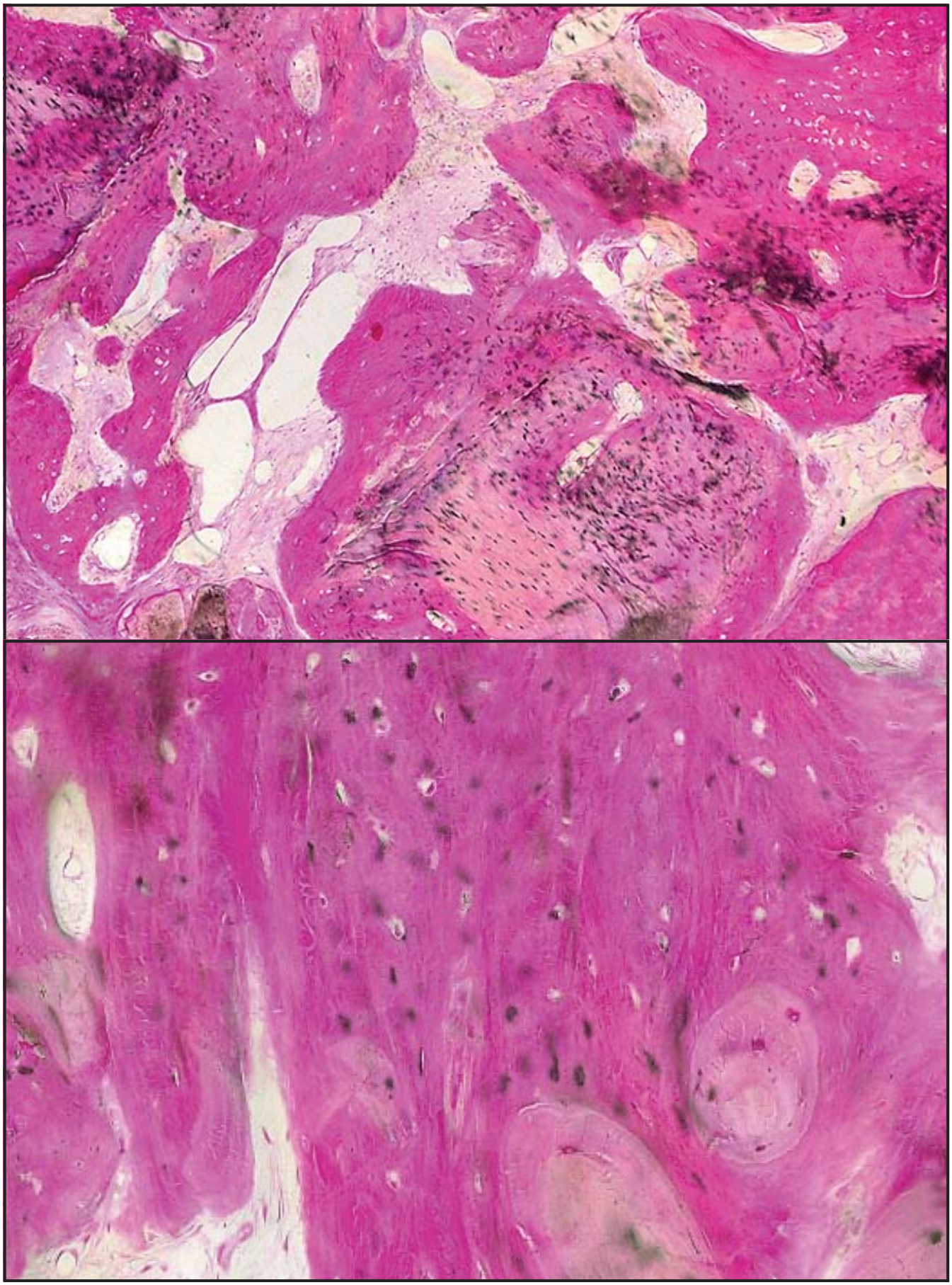
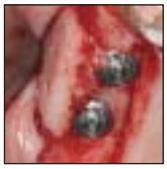


Clinical and Histologic Evaluation of Fresh Frozen Human Bone Grafts for Horizontal Reconstruction of Maxillary Alveolar Ridges



