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A histomorphometric comparison of anorganic bovine bone (ABB) and calcium sulfate (CaS) used in sinus augmentation procedures: a study in sheep

ABSTRACT

Aim The selection of an appropriate grafting material is one of the factors that are important in achieving adequate bone formation following sinus grafting. Histologic and histomorphometric examination is the best method for evaluating the outcome of a sinus augmentation procedure as one can evaluate both the degree of vital bone formation and the implant-bone interface. The aim of the present study was to perform a histologic and histomorphometric comparison between anorganic bovine bone (ABB) and calcium sulfate (CaS) in sinus augmentation procedures in sheep.

Materials and methods Twelve adult female sheep were used in the present study. In each animal one sinus was randomly selected to receive ABB, whereas the contralateral side received CaS. An equal volume of graft material was used (3,5 cm³) within each sinus cavity. At 3 and 6 months, following implant placement, a group of 6 animals was euthanized, and specimens retrieved with a 5 mm trephine bur to be processed for histology.

Results New bone formed directly on the surface of the ABB particles without gaps or formation of fibrous connective tissue. The graft particles served as a scaffold for the new bone formation and the material appeared to be highly osteoconductive. There was an increase in the amount of newly formed bone from 3 months (21%) to 6 months (39%) with a corresponding decrease in the amount of residual grafted material from 39% to 32%. Also in the CaS augmented sinuses there was an increase from 3 months (19%) to 6 months (37%); the decrease in the residual grafted material was from 27% at 3 months to 9% at 6 months. The bone-implant contact (BIC) increased over time for both materials, reaching 45% for ABB and 40% for CaS at 6 months.

Conclusions The regenerated bone appeared to be able to grow in close apposition to dental implants. Moreover, ABB complete resorption did not seem to be a prerequisite for new bone formation and implant integration. On the other hand, CaS quick resorption processes did not seem to prevent the formation of bone in tight contact with the implant surface. Both materials seemed to be suitable for sinus augmentation procedures.

KEYWORDS Calcium sulfate; Organic bovine bone; Sinus augmentation procedure.

INTRODUCTION

The goal of a successful sinus augmentation procedure is the formation of a sufficient bone matrix for both the mechanical support and the biological integration of endosseous dental implants (1, 2). Histological analysis of the regenerated tissues in grafted sinuses will provide useful information on the nature and amount of newly-formed bone (3). The timing for the resorption and ultimate replacement of the graft materials is not completely fully understood (3). The sheep model has proven to be an appropriate animal model: the sheep presents a maxillary sinus of an adequate size and the bone physiology and structure are reportedly similar to those of man (4).

Anorganic bovine bone (ABB) is a deproteinized sterilized bovine bone with 75% to 80% porosity and a crystal size of approximately 10 µm in the form of cortical granules. Many animal and human clinical and histological reports of ABB used for maxillary sinus floor elevation have been published (5-15). ABB has been shown to be able to up-regulate some functional activities of osteoblast-like cells, e.g. cell cycle regulation, signal transduction, apoptosis, and vesicular transport (16). In human histological studies of specimens retrieved from sinuses augmented with ABB a close spatial relationship was found between angiogenesis and osteogenesis (17, 18). The ability of dental implants to survive in sinuses grafted with ABB has been documented in some recent reviews (19-21). Different opinions have been expressed about the

resorption capabilities of ABB. No osteoclastic activity was found (11) and ABB did not seem to be affected by resorption and remodeling (22). Other researchers found, on the contrary, that the ABB underwent osteoclastic resorption (9, 23, 24). In an in vitro study it was found that osteoclasts formed on ABB particles, and that these osteoclasts were able to attach and to resorb the material particles (25).

