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Histologic and histomorphometric evaluation of anorganic bovine bone (ABB) retrieved 7 years after a sinus augmentation procedure

Keywords Sinus lift, histology, anorganic bovine bone, long term studies.

ABSTRACT

Background Anorganic bovine bone (ABB) has osteoconductive properties and no inflammatory or adverse responses. In spite of these successful results, histological data in humans over the long-term period are scarce. The purpose of this study was to evaluate a bone specimen retrieved from a sinus augmented with ABB 7 years after surgery.

Materials and methods The histological examination was performed in 5 thin sections of the specimen, comparing histomorphometric measures for newly formed bone, marrow spaces, residual ABB particles. The investigation was carried out under light microscopy.

Results Trabecular bone with many remodeling areas was present. Grafted ABB particles were almost completely surrounded by bone, and in some cases they were found to be in contact with secondary osteons. Some particles were united by newly formed bone trabeculae. In other areas, the particles seemed to be almost completely resorbed and substituted by bone. No gaps were present at the bone-biomaterial interface. No inflammatory tissue infiltrate was present. Histomorphometry showed that bone represented 46% ± 3.8%, ABB particles 9% ± 1.2%, marrow spaces 45% ± 3.2%.

Conclusions After 7 years the tissue pattern appeared composed of residual particles of the grafted biomaterial in close contact to the new bone. The mineralized matrix of the bone around the particles had collagen fibers randomly oriented and significantly more osteocytes embedded. The results demonstrate both a high level of osteoconductivity and a "biomimetic" behaviour of the biomaterial over time.

INTRODUCTION

Different opinions have been reported about the resorption capabilities of anorganic bovine bone (ABB): no osteoclasts on the surface of the material were observed in some studies (1-6), while, on the contrary, a resorption has been observed in other studies (7-15). It has been reported that a biomaterial should be resorbed with time and be replaced by newly formed bone (5). Moreover, a bone substitute material should be osteoconductive and biocompatible. The ultimate long-term fate of ABB is still not completely known (5).

In previous studies performed in our laboratory we found different values of residual ABB in specimens retrieved from human sinuses: 31-39% at 6 months, 29% at 20 months, 12% at 5 years, 16% at 9 years (16-19). The slow resorption of ABB could be an advantage in that it helps in keeping the dimensions of the augmented sites (20), in contrast to what has been observed to happen with autologous bone, where, in some instances, a resorption of more than 50% of the original volume of the grafted material was observed (20). Furthermore, a predictable

osseointegration of dental implants can be obtained even in sites where the ABB grafted material is not completely resorbed over time (18, 21-23). Human histologic reports on the ABB performance over the long-term period are present in the literature, but are quite rare. Long-term period results in humans are important to understand if the continued presence of the graft particles can produce an interference with the prognosis of dental implants inserted into this bone composite (4).
Aim of the present study was a histological and histomorphometrical evaluation of a specimen retrieved 7 years after a sinus augmentation procedure with ABB.

MATERIALS AND METHODS

A 52-year-old patient with a monolateral maxillary edentulism involving the premolar/molar regions underwent a sinus augmentation procedure. The preoperative mean height of the subantral bone was 3 mm. The patient was a non smoker and had a non contributory past medical history. In the surgical procedure 100% of ABB was used. After a 6 months healing period, 3 dental implants (XiVE®, Dentsply-Friadent, Mannheim, Germany) were inserted and restored with a fixed prosthesis. The restauration was loaded and the implants appeared to be successfully osseointegrated.
After a little more than 7 years, one of the implants had to be substituted due to periimplantitis. During the removal of the implant and the insertion of a new implant, a bone core of the regenerated area was obtained with a trephine bur.

Processing of specimens

The bone core was stored immediately in 10% buffered formalin and processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy) (25). The specimen was dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200, VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimen was sectioned longitudinally along the major axis with a high-precision diamond disc at about 150 µm and ground down to about 30 µm. Three slides were obtained. The slides were stained with acid fuchsin and toluidine blue.

Histomorphometry

Histomorphometry of the percentages of newly-formed bone, residual grafted particles and marrow spaces was carried out using a light microscope (Leitz Laborlux, 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, Media Cybernetics Inc., Immagini e Computer Snc, Milano, Italy).

