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Case Series

Clinical, Histologic, and Histomorphometric Analyses of Regenerated Bone in Maxillary Sinus Augmentation Using Fresh Frozen Human Bone Allografts

Claudio Stacchi,* Giovanna Orsini,† Donato Di Iorio,‡§ Lorenzo Breschi,* and Roberto Di Lenarda*

Background: *The purpose of the present study was the clinical and the histologic evaluation of fresh frozen human bone (FFB) allografts used for maxillary sinus-augmentation procedures.*

Methods: *Ten subjects were treated with maxillary sinus augmentations using FFB. Radiologic measurements were recorded on computed tomography scans preoperatively and 5 months after the sinus surgeries. At 5 months, during implant placement, 10 core biopsies were retrieved and processed for histomorphometric evaluation under light microscopy (LM). Clinical and histomorphometric measurements are presented as mean ± SD.*

Results: *At baseline, the height of the alveolar ridge measured 4.3± 1.3 mm (mean); after augmentation procedures, at implant positioning, it had a mean height of 16.0± 1.8 mm. All 22 dental implants were clinically healthy after 5 months. LM showed that most of the specimens presented newly formed bone that was completely integrated with preexisting bone. The interface areas between new and old bone were not discernible. Woven bone was present in some areas of the biopsies; however, in the majority of the examined regions, there was mature osseous tissue presenting features of trabecular bone. There was no evidence of an acute inflammatory infiltrate. Histomorphometry revealed that the percentage of bone was 48.15%± 14.32%, whereas marrow spaces occupied the rest of the area.*

Conclusion: *FFB is a biocompatible material that can be successfully used for maxillary sinus augmentations without interfering with normal reparative bone processes.* J Periodontol 2008;79:1789-1796.

KEY WORDS

Case series; histology; regeneration.

* Department of Biomedicine, University of Trieste, Trieste, Italy.
† Currently, Department of Restorative Dentistry, Institute of Stomatology, Polytechnic University of Marche, Ancona, Italy; previously, Center for Excellence on Aging, University of Chieti-Pescara, Chieti, Italy.
‡ Center for Excellence on Aging, University of Chieti-Pescara.
§ Department of Stomatology, University of Chieti-Pescara.

Insufficient bone volume in the posterior maxilla can be one of the major problems accompanying dental implant insertion. One of the goals of sinus-augmentation procedures is the creation of vital bone to provide a sufficient volume of osseous tissue to obtain the osseointegration of implants placed in the posterior maxilla.[1-3]

The goals of biomaterial research for bone regeneration are the continuous development and improvement of biocompatible substances that induce a predictable and rapid healing of the tissues at the interface with dental implants.[4] The integration of bone substitutes implicates a series of biologic events critical for long-term clinical success, some of which take place at the bone–biomaterial interface, mainly during the first period of bone healing.[4]

Different materials have been proposed for sinus-augmentation procedures, but it is still not clear which graft materials are most clinically suitable for bone regeneration.[5-11] Although autogenous bone has long been considered the gold standard for sinus-augmentation procedures, its disadvantages include limited intraoral availability, a tendency to undergo partial resorption, the need for an additional surgery in case of extraoral donor sites, and associated morbidity.[5] Moreover, recent literature reviews[9,12,13] demonstrated that xenografts exhibited better results in terms of implant survival rate and stability of the graft in augmented maxillary sinuses. Nevertheless, autogenous bone has some important advantages: there have been more published reports on autogenous bone than on any other biomaterial, the healing of the autograft is faster, and its bioabsorbability makes it much safer in case of accidental dissemination of the material into the sinus cavity.[12]

Among human bone grafts, impacted morselized bone grafts have been successfully used to treat the bone loss in revision total hip arthroplasties.[14-16] For this technique, femoral heads or iliac crests are most often used from a bone bank according to

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the standards of the Musculoskeletal Council of the American Association of Tissue Banks (AATB) and the European Association of Musculo Skeletal Transplantation (EAMST).[17,18] Immediately after removal, the bone tissue is stored at –80°C for ≥6 months; if there are no contraindications emerging from the results of the screening procedures, the fresh frozen bone (FFB) can be used for implantation.[19]

Newly formed bone occurs in the graft by means of revascularization, bone resorption, and formation of woven bone and is then replaced by lamellar bone. All of these phases are mediated by the influence of loading.[15,16] Previous studies[20,21] showed that the processing and storage of FFB kills eukaryotic and prokaryotic cells through disruption of the cell membranes as a result of ice crystal formation. Conversely, there is general concern about the possibility of introducing viral, bacterial, and/or oncogenetic contamination with the donor bone.[22] Despite the high standards of screening tests and procurement procedures for donors, there have been several reports[23-27] on the development of infectious diseases due to the transplantation of contaminated musculoskeletal allografts. However, allograft transplantation has been demonstrated to be safe because recent protocols have reduced graft antigenicity while preserving cell viability.[19,28,29] Fast freezing using the cryoprotective substance dimethyl sulfoxide (DMSO) has proven to be a promising means to improve immune tolerance of allograft bone and to enhance the biologic function by maintaining viable cells capable of giving rise to cell growth.[28-30]

The successful use of FFB in orthopedic surgery[19,28-31] has paved the way to introduce this procedure in oral-surgery and dental-regenerative techniques to augment maxillary sites in case of insufficient bone volume before dental implant placement.

The aim of the present study was to report the clinical outcomes together with histologic and histomorphometric results in specimens retrieved 5 months after maxillary-sinus augmentation using human FFB allografts.

