialit-2 implants showed good bone-to-implant contact in the area of original bone by means of light microscopy (mean 70 ± 18.79%). The implant portion penetrating the Bio-Oss was partially separated from the bone substitute by connective tissue. Regarding the bone-to-implant contact, approximately 52.16% (original bone 70 ± 18.79%; augmented sinus 34.31 ± 9.65%) of the surface was covered by bone. The reduction in volume of the augmented area in the histologic findings, in comparison to the original defect size, showed a mean value of 14.60 ± 4.4%.

In the group treated with autogenous bone, fibrous connective tissue could be found in all specimens. In 1 animal, necrotic bone particles could be seen and in another animal, the identification of native bone versus bone graft could not be made. All Frialit-2 implants showed a good bone-to-implant contact in the area of native bone by means of light microscopy (mean 60.21 ± 11.46%). The implant portions penetrating the augmented bone were partially separated from the grafted bone by connective tissue. Regarding the bone-to-implant contact, 60.21% (original bone 65.42 ± 5.60%; augmented sinus 58.13 ± 4.56%) of the surface was covered by bone. The volume of the augmented area in comparison to the original defect had been reduced by 3.8 ± 2.5%.

Results at 180 Days

Bio-Oss showed osseous ingrowth in all specimens. In 1 animal, residual fibrous tissue could be found. Regarding the bone-to-implant contact, approximately 63.43% (original bone 69.64 ± 10.56%; augmented sinus 57.64 ± 9.56%) of the surface was covered by bone. The volume of the augmented area in the histologic findings in comparison to the original defect was 16.5 ± 8.67% (Fig 7a).

In the group treated with autogenous bone, the interface of the graft to original bone could no longer be distinguished in 3 animals. The bone-to-implant contact showed 42.22% (original bone 52.31 ± 4.87%; augmented sinus 32.13 ± 14.46%) coverage by bone. The volume of the augmented area in the histologic findings in comparison to the original defect was 39.80 ± 16.14% (Fig 7b).

DISCUSSION

Implant-based prosthetic rehabilitation of the posterior edentulous maxilla is difficult in many cases because of the extension of the sinus. Incorporation of endosseous implants in augmented sinuses, whether placed simultaneously or as a 2-stage procedure, has been clinically proven as a predictable surgical treatment method.[3–5,9,15,20,25,26] Still in question is the permanence of the grafted material. The aim of this research was the validation of autogenous bone compared to the bone substitute Bio-Oss regarding bioacceptance and the question of resorption using a split-mouth animal design as a model.

The advantage of autogenous bone as a graft material is the fast angiogenic ingrowth of vessels from the surrounding original bone. This revitalizes parts of the graft and its cells, which subsequently will participate in the local metabolism, meaning osteoclastic resorption and functional oriented osteoblastic remodeling. Resorption includes the liberation of growth factors, such as platelet-derived growth factor and transforming growth factor-beta, involved with the forming of new capillary sprouts, the proliferation of stem cells, and the activation of macrophages.[12,27] In addition, autogenous bone used as a graft needs the stimulation of functional loading (for example, via implants) to respond with a balanced cascade of resorption and formation of new bone. In the present model the implants remained unloaded. The autogenous graft lost approximately 4% of its volume after 90 days and a considerably larger percentage of 40% after 180 days. Taking the biologic metabolism of the beagle into consideration, the implants remained unloaded for a period far exceeding the 6 months in humans described by Boyne and James.[1]

Fig 7a Histologic specimen augmented with Bio-Oss at 180 days shows minimal resorption (red arrows indicate resorption zones; magnification ×1.25).

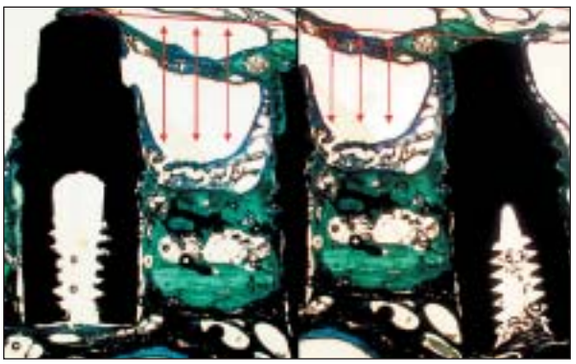


Fig 7b Specimen augmented with autogenous bone at 180 days showing maximal resorption (red arrows indicate resorption zones; magnification ×1.25).

On the other hand, the xenogenous Bio-Oss was nearly undisturbed by resorption and measured a loss of approximately 15% after 90 and 180 days. The histologic analysis did not demonstrate any signs of resorption of the Bio-Oss scaffold; only the ingrown native bone seemed to participate in the remodeling process. The initial volume reduction must be explained by shrinkage of the connective tissue and its conversion process in native bone. However, Bio-Oss will not be resorbed, and native bone does integrate into the material through penetrating the scaffold and forming vital bone layers on the avital trabeculae of the Bio-Oss structure.[11,18–21] The interconnecting pores of the material allow complete camouflage of this foreign body, and the ingrown vital bone layers participate in the turnover involved in the remodeling process.

CONCLUSION

Considering the outcome of this limited study, Bio-Oss can be successfully used as a material for sinus augmentation when one does not need full bony regeneration of the augmented area. This cannot be achieved using Bio-Oss alone because of its material properties. But the use of Bio-Oss can prevent unwanted early resorption of the augmentation area in the sinus.

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