Calcium sulfate (CaS) is an highly biocompatible material which has the characteristic of being one of the simplest as well as one of the synthetic bone graft materials with the longest clinical history, spanning more than 100 years. It has been successfully used to treat periodontal disease, endodontic lesions, alveolar bone loss, maxillary sinus augmentation, and orthopedic lesions (26-29). CaS rapidly resorbs leaving a calcium phosphate lattice which promotes osteogenic activity, mimics the mineral phase of bone and is resorbed at the rate of bone formation (30). Concern has been expressed about this material, due to its fast resorption (31). Complete CaS resorption has been reported in 6 weeks in rabbits (32) and 13 weeks in dogs (33). In humans, CaS is almost completely resorbed after 6-8 months (34-36). In a histologic and histomorphometric study on sinus augmentation procedure with several different grafting materials it was found that with CaS the newly formed bone was $38 \pm 3.2\%$, marrow spaces $45 \pm 1.3\%$, and residual graft particles $13 \pm 2.1\%$, while, with ABB newly formed bone was $39 \pm 1.6\%$, marrow spaces $34 \pm 1.6\%$, residual material $31 \pm 1.4\%$ (14). No differences were found in the amount of bone regenerated in sinus augmentation using CaS or ABB while a significant difference was found in the amount of residual grafted material (14).

Histologic examination is the only mean whereby it is possible to evaluate the outcome of a sinus augmentation procedure by studying the events at the implant-bone interface (11).

The aim of the present study was to perform a histologic and histomorphometric comparison of ABB and CaS in sinus augmentation procedures in sheep. Vital bone formation and bone to implant contact will be evaluated at both 3 and 6 months.

MATERIALS AND METHODS

Twelve adult female sheep (approximately 20 to 36 months old) were used in the present study. Surgical procedures were performed in a strict sterile environment under general anesthesia. Sedation was achieved with xylazine 0.2 mg/kg i.m. (Rompum®; Bayer), followed after 10 minutes by diazepam 0.2 mg/kg i.v. (Diazepam® 0.5; Intervet) and atropine sulfate 6 mg i.m. (Atropina Solfato; Fort Dodge). Anesthesia was induced with ketamine 10 mg/kg i.m.

(Ketavet® 100; Intervet). The sheep were intubated, and general anesthesia maintained by inhalation of 2.5% halothane (Halotane®; Merial) in a mix of oxygen.

Surgical protocol

The surgical field was prepared to include the main landmarks, namely, the angular vein of the eye and the transverse artery of the face. The sheep were prepped and draped in a customary manner for a sterile surgical procedure. An oblique extraoral incision, approximately 5 cm in length, was made over the most ventral aspect of the maxillary sinus with a #11 scalpel blade. The subcutaneous tissue and the masseter muscle were bluntly divided to expose the maxillary periosteum, which was incised and elevated dorsally. The lateral wall of the sinus was approached with a #6 surgical rotating tungsten bur to perform a rectangular surface flap for antrostomy under abundant irrigation with saline solution. The bone of the rectangular flap was removed with a chisel instrument along the osteotomy line. Maxillary sinus elevation was performed bilaterally in each sheep. The elevation of the schneiderian mucosa was performed bilaterally in each sheep with special care to avoid membrane perforation. In each animal, one sinus was randomly selected to receive ABB (Geistlich Bio-Oss, Geistlich, Wohlen, Switzerland), whereas the contralateral side received the CaS (SurgiPlaster Sinus, Ghimas, Casalecchio di Reno, Bologna, Italy). An equal volume of graft material was used (3.5 cm^3) within each sinus cavity. In the sinus filled with ABB a collagen membrane was positioned to cover the bony window, while, in the sinus filled with CaS, calcium cement was positioned to cover the bony window. Two $4 \times 13 \text{ mm}$ implants (Biolog International, Boca Raton, FL, USA) were placed at a distance of 2 mm from the bony window and at a distance of 3-4 mm from the residual sinus wall. The two implants were located approximately 2 mm posteriorly to the bony window. In this study were used tapered implants, since they offer a natural resistance against displacement (into the sinus) as they are wide at the alveolar crest and narrow at the apical end. A very good primary implant stability was obtained in all cases. The implants were in contact with only 2 mm of native bone while the remaining area of the implants was in contact only with the biomaterial. Deep and superficial fasciae of the masseter muscle were sutured with a 3-0 multifilament resorbable suture (Vicryl® - Ethicon Inc.) in a simple continuous pattern. The animals were given IV 20 mg/kg of Ampicillin (Vetamplius® - Fatro) every 12h for 3 days postoperatively. No postoperative complication were present.

At 3 and 6 months following implant placement, a group of 6 animals was euthanized using an overdose

of thiopental (Pentothal Sodium - Intervet) and embutramide (Tanax® - Intervet).

