Maxillary Sinus Augmentation in Humans Using Cortical Porcine Bone: A Histological and Histomorphometrical Evaluation After 4 and 6 Months

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ABSTRACT

Background: Bone substitutes, such as allografts, xenografts, and alloplasts, have been proposed in several augmentation procedures.

Purpose: The aim of the present study was a histologic and histomorphometric evaluation of specimens retrieved 4 or 6 months after sinus augmentation using cortical porcine bone augmentation material.

Materials and Methods: A total of 77 specimens, retrieved after 4 and 6 months from augmented sinuses, were used in this study. The specimens were processed to be observed under light microscopy. Histomorphometric measurements were presented as means ± standard deviations.

Results: Most of the particles were surrounded by newly formed bone with large osteocyte lacunae. Histomorphometry showed that, after 4 months, the newly formed bone represented 28%, marrow spaces 36%, the residual graft material 37%, while, after 6 months, the newly formed bone represented 31%, marrow spaces 34%, while the residual graft material was 37%.

Conclusion: The present results show that cortical porcine bone is a biocompatible, osteoconductive biomaterial that can be used for maxillary sinus augmentation procedures without interfering with the normal reparative bone processes.

KEY WORDS: bone regeneration, porcine bone-derived biomaterial, sinus augmentation procedures

Autogenous bone has been reported to be the golden standard in bone regeneration procedures because it contains viable osteoblasts, organic and inorganic matrices, and biological modifiers.[1] However, the use of autogenous bone has several disadvantages, that is, a limited availability, a tendency to partially resorb, the

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need for an additional surgery, and the increased morbidity. Bone substitutes, such as allografts, xenografts, and alloplasts, have been proposed in several augmentation procedures.[1,2] Maxillary sinus augmentation procedures have been used to obtain a sufficient volume of bone to allow implant placement. Different biomaterials have been used for this procedure, but there are still differences about which graft material is the most suitable. Most bone substitutes are believed to be osteoconductive, serving as scaffold for bone formation.[2–11] Recent systematic reviews of the literature have shown a higher implant survival/success rate using xenografts as compared to autogenous bone.[12–14] Apatos® (Tecnoss, Turin, Italy) is a xenogeneic bone substitute consisting of sterilized cortical porcine bone in form of particles with a high porosity and with a diameter ranging from 250 to 1,000 μm. This biomaterial is similar to human bone,

and it has been reported, in humans, to be osteoconductive, well integrated in the host site and incompletely resorbed after 5 months,[15] and with no signs of adverse reactions in a rabbit study.[2] In a previous light microscopy and transmission electron microscopy study from our laboratory on specimens retrieved from human sinuses augmented with this biomaterial, it was found that the material was biocompatible, well integrated in the host bone, and osteoconductive.[16] It was thought important to confirm further, in a larger number of human specimens, these results after different time periods. The aim of the present study was a histologic and histomorphometric evaluation of specimens retrieved 4 or 6 months after sinus augmentation using the same porcine bone material.

MATERIALS AND METHODS

One hundred twenty-one healthy patients with noncontributory past medical history (71 women and 50 men, all nonsmokers, mean age 54 years, range 51–63 years) were included in this study. All the patients had been treated in the Outpatient Clinic of the Department of Oral Sciences of the University of Chieti-Pescara, Chieti, Italy. All the patients had been enrolled over a 4-year period. The protocol was approved by the Ethical Committee of the University of Chieti-Pescara. All the patients were candidates for augmentation in the posterior maxilla, scheduled to receive fixed prosthesis or crown restorations, and signed a written informed consent. The clinical results of this study will be reported in another manuscript (unpublished data).

The sinus augmentation was performed in all patients using cortical porcine bone particles (Apatos) mixed with sterile saline solution and some blood, and carefully packed in the sinus cavity; the quantity of Apatos needed for each augmentation varied from 1 to 3 g, with a mean of 2.0 to 2.5 g. Only in a few cases of very large sinuses it was necessary to use about 4.0 g. A resorbable membrane (OsteoBiol®, Tecnoss) was positioned against the packed sinus window. Titanium dental implants (3i, Implant Innovations, West Palm Beach, FL, USA) were inserted 4 and 6 months after the maxillary sinus augmentation procedures. At the time of implant surgery, bone cores were harvested from the lateral wall using a 3.5×10 mm-diameter trephine under a cold sterile saline solution irrigation. The retrieved bone cores were processed for light microscopy. All the biopsies were harvested after 4 and 6

months exactly, ±1 week. The protocol called for implant insertion after 4 months, but a large number of patients (32 patients) came back and were treated at a later period (6 months).