RESULTS

At low power magnification, trabecular bone with wide marrow spaces was present (Fig. 1). Grafted ABB particles were almost completely surrounded by bone, and in some cases they were found to be in contact with secondary osteons (Fig. 2). The newly-formed bone had grown in direct contact with the ABB particles which appeared to be, in most instances, totally incorporated in bone with no gaps or connective, fibrous tissue at the bone-bioma-

Fig. 1 At low power magnification, trabecular bone with many marrow spaces was present. Few residual grafted particles (asterisk) are evident. Acid fuchsin-toluidine blue 12X.

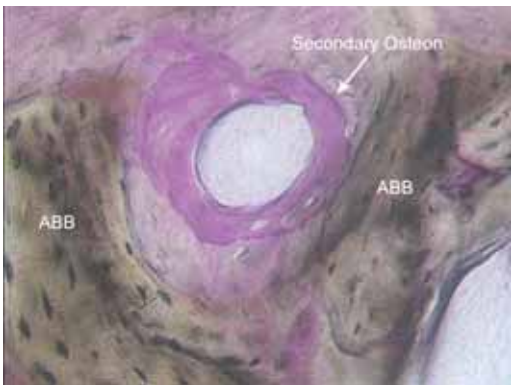

Fig. 2 Grafted ABB particle in close contact with a secondary osteon. Acid fuchsin-toluidine blue 200X.

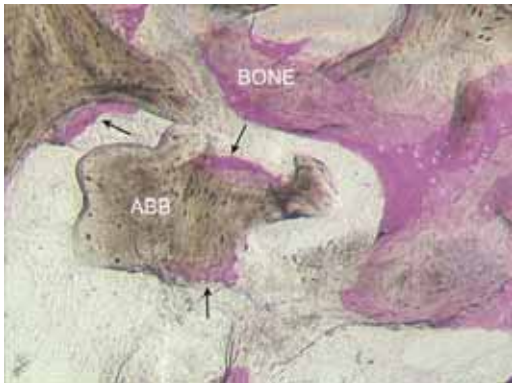

Fig. 3 The newly-formed bone (arrows) had grown in direct contact with the ABB particles with no gaps at the bone-biomaterial interface. Acid fuchsin-toluidine blue 100X.

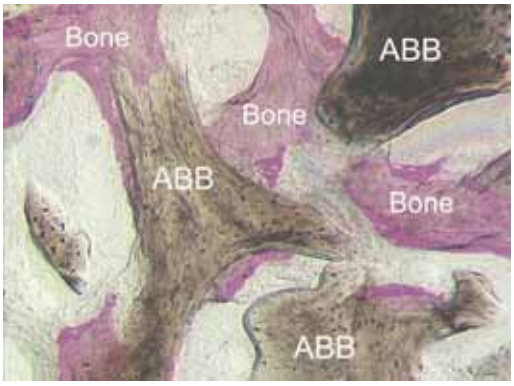

Fig. 4 Bone bridges connected some ABB particles. Acid fuchsin-toluidine blue 100X.

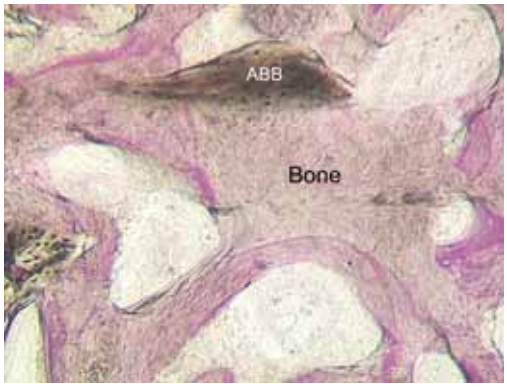

Fig. 5 An ABB particle seemed to be partially resorbed and substituted by bone tissue. Acid fuchsin-toluidine blue 100X.

terial interface (Fig. 3). Some ABB particles were bridged by newly formed bone trabeculae (Fig. 4). In other areas, the particles seemed to be partially resorbed and substituted by bone tissue (Fig. 5). Around some particles it was possible to see the presence of a rim of osteoblasts that were in the active process of deposing osteoid matrix (Fig. 6a-b). No osteoclasts were visible. No inflammatory tissue infiltrate or foreign