Histologic examination

Each maxilla was separated from the skull, and gross sectioning of the specimen was performed. Sinuses and surrounding tissues were washed in saline solution and immediately fixed in 4% para-formaldehyde and 0.1% glutaraldehyde in 0.15 M cacodylate buffer at 4°C and pH 7.4. The specimens were processed using the Precise 1 Automated System (Assing, Rome, Italy) (37). The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization the specimens were sectioned, along the longitudinal axis of the implants, with a high-precision diamond disc at about 150 µm and ground down to about 30 µm with a specially designed grinding machine. Three slides were obtained for each implant. These slides were stained with acid fuchsin and toluidine blue and examined with transmitted light Leitz Laborlux microscope (Leitz, Wetzlar, Germany).

Histomorphometry was carried out using a light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high resolution video camera (3CCD, JVC KY-F55B, JVC®, Yokohama, Japan) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, Intel®, Santa Clara, CA, USA). This optical system was associated with a digitizing pad (Matrix Vision GmbH, Oppenweiler, Germany) and a histometry software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc Milano, Italy).

Statistical analysis

The values for bone-implant contact (BIC), marrow spaces, residual graft material and newly-formed bone were recorded and the mean values calculated. Statistical significance was evaluated with a parametric test, the Student-Neuman-Keuls test for multiple comparisons. The percentage of bone-implant contact, marrow spaces, residual grafted material and new bone have been expressed as a mean ± standard deviation and standard error. Statistically significant differences were set at $p < 0.05$. The analyses were performed using SPSS 8 for Windows.

RESULTS

ABB and CaS at 3 months

Newly-formed bone was present around the perimeter of the implant, and was characterized by a strong affinity for acid fuchsin, and the presence of wide osteocyte lacunae in the ABB (Fig. 1) and CaS

groups (Fig. 2). Some grafted particles of ABB and CaS were lined by newly-formed bone (Fig. 3, 4). In these areas there was a complete absence of osteoblasts. Only few small diameter capillaries were present in the ABB group, while many capillaries were present in the CaS group. In some areas it was possible to observe bone remodelling units (BMU) with vessels, osteoblasts and osteoclasts. No contact was observed between ABB and CaS particles, and the surface of the implants. No acute or chronic inflammatory reaction was present in the two groups. In particular at the periphery of each granule of CaS there was a diffuse band of material that appeared greyish in color, in close contact with a region positive for acid fuchsin, representing the newly formed bone. In the center of each bead, a greyish granular polycrystalline material was evident; there were small patches of acid fuchsin positivity, showing osteoid matrix.

Histomorphometry in the ABB group showed that newly-formed bone represented $21 \pm 1.2\%$, marrow spaces 40 ± 3.1 , while the residual grafted material was $39 \pm 3.2\%$. Bone to implant contact percentage (BIC) was $20.5 \pm 2\%$. Histomorphometry in the CaS group showed that newly-formed bone represented $19 \pm 2.2\%$, marrow spaces 45 ± 3.3 , while the residual graft material $27 \pm 1.9\%$. BIC percentage was $14 \pm 2\%$.

ABB and CaS 6 months

Mostly mature bone was present at the interface with the implant in the two group (Fig. 5, 6). Lamellar and woven bone were separated by a well-defined irregular



Fig. 1 ABB 3 months. Newly-formed bone was present the perimeter of the implant. Acid fuchsin-Toluidine blue (8X).

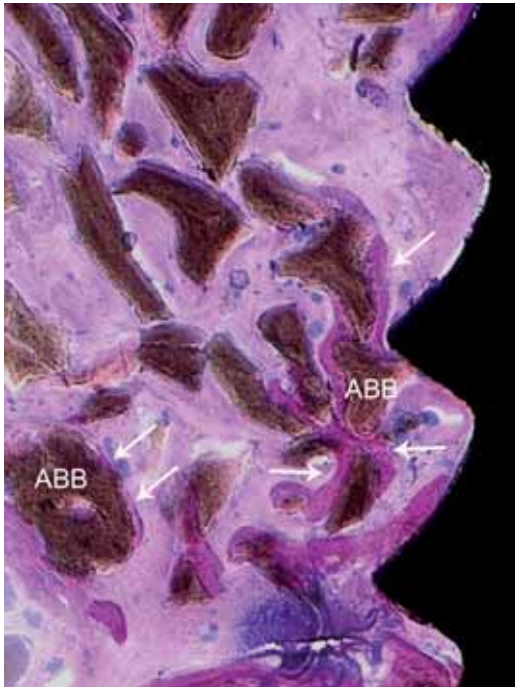


Fig. 2 ABB 3 months. At higher magnification it was possible to observe that some ABB particles (ABB) were surrounded by newly-formed bone (arrows). Acid fuchsin-Toluidine blue (40X).