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The aims of the present study were to clinically and histologically evaluate human fresh frozen bone (FFB) grafts used to treat severe maxillary horizontal defects prior to dental implant placement. Ten patients were treated with FFB onlay grafts. Measurements using computed tomography scans were recorded preoperatively and at 5 months. Six core biopsies were retrieved and processed for light microscopy. At baseline, thickness of the maxillary alveolar ridge measured 2.3 ± 0.4 mm; it measured 6.8 ± 0.5 mm after reconstruction. All implants were successful after 24 months. Histologic results showed that FFB blocks and new bone were integrated perfectly. Histomorphometry revealed a mean percentage of bone of 57.5% ± 24.7%. (Int J Periodontics Restorative Dent 2011;31:535–544.)

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Placement of endosseous dental implants requires sufficient bone volume for complete implant coverage. Autologous, alloplastic, and xenogenous materials are used for different bone augmentation procedures. In bone block grafting techniques, autologous bone is considered to be the gold standard,[1,2] and bone grafts from intraoral sources can be recommended in cases of short-span reconstructions.[3] However, when harvesting autologous bone, donor site morbidity has to be taken into consideration. Another disadvantage for cases of severe atrophy is the limited availability of autologous bone when taken from intraoral donor sites. Possible origins for extraoral autogenic bone include the calvarium, tibia, and iliac crest.[4,5] Although these techniques are used in major arch reconstructions, they are not always recommended because of their morbidity and the need for general anesthesia and hospitalization. Therefore, the possibility of using human fresh frozen bone (FFB) allografts has recently gained attention, particularly in orthopedic surgery.

Table 1 **Description of patients enrolled in the present study: Pre- and postoperative measurements of the maxillary grafted sites (mm), implant dimensions (mm), and sites of insertion**

Patient	Sex	Age (y)	Preoperative	Postoperative	Implant dimensions (tooth*)
1	F	57	2.8	7.6	4 × 13 (14), 4 ×13 (15)
2	F	52	2.4	6.2	4 × 13 (14)
3	M	54	1.8	6.7	4 × 13 (23), 4 × 13 (24)
4	F	38	2.6	6.8	4 × 13 (11)
5	F	69	1.5	6.7	4 × 11.5 (25), 4 × 11.5 (26)
6	M	46	2.2	6.1	4 × 11.5 (14), 4 × 13 (15)
7	F	55	2.6	6.3	4 × 11.5 (25), 4 × 11.5 (26)
8	M	19	2.4	6.2	4 × 13 (12)
9	M	51	2.8	6.9	4 × 13 (11)
10	M	57	2.5	Removed	–

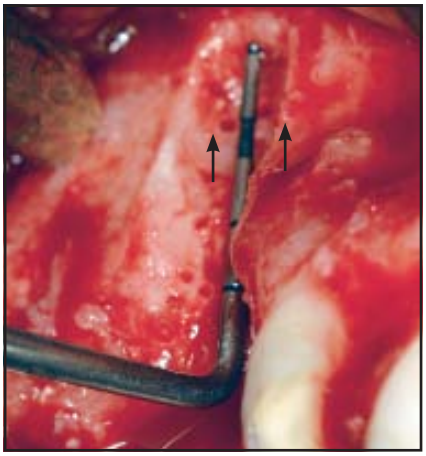
F = female; M = male.
*FDI tooth-numbering system.

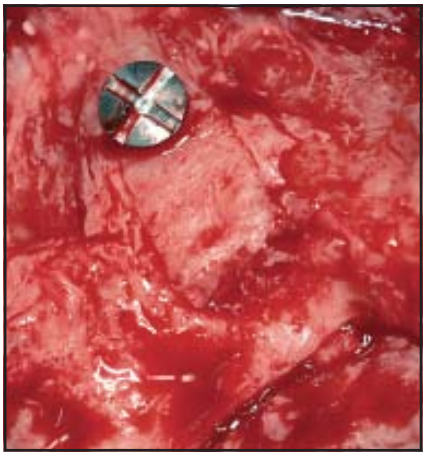
FFB grafting has been successfully used to treat bone loss in revision total hip arthroplasties.[6–8] Femoral heads or iliac crests from bone banks are most often used for this technique, according to the standards of the Musculoskeletal Council of the American Association of Tissue Banks and the European Association of Musculo Skeletal Transplantation.[9,10] Immediately after removal, the bone grafts are stored at –80°C for at least 6 months, and if no contraindications arise, the FFB is then suitable for implantation.[11] The successful use of FFB in orthopedic surgery has paved the way to introduce this procedure in oral surgery and regenerative applications, not only to augment bone in maxillary sinus procedures,[12] but also for alveolar ridge reconstruction in cases of horizontal insufficient bone volume before dental implant placement. Onlay bone grafting techniques have been used in situations with normal or acceptable maxillomandibular relationships. The onlay integration implicates a series of biologic events critical for long-term success, some of which take place at the bone-onlay interface, mainly in the first period of the healing process.[13]