Specimen Processing

A total of 77 specimens were used in this study. Only the biopsies where it was possible to see all the specimens without fractures or other types of damages were used; all the other biopsies were excluded from the evaluation. In all cases, the whole specimen was evaluated. All specimens were immediately fixed in 10% buffered formalin, and processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy).[17] 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 their longitudinal axis, with a high-precision diamond disk at about 150 µm, and ground down to about 30 µm with a specially designed grinding machine. The slides were stained with acid fuchsin and toluidine blue. The slides were observed in normal transmitted light under a Laborlux light microscope (Leitz, Wetzlar, Germany). The histomorphometry was performed using the light microscope 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).

RESULTS

Clinical Observations

In a few cases, there were complications, such as a retard in the wound healing (in five cases) and a loss of the graft material (in one case). No postoperative complications were present at the time of the implant surgeries. No differences were found between men and women.

Histological Results

Four Months. A trabecular bone pattern was observed in the augmented area. In all biopsies, trabecular bone
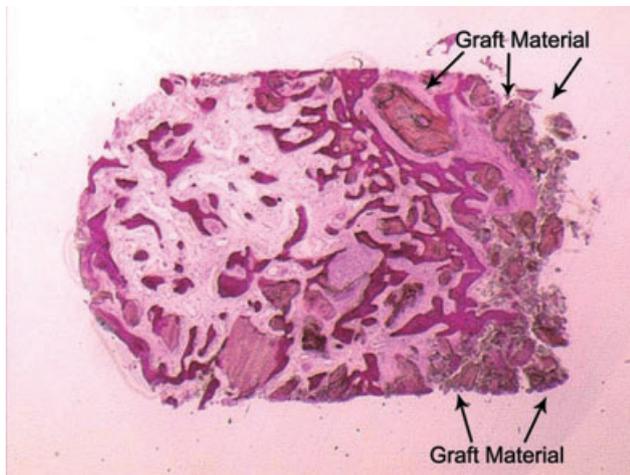
Figure 1 Trabecular bone with wide marrow spaces is present. Graft material remnants are present (*arrows*) and shows a lesser dye affinity than newly formed bone. A higher density of newly formed bone is present toward the internal portion of the sinus (*right portion of the slide*). Acid fuchsin–toluidine blue 12×.

Figure 2 No gaps were present at the bone–particle interface, and newly formed bone was always in close contact with the particles. Acid fuchsin–toluidine blue 40×.

was formed over the entire grafted area; grafted material particles were present in all specimens. Graft material granules could still be recognized in and between the trabecular bone (Figure 1). In many areas, the osteocyte lacunae of the graft material were empty. Many active osteoblasts, lining the layers of osteoid matrix, were observed around the biomaterial particles. Most of the particles were surrounded by newly formed bone with large osteocyte lacunae. This bone was woven and presented a structure with well-organized trabeculae. No gaps were present at the bone–particle interface, and the bone was always in close contact with the particles (Figure 2). No significant inflammatory cell infiltrate was present around the particles or at the interface with bone.

Some of the particles appeared to be cemented by this newly formed bone. At higher magnification, the bone presented wide osteocyte lacunae (Figure 3). No Haversian canals were observed. Many bone trabeculae were in the process of being remodeled, as shown by the thick osteoid layer lined with osteoblasts at one side while at the opposite site osteoclasts in resorption lacunae were observed. No osteoclasts were observed around the graft particles. Histomorphometry showed that newly formed bone represented $28.2 \pm 2.1\%$, marrow spaces $36.8 \pm 1.9\%$, while the residual graft material $37.3 \pm 3.1\%$. All the newly formed bone was woven bone; no lamellar bone was present. The contact between newly formed bone and graft particles was $25.4 \pm 3.1\%$.