MATERIALS AND METHODS

Ten subjects (seven males and three females; age range: 41 to 69 years; mean age: 60.2 ± 8.8 years) requiring unilateral maxillary sinus augmentation participated in this study. Subjects were enrolled between October 2004 and October 2005. The protocol was approved by the Ethics Committee of the University of Trieste, and all subjects signed a written informed consent form. Inclusion criteria were a maxillary partial (unilateral) edentulism involving the premolar/molar areas and the presence <6 mm of crestal bone between the sinus floor and the alveolar ridge. General exclusion criteria were acute myocardial infarction within the past 6 months, uncontrolled coagulation disorders, uncontrolled metabolic diseases (diabetes mellitus and bone pathologies), radiotherapy to the head/neck within the past 24 months, treatment with intravenous bisphosphonates, psychologic or psychiatric problems, heavy smoking (>10 cigarettes/day), and alcohol or drug abuse. Local exclusion criteria were maxillary sinus pathologies, oral infections, and uncontrolled periodontal disease. At the initial visit, all subjects underwent a clinical and occlusal examination, periapical and panoramic radiographs, and study models. Then a prosthetic evaluation with diagnostic waxing was done, and computed tomography (CT) scans with a template were performed to study the programmed implant sites as well as to evaluate the morphology of the bony walls and possible intrasinusal pathologies.

Surgical Protocol

Each subject was draped to guarantee maximum asepsis. The skin was disinfected using iodopovidone 10%,‖ and the subjects were asked to rinse with chlorhexidine mouthwash 0.2%¶ for 30 seconds. Under local anesthesia with ropivacaine HCl, 2 mg/ml,# a crestal incision, slightly toward the palatal aspect throughout the length of the edentulous segment, was performed supplemented by buccal releasing incisions mesially and distally. A full-thickness flap was elevated to expose the alveolar crest and the lateral wall of the maxillary sinus. Using a piezoelectric device,** a trapdoor was made in the lateral sinus wall, scraping off the bone until reaching the sinus membrane.[32] The membrane was elevated using a piezoelectric-specific insert and manual sinus curets of different shapes until it became completely detached from lateral, medial, and lower walls of the sinus.

Cortical chips of FFB†† were carefully packed into the narrow and undercut areas of the sinus, then a cortico-cancellous FFB block was inserted and fixed to the sinus floor using titanium miniscrews.‡‡ The packing was completed with additional cortical FFB chips (Fig. 1).

A bioabsorbable membrane§§ was positioned against the packed sinus window. The mucoperiosteal flap was replaced and sutured with multiple horizontal mattress sutures. Amoxicillin/clavulanate potassium (875 + 125 mg) tablets‖‖ (one tablet twice a day) and ibuprofen¶¶ (600 mg, twice a day) were prescribed for 1 week. Sutures were removed 10 days after surgery. Post-surgical visits were scheduled at monthly intervals to check the course of healing. After 5

‖ Betadine, Purdue Pharma, Stamford, CT.
¶ Corsodyl, SmithKline Beecham, Brentford, Middlesex, U.K.
# Naropin, AstraZeneca, London, U.K.
** Piezosurgery, Mectron, Carasco, Italy.
†† Banca dei Tessuti della Regione Veneto, Treviso, Italy.
‡‡ SQ 17, Nuova Geass, Pozzuolo del Friuli, Italy.
§§ Gore Resolut Adapt, W.L. Gore & Associates, Flagstaff, AZ.
‖‖ Augmentin, GlaxoSmithKline, Brentford, Middlesex, U.K.
¶¶ Brufen, Abbott Laboratories, Abbott Park, IL.

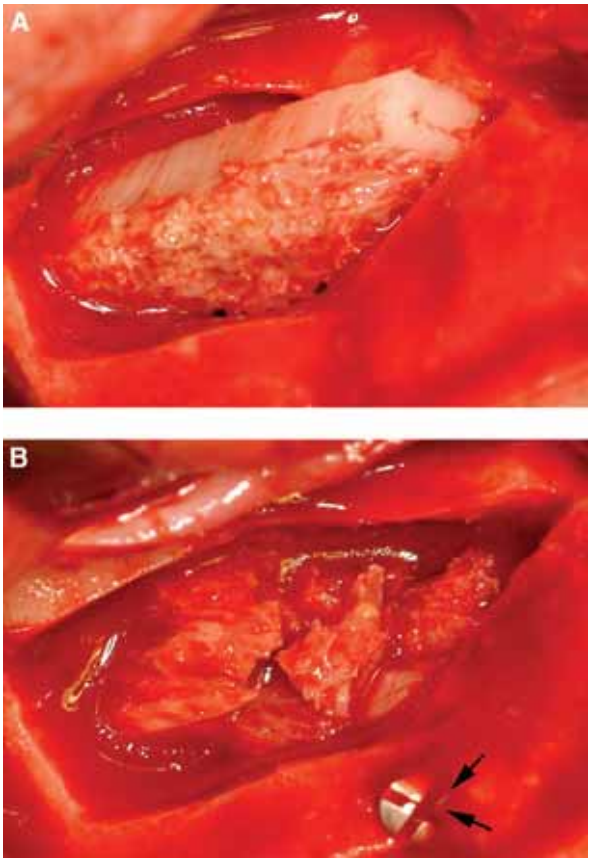

Figure 1.
A) *After the membrane elevation and the packing of the undercut areas of the sinus with FFB cortical chips, a properly shaped FFB corticocancellous block was inserted into the cavity with the cortical side apically oriented.* ***B)*** *The block was fixed with a crestal titanium miniscrew (arrows), and the packing of the cavity was completed with more FFB cortical chips.*

months, a second CT scan with the template was performed, and the dimension of the regenerated osseous tissue was evaluated. At the time of implant surgery, 10 bone cores were harvested from the alveolar crest using a 3.5×10-mm-diameter trephine under cold, sterile saline solution irrigation. The 10 retrieved bone cores were marked with a notch on the crestal side for a correct orientation and processed for light microscopy. Then the osteotomies in the biopsy sites were completed to permit the insertion of titanium dental implants with a dual acid-etched surface.[##] Implants were buried, and the second-stage surgery was carried out after an additional healing period of 5 months.