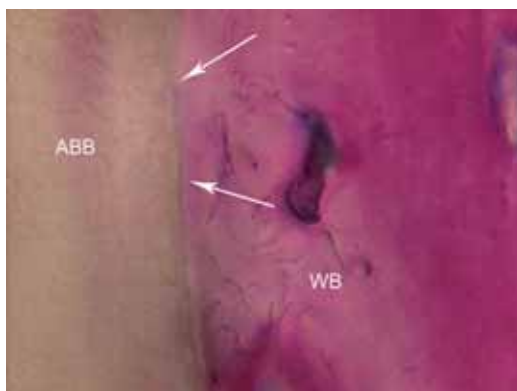


Fig. 4 ABB 6 months. At higher magnification it was possible to observe a particle of ABB (ABB) in direct contact with newly-formed bone (WB). No gaps were observed at the interface (arrows). Acid fuchsin-Toluidine blue (400X).

cement line. Small marrow spaces or small resorption lacunae were located at the tips of all the threads of the implants. The bone surrounding these lacunae presented a strong affinity for the dyes, wide osteocytic lacunae and it appeared to be undergoing remodelling. In this area, bone lamellae were organized in a concentric way around the point of the thread. No gaps or fibrous tissue were present at the interface between ABB particles and bone (Fig. 7). In the CaS group newly formed bone in close contact to the implant surface was present and large marrow spaces surrounded by osteoblasts actively depositing osteoid matrix were observed (Fig. 8). CaS was almost completely resorbed,



Fig. 3 ABB 6 months. Two implants were surrounded by mature bone. Acid fuchsin-Toluidine blue (8X).



Fig. 5 CaS 3 months. Newly-formed bone with large marrow spaces was present around the implants. Acid fuchsin-Toluidine blue (8X).

and newly formed bone was present in close contact with some residual particles. Some of the marrow spaces abutted on the implant surface and some of the Haversian systems were in direct contact with the implant surface. No acute or chronic inflammatory cell infiltrate was present. No contact was observed between the grafted particles and the surface of the implants, and newly-formed bone was always interposed between these two structures. Histomorphometry in the ABB group showed that newly-formed bone represented $39 \pm 3.3\%$, marrow spaces $42 \pm 3.5\%$, while the residual grafted material was $32 \pm 2.5\%$. BIC percentage was $45 \pm 2\%$. Histomorphometry in the CaS showed that newly-formed bone represented $37 \pm 2.1\%$, marrow spaces



Fig. 6 CaS 3 months. At higher magnification it was possible to observe newly-formed trabecular bone (WB) and new vessels (arrows) in the CaS. Acid fuchsin-Toluidine blue (100X).



Fig. 7 CaS 6 months. Mature bone with marrow spaces was present around the implants. No residual particles of CaS were observed. Acid fuchsin-Toluidine blue (8X)

N = 12	% newly formed bone	% ABB and CaS	% marrow spaces	BIC %
ABB 3 months	21±1.2	39 ±3.2	40 ±3.1	20
ABB 6 months	39 ±3.3	32 ±2.5*	42 ±3.5*	45
CaS 3 months	19 ±2.2	27 ±1.9	45 ±3.3	14
CaS 6 months	37 ±2.1	9 ±3.1*	59 ±4.1*	40

Table 1 Percentages of newly-formed bone, residual grafted materials, marrow spaces, and bone to implant contact (BIC) for both materials.

59±4.1, while the residual graft material was 9±3.1%. BIC percentage was 40±2% (Table 1).