Six Months. All biopsies contained mineralized material. At low-power magnification, it was possible to observe newly formed bone around the grafted material partcles (Figure 4). The border between newly formed bone and graft material was easily observed. The porcine bone particles presented marked staining differences from the host bone and had a lower affinity for the stains (Figure 5). In a few fields, osteoblasts were observed in the process of apposing bone directly on the particle surface (Figure 6). The most peripheral osteocytic lacunae present in the biomaterials appeared to be always filled by osteocytes, while the most central ones appeared to be filled by small cells with morphologic
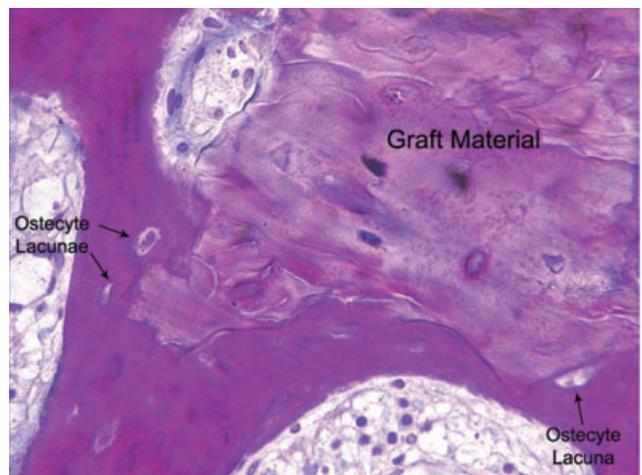

Figure 3 At higher magnification, the bone presented wide osteocyte lacunae in vicinity and at close contact with the graft material. Acid fuchsin–toluidine blue 200×.
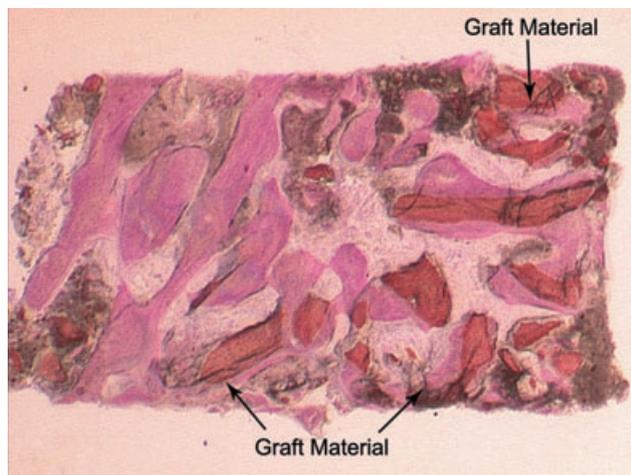
Figure 4 At low-power magnification, it was possible to observe newly formed bone around the grafted material particles (*arrows*). Acid fuchsin–toluidine blue 12×.

and staining features different from the osteocytes. Only in a few cases the osteocyte lacunae were empty. No significant inflammatory cell infiltrate was present. Only in a few areas it was possible to see multinucleated giant cells, and bone remodeling was apparent by the presence of osteoclasts in Howship's lacunae. Few osteoclasts could also be seen at the periphery of some of the porcine bone granules. In some areas, gaps were present at the interface between newly formed bone and biomaterial particles. Inside some marrow spaces, the graft particles appeared to be surrounded by capillaries and cells: in some of these marrow spaces, it was possible to observe the presence of acid fuchsin positive not yet mineralized material lining their inner surface.
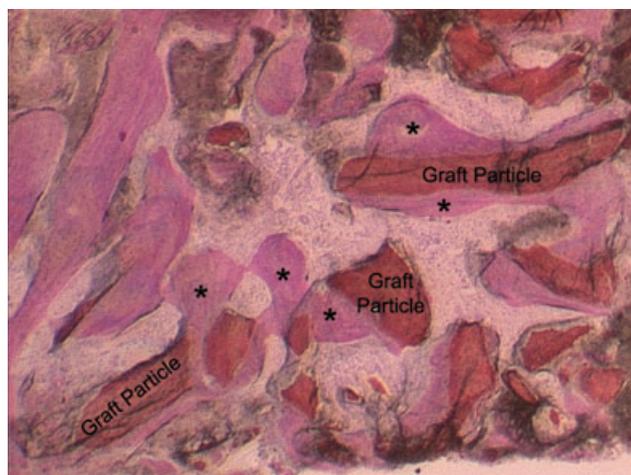