FFB allograft preparation was performed in accordance with the approved guidelines of UNI EN ISO9001:2000IQ-NETIT/25398. The bone-banking procedures are described in detail.

The potential donors gave their informed consent and were screened by a questionnaire with regard to their medical, social, and sexual history (following the AATB and EAMST guidelines).[17,18] The objective was to ensure that tissues retrieved from donors were of acceptable quality without posing unacceptable risks for recipients. Medical history and clinical, hemodynamic, biochemical, and pharmacologic parameters were fundamental prerequisites to assess the general suitability of the deceased persons as tissue donors. Microbiologic and serologic tests were performed to minimize the risk for transmission of infectious disease.

The microbiology monitoring included histologic and liquid collection in every phase of the processing procedure, searching for contamination with aerobic or anaerobic bacteria, mycobacteria, or fungi. The following serologic tests were performed: hepatitis B surface antigen (HBsAg), anti-HB core antigen antibody (anti-HBc Ab), anti-HBs Ab, anti–hepatitis C virus Ab (anti-HCV Ab), anti–human immunodeficiency virus (HIV) (1/2) Ab, anti–human T-lymphotropic virus (anti-HTLV) (1/2) Ab, anti-cytomegalovirus (anti-CMV) Ab (immunoglobulin G [IgG] and M [IgM]), anti-toxoplasma Ab (IgG and IgM), and a syphilis test. Finally, a polymerase chain reaction test for HIV, hepatitis B virus (HBV), and HCV RNA was performed. If any test indicated an active or previous disease, the donor bone was discarded. Then the bone was disinfected in a medium with a multiantibiotic solution for 72 hours, packed, and frozen using a cryoprotectant (10% DMSO).

Specimen Processing

Light microscopy. The 10 maxillary sinus bone biopsies were immediately fixed in 10% buffered formalin and processed to obtain thin ground sections with a specially designed system.[***] The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a London resin.[†††] After polymerization, the specimens were sectioned, along their longitudinal axis, with a high-precision diamond disk at ~150 μm and ground to ~40 μm with a specially designed grinding machine. The slides were stained with acid fuchsin and toluidine blue or with acid fuchsin and a mixture of methylene blue and Azzurro staining.[‡‡‡] The slides were observed under normal transmitted light with a light microscope.[§§§] The histomorphometry was performed using the light microscope connected to a high-resolution video camera[||||||] and interfaced to a monitor and personal computer.[¶¶¶] This optical system was associated with a digitizing pad[###] and a histometry software package with image-capturing capabilities.[****]

Osseotite, Implant Innovations, Palm Beach Gardens, FL.
*** Micromet, Remet, Casalecchio di Reno, Italy.
††† LR White Resin, London Resin, Theale, Berkshire, U.K.
‡‡‡ Azzurro II, Merck, Darmstadt, Germany.
§§§ Leitz Laborlux, Leica Microsystems, Wetzlar, Germany.
|||||| 3CCD JVC KY-F55B, JVC, Yokohama, Japan.
¶¶¶ Intel Pentium III 1200 MMX, Intel, Santa Clara, CA.
Matrix Vision, Oppenweiler, Germany.
**** Image-Pro Plus 4.5, Media Cybernetics, Silver Spring, MD; Immagini & Computer, Milan, Italy.

Statistical Analysis

Baseline and post-treatment measurement data of newly formed bone were presented as means ± SD using a statistical software package.††††

RESULTS

Clinical Observations

There were no dropouts during the entire period of observation. In one case, a 3.0-mm-wide perforation of the sinus membrane occurred during the antrostomy and membrane-elevation procedures (7.14% prevalence); however, after covering it with a bioabsorbable membrane,‡‡‡‡ the grafting of the sinus was completed normally. No other intra- and postoperative complications were present during the sinus-augmentation procedures or at the time of implant surgeries.

On baseline CT scans, the height of the alveolar ridge coronal to the floor of the maxillary sinus ranged from 1.8 to 6.0 mm, with a mean of 4.3 ± 1.3 mm (Fig. 2). At the time of implant positioning, the heights on CT scans varied from 13.1 to 19.2 mm (average, 16.0 ± 1.8 mm), with a mean bone height gain of 11.7 ± 1.7 mm (Fig. 3). In addition, we observed that if the Schneiderian membrane was hypertrophic at baseline, the hypertrophy was very often significantly reduced 5 months after the grafting. After 5 months of healing, all 22 implants with a dual acid-etched surface (4.0 × 13 mm)§§§§ appeared osseointegrated at clinical examination and on radiographs. After the abutment connection, all subjects received provisional fixed acrylic resin prostheses and, after 6 months, they underwent definitive prosthetic rehabilitation with cemented metal-ceramic crowns. All subjects were followed for a minimum of 1 year after abutment connection; at this time, all implants were successful.