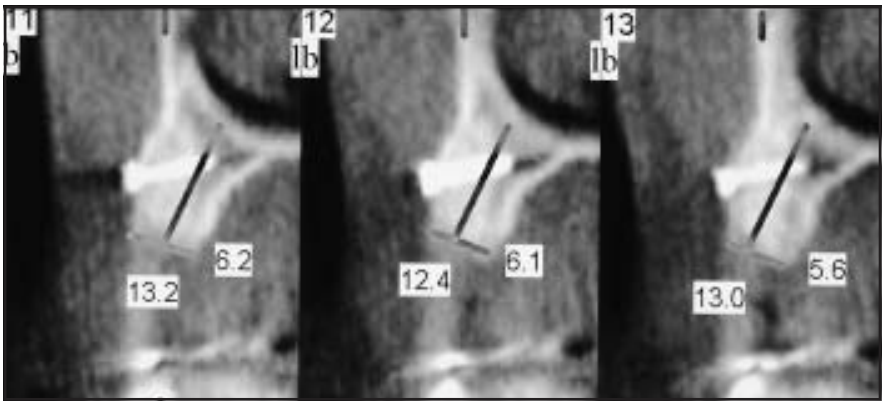
The aims of the present study were to report clinical, histologic, and histomorphometric results of horizontal augmentation procedures using human FFB allografts in cases of severe maxillary ridge defects.

Method and materials

Ten patients (five men, five women; age range, 19 to 69 years; mean age, 49.0 ± 14.0 years) requiring implant-supported maxillary rehabilitations participated in this study (Table 1). The protocol was approved by the Ethics Committee







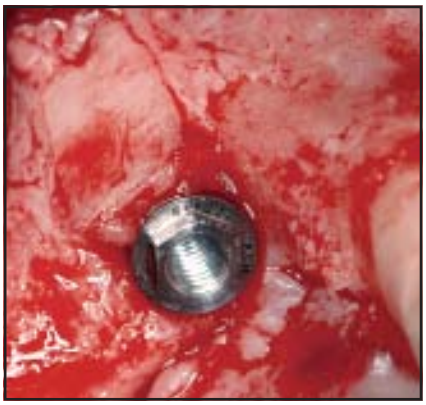


Fig 1a (left) *Horizontal defect after piezoelectric debridment, shaping, and cortical perforations* (arrows).

Fig 1b (right) *At 5 months, the FFB block fixed with a titanium miniscrew was perfectly integrated.*

Fig 1c (bottom left) *Postoperative CT scan of the regenerated horizontal defect at 5 months, with the fixation screw in place, showing good integration between the FFB graft and native bone.*

Fig 1d (bottom right) *The miniscrew was removed and a 4.0-mm-diameter implant was inserted in the augmented zone.*

of the University of Trieste, Trieste, Italy, and all patients signed a written informed consent form. Inclusion criteria were maxillary partial edentulism, to be treated with the insertion of one or more implants, associated with severe horizontal ridge defects (Cawood and Howell Class IV[14] with residual bone thickness less than 3 mm). General exclusion criteria were acute myocardial infarction within the past 6 months, uncontrolled coagulation disorders, uncontrolled metabolic diseases, patients treated with radiotherapy to the head or neck region within the past 24 months, patients treated with bisphosphonates, patients with psychologic or psychiatric problems, heavy smokers, and alcohol or drug abusers. Local exclusion criteria were oral infections and uncontrolled periodontal disease. At the initial visit, all patients underwent a clinical and occlusal examination, prosthetic evaluation with diagnostic waxing, periapical and panoramic radiographs, and computed tomography (CT) scanning to study the planned implant sites as well as to evaluate the morphology of the alveolar ridge.