Statistical analysis

At 3 months, statistical analysis of the differences between the two biomaterials was not significant for newly formed bone ($p=0.020$) and for percentage of marrow spaces ($p=0.02$). The differences were, however, significant for residual grafted biomaterials present ($p=0.001$) and for the bone-implant contact percentages ($p=0.009$). At 6 months, statistical analysis of the differences between the two biomaterials was not significant for percentages of newly-formed bone ($p=0.239$) and bone implant contact ($p=0.011$). Statistically significant differences were present for the residual grafted biomaterials ($p=0.0001$) and for the marrow spaces ($p=0.001$).

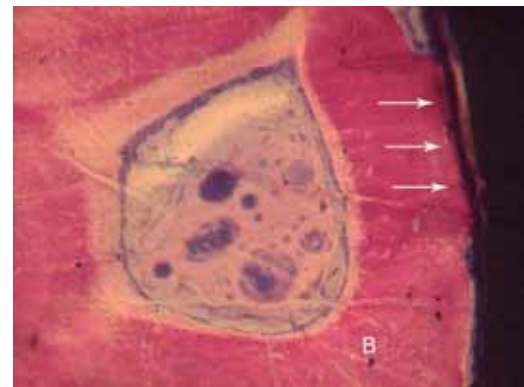


Fig. 8 CaS 6 months. Mature bone (B) and in direct contact with the implant surface. No gaps were present at the interface (arrows). Acid fuchsin-Toluidine blue (200X).

DISCUSSION

In some histologic studies of implants retrieved from human sinuses augmented with ABB it was found that the continued presence of ABB particles did not jeopardize the successful implant integration, and no contact was ever observed between the residual grafted particles and the implant surface with a layer of newly formed bone always interposed between the implant surface and the graft particles (8, 38-40). In a series of retrieved human biopsies from sinuses augmented with ABB, a decrease of the percentage of residual grafted particles was observed over time: 31-39% at 6 months, 29% at 20 months, 16% at 9 years (14, 41, 42). In two human studies (31, 43) on sinus specimens retrieved after 4 and 6 months it was shown that CaS was almost completely resorbed and

substituted by calcium phosphate, in a way similar to that already reported in rabbits and dogs (30, 44). In a histological evaluation of an implant retrieved, after 7 months, from a sinus augmented with CaS it was shown that newly-formed bone was found at the interface of the implant, with a very high bone-to-implant contact percentage (55%) (34).

In the present study, newly formed bone was formed directly on the surface of the ABB particles with no intervening gaps or formation of fibrous connective tissue. The graft particles served as a scaffold for the new bone formation and the material appeared to be highly osteoconductive. All the ABB particles appeared to be integrated into the newly formed bone. There was an increase in the amount of newly formed bone from 3 months (21%) to 6 months (39%) with a corresponding decrease in the amount of residual grafted material from 39% to 32%. Also in the CaS augmented sinuses there was an increase from 3 months (19%) to 6 months (37%); the decrease in the residual grafted material was from 27% at 3 months to 9% at 6 months. The values for the residual grafted materials at 6 months both for the ABB are strikingly similar to the values found in human augmented sinuses, where the values were respectively 31% for ABB and 13% for CaS. The BIC increased over time for both materials, reaching 45% for ABB and 40% for CaS at 6 months. The newly formed bone appeared to be closely adapted to the implant surface; no gaps or connective tissue was present at the interface. No foreign body giant cells were visible at the bone-implant interface. It must be mentioned that a confounding variable could be the use of a collagen membrane on one side and of calcium cement on the other; a resorbable barrier membrane has been shown to have a positive effect while the effect of a calcium cement barrier is unknown.

In conclusion, the present study demonstrated that the regenerated bone appeared to be able to grow in close apposition to dental implants. No statistically significant differences were present in the bone to implant contact percentages at 6 months between the two groups. The newly formed bone was in direct contact with a large portion of the implant surface. Moreover, the complete resorption of ABB did not seem to be a prerequisite for new bone formation and implant integration and the presence of residual grafted particles did not seem to interfere with the bone healing processes. On the other hand, the quick resorption of the CaS did not seem to prevent the formation of bone in tight contact with the implant surface.

Both materials seemed to be suitable for sinus augmentation procedures and showed a high biocompatibility and osteoconductivity. Long-term studies are needed to better understand the ultimate fate of the graft materials.

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