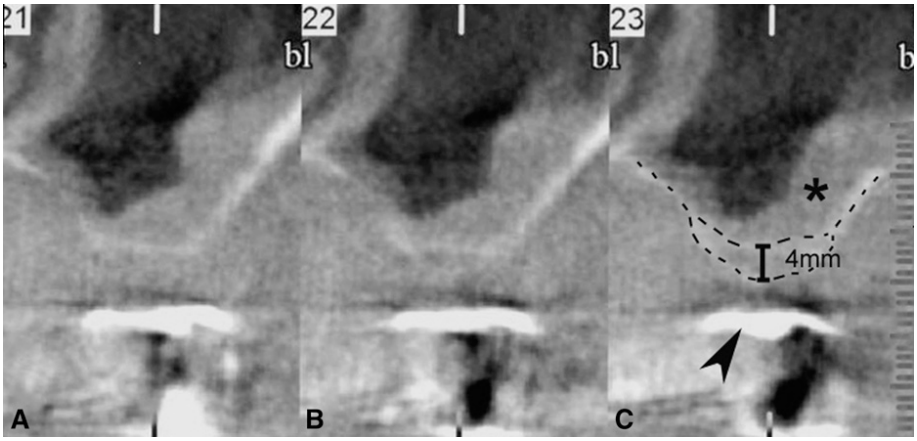

Figure 2.
***A** and **B)** Preoperative CT scan with a template. **C)** The template (arrowhead) was helpful to evaluate native crestal bone, sinus anatomy, and possible sinus pathologies. The height of the alveolar ridge measured ~4 mm. The asterisk shows a slightly hypertrophic Schneiderian membrane.*

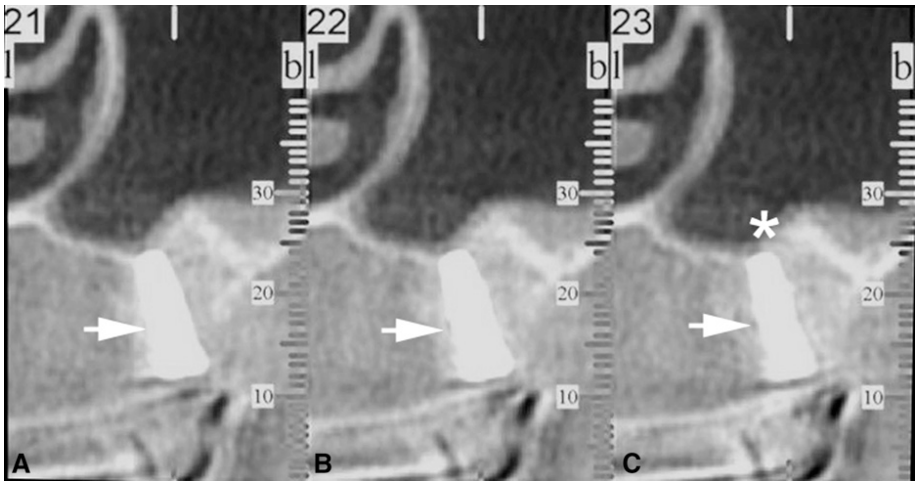

Figure 3.
***A through C)** CT scan at 5 months after implant positioning (arrows) to evaluate quantity of regenerated tissue and sinusal reactions to the grafting. *Reduction of the Schneiderian membrane hypertrophy.*

Light Microscopy

Most of the FFB allograft and chips were fused and not distinguishable from the minimal preexisting bone, and active osteogenesis was observed in the outer layer of the allograft (Fig. 4A). The newly formed bone was strongly stained by acid fuchsin and was lined by a rim of osteoblasts that were actively depositing it (Fig. 4B). Many osteocytes trapped in their mineralized matrix were present in the newly formed mature osseous tissue. The FFB allografts were completely integrated, and it was not possible to discern the residual FFB from the preexisting bone. The newly formed bone mainly surrounded the preexisting bone and/or residual FFB, presenting features of mature bone, with well-organized lamellae and numerous small osteocytic lacunae (Fig. 5). In some regions, osteonic structures were also present (Fig. 5A). The lamellae

†††† Primer 4.02, McGraw Hill, New York, NY.
‡‡‡‡ Gore Resolut Adapt, W.L. Gore & Associates.
§§§§ Osseotite, Implant Innovations.