Figure 5 The porcine bone particles presented marked staining differences from the host trabecular bone and had a lower affinity for the stains. Acid fuchsin–toluidine blue 20×.
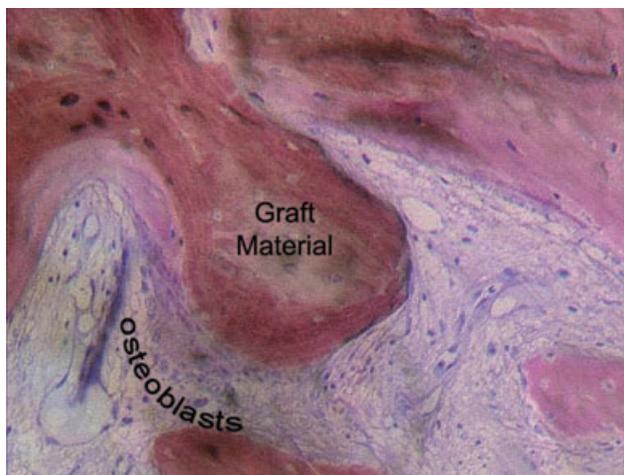

Figure 6 Osteoblasts were observed in the process of apposing bone directly on the particle surface. The graft particle appeared to be completely surrounded by newly formed bone. Acid fuchsin–toluidine blue 100×.

Histomorphometry showed that newly formed bone represented $31.4 \pm 2.6\%$, marrow spaces $34.3 \pm 3.1\%$, while the residual graft material $37.6 \pm 2.2\%$. Woven bone represented about $92.2 \pm 8.1\%$ of the newly formed bone. The contact between newly formed bone and graft particles was $28.4 \pm 2.4\%$.

DISCUSSION

Maxillary sinus augmentation surgical techniques as well as osteoconductive potential of various bone substitutes have greatly evolved over the last few years, allowing predictable placement of dental implants in the regenerated maxillary premolar and molar areas.

The present results confirm the good biocompatibility and high osteoconductivity of this porcine biomaterial. Most of the grafted biomaterial particles were surrounded by newly formed bone, and no gaps or connective, fibrous tissues were found at the biomaterial–bone interface. A slight increase in newly formed bone was found in the 6-month specimens (31%) as compared to the 4-month (28%) specimens. In a previous histological study from our laboratory, evaluating specimens retrieved from sinuses grafted with different materials, a similar percentage of newly formed bone was found after a 6-month period.[10] John and Wenz[18] reported that in some specimens retrieved after 3 to 4 months, from sinuses augmented using only Bio-Oss, a 28% newly formed bone was found.

Also, the percentage of contact between newly formed bone and graft particles compares favorably with that reported for anorganic bovine bone.[19]

Barone and colleagues[15] and Nannmark and Sennerby[2] did not find evidence of inflammatory infiltrate, necrosis, foreign body reaction, or other signs of adverse reaction when using porcine bone. Also in a previous histologic and ultrastructural study from our laboratory, we found no inflammatory or other adverse reactions in the bone formed in sinus augmentation procedures.[16] Porcine bone was found to promote bone formation and did not interfere with bone regeneration.[16]

Another aspect that should be taken into consideration is the resorption rate of this biomaterial. Our histologic results show that, after 4 and 6 months, no evidence of graft resorption was present. Only a few osteoclasts were observed in the 6-month specimens. The percentage of the residual graft material was the same after 4 and 6 months (about 37%). On the contrary, Barone and colleagues,[15] in a human study, found partial resorption of the material after 5 months in 18 patients, and Nannmark and Sennerby,[2] in a rabbit study, found clear signs of resorption of the porcine bone with resorption lacunae at the surface of the particles, after a healing period of 4 and 8 weeks.

Different opinions exist about the resorption capability of other biomaterials used in sinus augmentation procedures. No osteoclastic activity was found, and anorganic bovine bone did not seem to be affected by resorption and remodeling.[19,20] Other researchers found, on the contrary, that the anorganic bovine bone underwent resorption.[21,22] 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.[23] Optimal implant osseointegration has been found in sinus where the graft material (anorganic bovine bone) was still present after several years from the surgical procedure.[4,24–26] No untoward effects were observed from the presence of the biomaterial particles.[4,24–26] The evaluation of specimens retrieved from sinuses augmented with a porcine bone substitute after longer time periods will be necessary to understand in a more complete way the resorption processes of this biomaterial.[27] The long-term cumulative success rate in implants inserted in sites augmented with a xenograft is still unknown.[8]

This is a histological and histomorphometrical study only, and the long-term outcome that will be reported in a separate manuscript (unpublished data) is satisfactory and it compares well with other studies using other graft materials.

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