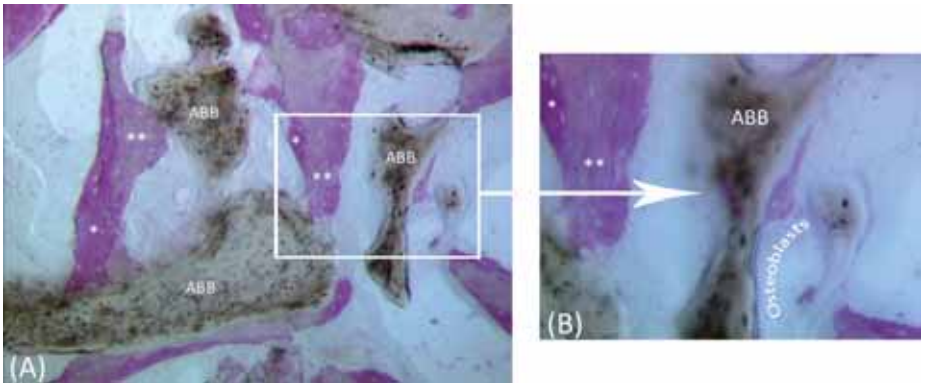

Fig. 6a ABB particles were surrounded and connected by bone bridges of mature(**) and newly formed (*). Bone remodeling areas are present. Acid fuchsin-toluidine blue. 100 X.

Fig. 6b Newly formed bone trabecula with a rim of osteoblasts actively secreting bone matrix between two ABB particles. Acid fuchsin-toluidine blue 200X.

body reaction was present. Histomorphometry showed that bone constituted 46% ± 3.8%, ABB particles 9% ± 1.2%, while marrow spaces represented 45% ± 3.2%.

DISCUSSION

ABB is almost identical to human bone mineral from a chemical and physical point of view. ABB has a compressive strength of 35 MPa and its porous nature (75% of the total volume) serves to greatly increase its biointegration capability. Histological evaluation of the newly formed tissues in sinus augmentation procedures will be very helpful to understand the nature and amount of newly formed bone and remnants of grafting material.

Resorption of ABB is still a subject of controversy (1) and according to some authors no definitive conclusions can be obtained from the literature if ABB will be resorbed over time (5). Resorption lacunae and osteoclasts were absent in sinus augmentation specimens after 6 and 12 months (1). Also Yildirim et al. (2) did not find signs of osteoclast activity. Ewers et al. (26) have found ABB particles still present after more than 4 years after grafting procedures and no evident signs of resorption.

Different results have been reported by several investigators (7-11). All these Authors have found the presence of active osteoclasts on the surface of the ABB particles, and a decrease of the density of ABB over time. In some cases, it can be very advantageous to use a material that shows very little degradation such as ABB. Osteoclasts are regulated by the events occurring in the microenvironment of the osteoclast-substratum interface. In this sealed compartment osteoclasts produce a release of mineral ions from the substratum surface leading to an increase of Ca^{2}+ ions in this area. This high concentration of Ca^{2}+ ions determines an increase of the intracellular calcium levels with a resulting inhibition of osteoclastic bone resorption and the osteoclast detachment from the bone surface (28).

The incomplete resorption of the ABB particles, found in the present case 7 years after the placement of the graft, could then be explained by the high calcium concentration present on the biomaterial surface which could inhibit the osteoclastic resorption. After 7 years, most of the the graft particles were found to be incorporated within newly formed bone; a cancellous network that might strengthen the osseous tissue mass is then created, and the ability of newly formed bone to withstand implant loading could be improved. Another mechanism explaining the long-term presence of the grafted particles could be a bonding mechanism that helps to maintain a biomechanical integrity of the newly-formed bone/grafted material during the remodeling/repairing processes.

In conclusion, the present histologic results confirm the high biocompatibility and osseoconductivity of ABB: most of the grafted particles were embedded into newly formed bone, and newly-formed bone, in several instances, tended to bridge the biomaterial particles. A favorable long-term response was observed around the ABB particles with no inflammatory cell infiltrate present, and a long-term stability of the tissues seemed to have been reached through the incorporation of the grafted particles in newly-formed bone. The residual grafted ABB particles did not appear to have interfered with bone healing.

ACKNOWLEDGMENTS

This work was partially supported by the

National Research Council (CNR), Rome, Italy, by the Ministry of Education, University and Research (MIUR), Rome, Italy.

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