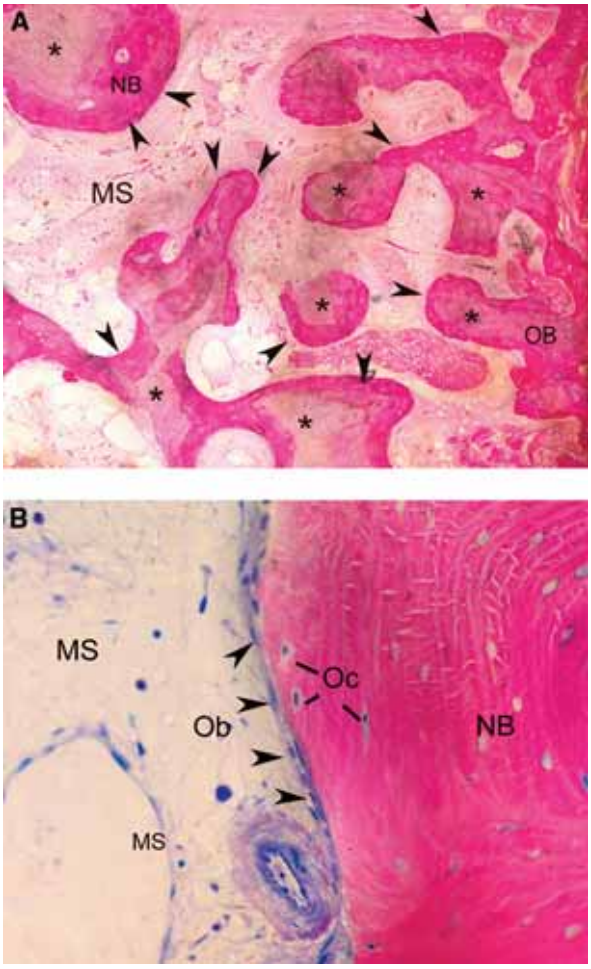

Figure 4.
A) *The histologic specimen was characterized by trabecular bone with large marrow spaces (MS). Most of the FFB chips (*) were fused and almost not distinguishable from the minimal preexisting bone (old bone [OB]). Newly formed bone (NB) was observed at the periphery of the FFB particles forming bone bridges connecting them (arrowheads).* ***B)*** *The newly formed bone (NB) was strongly stained by acid fuchsin and lined by a rim of osteoblasts (Ob) that were actively depositing it. There were many osteocytes (Oc) trapped in their mineralized matrix. (Toluidine blue and acid fuchsin, A; acid fuchsin and Azzurro II, B; original magnification: A, ×5; B, ×40.)*

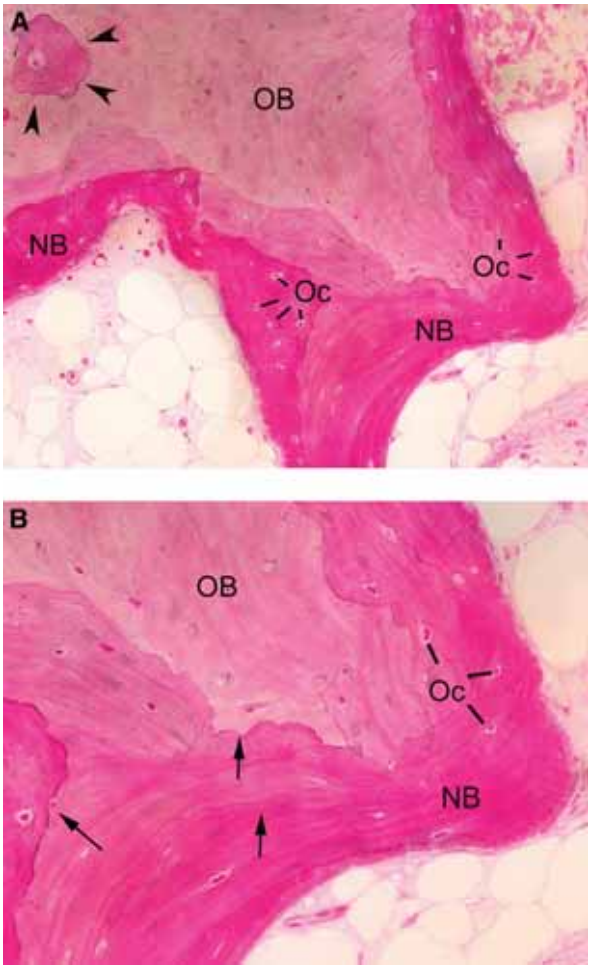

Figure 5.
A) *The newly formed bone (NB) was in close contact with the preexisting bone (old bone, OB) and presented features of mature bone, with well-organized lamellae, numerous osteocytes (Oc), and forming osteones (arrowheads).* ***B)*** *At higher magnification, it was possible to tell that the bone lamellae formed at different times were delimited by cement lines (arrows). (Toluidine blue and acid fuchsin; original magnification: A, ×20; B, ×40.)*

formed at different times were characterized by slightly different affinities for the fuchsin staining and were delimited by cement lines (Fig. 5B). In most fields, the majority of newly formed trabeculae were united by bone bridges delimiting marrow spaces (Fig. 6A), in which numerous newly formed vessels were present, a sign of intense angiogenesis (Fig. 6B). No acute inflammatory infiltrate or evidence of aberrant tissue reactions was present.

In summary, two different features of bone formation were observed in the specimens: 1) remodeling regions in which the preexisting bone was surrounded by newly formed bone that was already well-organized in multiple mature lamellae and 2) regions of trabecular newly formed bone that was not well organized, with numerous marrow spaces and signs of extensive angiogenesis. Some reactive connective tissue and a few inflammatory cells were observed in very limited fields of two retrieved specimens (data not shown).

Histomorphometry showed that the percentage of bone was 48.15% ± 14.32%, whereas the rest of the specimen was occupied by marrow spaces.

DISCUSSION

Maxillary sinus–augmentation surgical techniques as well as the osteoconductive potential of various bone substitutes have evolved greatly over the past few years, allowing the predictable placement of dental implants in the regenerated posterior maxillary region.[9-13]

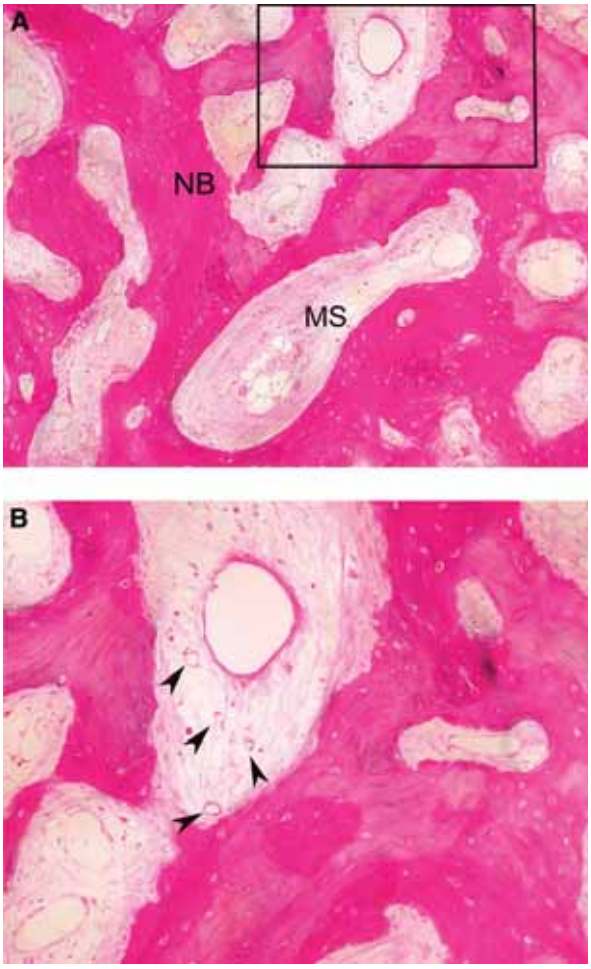

Figure 6.
A) *Areas of newly formed trabeculae (NB) were united by bone bridges delimiting numerous marrow spaces (MS).* ***B)*** *A high-magnification detail of the box in A shows a marrow space containing numerous small newly formed vessels, indicating intense angiogenesis (arrowheads). (Toluidine blue and acid fuchsin; original magnification: A, ×10; B, ×20.)*

The events occurring after biomaterials implantation consist of two components: the response of the host to the biomaterial and the behavior of the material in the host.[33] Light microscopy provides the most important information about the presence of bone or soft tissue contact, but it does not give additional information about the amount of osseous tissue formed.[34-37] Thus, the quantitative histomorphometric evaluation is important to define the effective regeneration that has occurred.[7,10,37] In our specimens, histologic and histomorphometric analyses were performed on non-decalcified core biopsies, and all samples showed newly formed bone in contact with preexisting bone, with cells in the osteocytic lacunae. The results of this study confirmed reports[1-13] in the literature, where in all of the cases in which regenerative procedures were associated with different allografts, that clinical and biologic responses were favorable and allowed dental implant placement. However, to the best of our knowledge, our work presents the first clinical data, supported by histologic findings, on the use of human FFB allografts in oral- and dental-regenerative procedures in maxillary bone. Despite some in vitro studies[19,28-30] on the accepted biocompatibility of these allografts in the recent scientific literature and a clinical report[31] in orthopedic hip surgeries, to the best of our knowledge no clinical study has been corroborated by histologic data on this human derived biomaterial in oral surgery. A recent systematic review[13] reported that implant survival in augmented sinuses varied from 92% for implants placed into autogenous and autogenous/composite grafts to up to 95.6% for implants placed into xenografts; in general, regardless of the graft material(s) used, favorable results using this regenerative technique can be achieved.

Other forms of human bone grafts, such as mineralized and demineralized freeze-dried bone, have been largely used in sinus-augmentation techniques.[2,10,38,39]

However, these latter human bone preparations, although possessing a more limited immunogenicity than fresh grafts and being easily incorporated,[10,38,39] have not been shown to contain vital cells, whereas, as shown by Heyligers and Klein-Nulend,[19] the fresh (banked) bone, obtained according to the standards of the AATB and the EAMST, contained living cells with growth capacity. The vital cells obtained from the allogenic bone fragments were derived from superficially located osteocytes, not from the bone marrow stroma.[40] A question that has to be asked is whether the vital cells from stored frozen allografts positively contribute to bone regeneration in impaction grafting. It was found that human allografts stimulated the release of factors that are capable of inducing osteoclastic bone resorption.[41] Graft proteins were shown to influence osteoconduction in an animal model.[42] It might also be possible that these cells stimulate the recruitment of blood vessels by chemotaxic agents. It is well documented that the process of new bone formation in impaction grafting starts with vascularization, followed by resorption of the grafted bone by osteoclasts and formation of woven bone by osteoblasts.[15,43]

A major possible negative consequence of the presence of viable cells is acute allograft-rejection responses. It was demonstrated that bone stored at –80°C has greater immunogenic capacity than bone stored at –20°C.[19] Egli et al.[28] reported that transplantation of frozen bone allografts was associated with significant local complications, such as graft infections, fatigue fractures, and non-unions. These complications mostly have been attributed to the remaining graft antigenicity and to the devitalized grafts

not being able to contribute to incorporation into the host tissue. However, Aho et al.[44] reported that subjects undergoing massive human osteoarticular allografts, with a follow-up of 11 years, might have shown a low to moderate cutaneous immune response but did not develop important signs of immunologic reaction, and no episode of clinical rejection was recorded.

The strategy to improve the clinical outcome of allograft transplantation should reduce graft antigenicity while preserving the biologic function and simultaneously maintaining graft viability.[28,29] The freezing protocol, in combination with DMSO, led to a selective depletion of the main source of antigen-presenting cells in the frozen graft, accompanied by a decrease in its immunogenicity.[28] DMSO has been used as a cryoprotectant in cryobiology and is still an important constituent of cryoprotectant vitrification mixtures used to preserve organs, tissues, and cell suspensions. Fast freezing blocked the biologic function of osteoblasts, chondrocytes, periosteum, and osteoclasts; however, if DMSO was present during the cryopreservation, some residual cell proliferation, and thus, partial viability, could be preserved within the marrow cavity of the osteochondral tissue.[28] Both effects, the inactivation of immune cells and the maintenance of cell viability, may be of importance in the revascularization of transplanted cryopreserved osteochondral grafts.[29,43,45] Therefore, it is possible that the proliferating cells observed in cryoprotectant dimethyl sulfoxide (cryoDMSO) rapidly frozen grafts are involved in the process of revascularization, a prerequisite for successful graft incorporation.[43-45]

The FFB allografts used in the current study were rapidly stored at –80°C and treated following the above-mentioned precautions. To our knowledge, no acute allograft rejection responses after impaction grafting in maxillary sinus augmentation have been published, and this phenomenon has not been seen in our clinical practice. The documented safety of this fresh-frozen graft,[28,29] associated with the previously mentioned advantages of human bone allografts and the fact that its cost is comparable to other biomaterials and that many subjects prefer human bone instead of animal bone, can render FFB a potential candidate for sinus augmentations as well as for large oral and maxillofacial reconstructions.

CONCLUSIONS

The present study showed that human FFB, when used alone, may promote bone formation and can be safely used because it does not interfere with bone-regeneration processes and implant osseointegration. These findings could increase the scientific knowledge of the clinician in understanding the biologic interactions occurring in the proximity of fresh-frozen bone substitute, showing that bone in contact with it presents all phases of bone formation and shows features similar to the preexisting osseous tissue, thus indicating the biocompatible properties of this graft. Further clinical trials and additional long-term histologic analyses are forthcoming.

ACKNOWLEDGMENT

The authors report no conflicts of interest related to this case series.

REFERENCES

1. Froum SJ, Tarnow DP, Wallace SS, Rohrer MD, Cho SC. Sinus floor elevation using anorganic bovine bone matrix (OsteoGraf/N) with and without autogenous bone: A clinical, histologic, radiographic, and histomorphometric analysis – Part 2 of an ongoing prospective study. *Int J Periodontics Restorative Dent* 1998; 18:528-543.
2. Valentini P, Abensur D. Maxillary sinus floor elevation for implant placement with demineralized freeze-dried bone and bovine bone (Bio-Oss): A clinical study of 20 patients. *Int J Periodontics Restorative Dent* 1997;17: 232-241.
3. Valentini P, Abensur D, Densari D, Graziani JN, Hämmerle CHF. Histologic evaluation of Bio-Oss in a 2-stage sinus floor elevation and implantation procedure. A human case report. *Clin Oral Implants Res* 1998;9:59-64.
4. Brunski JB, Puleo DA, Nanci A. Biomaterials and biomechanics of oral and maxillofacial implants: Current status and future developments. *Int J Oral Maxillofac Implants* 2000;15:15-41.
5. van den Bergh JP, ten Bruggenkate CM, Krekeler G, Tuinzing DB. Sinus floor elevation and grafting with autogenous iliac crest bone. *Clin Oral Implants Res* 1998;9:429-435.
6. Jensen OT, Shulman LB, Block MS, Iacono VJ. Report of the Sinus Consensus Conference of 1996. *Int J Oral Maxillofac Implants* 1998;13(Suppl.):11-45.
7. Artzi Z, Nemcovsky CE, Dayan D. Bovine-HA spongiosa blocks and immediate implant placement in sinus augmentation procedures. Histopathological and histomorphometric observations on different histologic stainings in 10 consecutive patients. *Clin Oral Implants Res* 2002;13:420-427.
8. Hallman M, Sennerby L, Lundgren S. A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. *Int J Oral Maxillofac Implants* 2002;17:635-643.
9. Wallace SS, Froum SJ. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. *Ann Periodontol* 2003;8: 328-343.
10. Scarano A, Degidi M, Iezzi G, et al. Maxillary sinus augmentation with different biomaterials: A comparative histologic and histomorphometric study in man. *Implant Dent* 2006;15:197-207.
11. Orsini G, Scarano A, Piattelli M, Piccirilli M, Caputi S, Piattelli A. Histologic and ultrastructural analysis of regenerated bone in maxillary sinus augmentation using a porcine bone–derived biomaterial. *J Periodontol* 2006;77:1984-1990.

12. Del Fabbro M, Testori T, Francetti L, Weinstein R. Systematic review of survival rates for immediately loaded dental implants. *Int J Periodontics Restorative Dent* 2006;26:249-263.
13. Aghaloo T, Moy PK. Which hard tissue augmentation techniques are the most successful in furnishing bony support for implant placement? *Int J Oral Maxillofac Implants* 2007;22(Suppl.):49-70.
14. van Biezen FC, ten Have BL, Verhaar JA. Impaction bone-grafting of severely defective femora in revision total hip surgery: 21 hips followed for 41-85 months. *Acta Orthop Scand* 2000;71:135-142.
15. Buma P, Donk S, Slooff TJ, Schreurs W. Bone graft incorporation after reconstruction of bony defects with impacted morselized bone graft. Histology of animals and patients. *Ortop Traumatol Rehabil* 2001;3:41-47.
16. van der Donk S, Buma P, Slooff TJ, Gardeniers JW, Schreurs BW. Incorporation of morselized bone grafts: A study of 24 acetabular biopsy specimens. *Clin Orthop Relat Res* 2002;396:131-141.
17. American Association of Tissue Banks. Standards for tissue banking. Available at: http://www.aatb.org. Accessed January 28, 2008.
18. European Association of Musculo Skeletal Transplantation. Common standard for musculo-skeletal tissue banking. Available at: http://www.eamst.org. Accessed January 28, 2008.
19. Heyligers IC, Klein-Nulend J. Detection of living cells in non-processed but deep-frozen bone allografts. *Cell Tissue Bank* 2005;6:25-31.
20. Urist MR. Physiologic basis of bone-graft surgery, with special reference to the theory of induction. *Clin Orthop* 1953;1:207-216.
21. Goldberg VM, Stevenson S. Natural history of autografts and allografts. *Clin Orthop* 1987;225:7-16.
22. Sugihara S, van Ginkel AD, Jiya TU, van Royen BJ, van Diest PJ, Wuisman PI. Histopathology of retrieved allografts of the femoral head. *J Bone Joint Surg Br* 1999;81:336-341.
23. Simonds RJ, Holmberg SD, Hurwitz RL, et al. Transmission of human immunodeficiency virus type 1 from a seronegative organ and tissue donor. *N Engl J Med* 1992;326:726-732.
24. Conrad EU, Gretch DR, Obermeyer KR, et al. Transmission of the hepatitis-C virus by tissue transplantation. *J Bone Joint Surg Am* 1995;77:214-224.
25. Cook SD, Salkeld SL, Prewett AB. Simian immunodeficiency virus (human HIV-II) transmission in allograft bone procedures. *Spine* 1995;20:1338-1342.
26. Pereira BJ, Milford EL, Kirkman RL, et al. Low risk of liver disease after tissue transplantation from donors with HCV. *Lancet* 1993;341:903-904.
27. Tomford WW. Transmission of disease through transplantation of musculoskeletal allografts. *J Bone Joint Surg Am* 1995;77:1742-1754.
28. Egli RJ, Sckell A, Fraitzl CR, et al. Cryopreservation with dimethyl sulfoxide sustains partially the biological function of osteochondral tissue. *Bone* 2003;33:352-361.
29. Egli RJ, Wingenfeld C, Hölzle M, et al. Histopathology of cryopreserved bone allo- and isografts: Pretreatment with dimethyl sulfoxide. *J Invest Surg* 2006;19:87-96.
30. Simpson D, Kakarala G, Hampson K, Steele N, Ashton B. Viable cells survive in fresh frozen human bone allografts. *Acta Orthop* 2007;78:26-30.
31. van Haaren EH, Heyligers IC, Alexander FG, Wuisman PI. High rate of failure of impaction grafting in large acetabular defects. *J Bone Joint Surg Br* 2007;89:296-300.
32. Vercellotti T, De Paoli S, Nevins M. The piezoelectric bony window osteotomy and sinus membrane elevation: Introduction of a new technique for simplification of the sinus augmentation procedure. *Int J Periodontics Restorative Dent* 2001;21:561-567.
33. Brunski JB. In vivo bone response to biomechanical loading at the bone-dental implant interface. *Adv Dent Res* 1999;13:99-119.
34. Wallace SS, Froum SJ, Tarnow DP. Histologic evaluation of the sinus elevation procedure. A clinical report. *Int J Periodontics Restorative Dent* 1996;16:46-51.
35. Landi L, Pretel RW, Hakimi NM, Setayesh R. Maxillary sinus floor elevation using a combination of DFDBA and bovine-derived porous hydroxyapatite: A preliminary histologic and histomorphometric report. *Int J Periodontics Restorative Dent* 2000;20:574-583.
36. Tapety FI, Amizuka N, Uoshima K, Nomura S, Maeda T. A histologic evaluation of the involvement of Bio-Oss in osteoblastic differentiation and matrix synthesis. *Clin Oral Implants Res* 2004;15:315-324.
37. Orsini G, Traini T, Scarano A, Perrotti V, Piccirilli M, Piattelli A. Maxillary sinus augmentation with Bio-Oss particles: A light, scanning and transmission electron microscopy study in man. *J Biomed Mater Res B Appl Biomater* 2005;74:448-457.
38. Cammack GV 2nd, Nevins M, Clem DS 3rd, Hatch JP, Mellonig JT. Histologic evaluation of mineralized and demineralized freeze-dried bone allograft for ridge and sinus augmentations. *Int J Periodontics Restorative Dent* 2005;25:231-237.
39. Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:299-303.
40. Sterck JG, Klein-Nulend J, Lips P, Burger EH. Response of normal and osteoporotic human bone cells to mechanical stress in vitro. *Am J Physiol* 1998; 274:E1113-E1120.
41. Mejdahl S, Hansen CA, Skjodt H, Reimann I. Human bone bank allografts stimulate bone resorption and inhibit proliferation in cultures of human osteoblast-like cells. *Acta Orthop Scand* 1998;69:63-68.
42. Tagil M, Aspenberg P. Impaction of cancellous bone grafts impairs osteoconduction in titanium chambers. *Clin Orthop* 1998;231-238.
43. Slooff TJ, Buma P, Schreurs BW, Schimmel JW, Huiskes R, Gardeniers J. Acetabular and femoral reconstruction with impacted graft and cement. *Clin Orthop* 1996;108-115.
44. Aho AJ, Eskola J, Ekfors T, Manner I, Kouri T, Hollmen T. Immune responses and clinical outcome of massive human osteoarticular allografts. *Clin Orthop Relat Res* 1998;346:196-206.
45. Wingenfeld C, Egli RJ, Hempfing A, Ganz R, Leunig M. Cryopreservation of osteochondral allografts: Dimethyl sulfoxide promotes angiogenesis and immune tolerance in mice. *J Bone Joint Surg Am* 2002;84-A: 1420-1429.

Correspondence: Dr. Giovanna Orsini, Department of Restorative Dentistry, Institute of Stomatology, Polytechnic University of Marche, Via Tronto 10/A, 60100 Ancona, Italy. Fax: 39-0862-27010; e-mail: g.orsini@unich.it.

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