Surgical Protocol

Under local anesthesia with ropivacaine HCl 2 mg/mL (Naropin, AstraZeneca), a full-thickness buccal trapezoidal flap was elevated, with releasing incisions placed at least 3 mm away from the defect. Using a piezoelectric device (Piezosurgery, Mectron), an accurate debridement of the ridge was made to provide better adaptation of the bone block to the recipient site. The extension of the defect was measured (Fig 1a), and a template was prepared using sterile paper. A corticospongious FFB block (Banca dei Tessuti

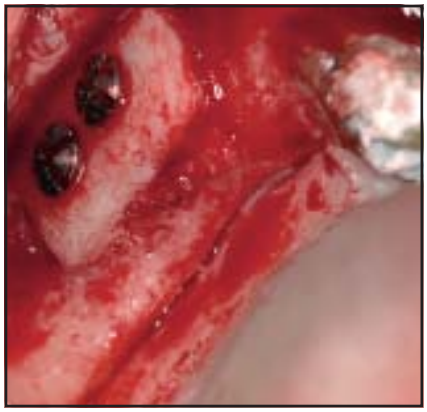
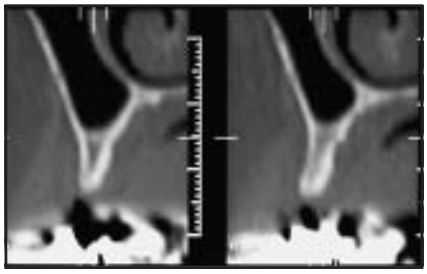
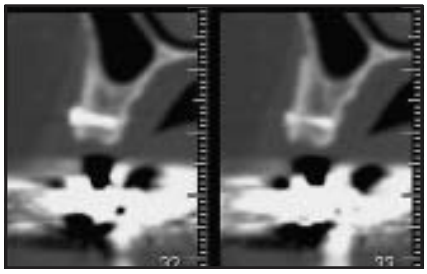


Fig 2a *An atrophic maxilla treated for horizontal augmentation with a buccal FFB block.*





Figs 2b and 2c *CT scan at* (left) *baseline and* (right) *after 5 months, with the onlay in place, before implant placement. At this time, the augmented thickness of the alveolar bone was recognizable.*

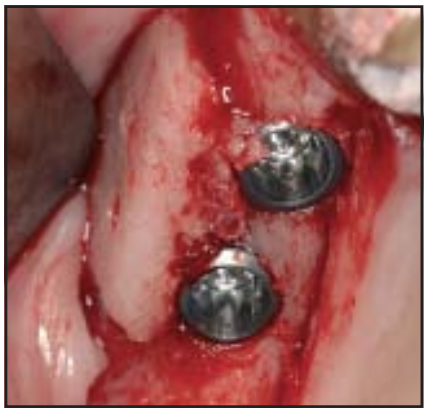


Fig 2d *After implant placement, the buccal walls of the implants were in contact with the FFB graft and the FFB bone chips.*

della Regione Veneto) was cut with the piezoelectric scalpel and modeled using the template, tested on the recipient site, and corrected until satisfactory adaptation was reached. To facilitate blood supply to the graft from the spongious native bone, the cortical aspect of the defect was perforated with a piezoelectric insert. Soft FFB chips were placed on the recipient site to complete the adaptation, and the block was fixed with titanium miniscrews SQ 17 (Nuova Geass) (Figs 1b and 2a). The flap was released with horizontal periosteal incisions, and the augmented zone was covered passively and sutured with multiple horizontal mattress and single sutures. Amoxicillin/clavulanate potassium (875 + 125 mg) tablets (Augmentin, GlaxoSmithKline; one tablet twice a day) and ibuprofen (Brufen, Abbott Laboratories; 600 mg twice a day) were prescribed for 1 week. Sutures were removed 10 days after surgery.

After 5 months, a second CT scan was performed (Figs 1c, 2b, and 2c); the dimensions of the graft were evaluated, and by means of a surgical template, titanium dental implants were placed using a staged approach (Osseospeed, Astra Tech and Osseogrip, Plan-1Health) (Figs 1d and 2d). Six bone cores (one from each of the first six patients enrolled) were harvested from the alveolar crest using a 3 × 10-mm diameter trephine under cold sterile saline solution irrigation during implant surgery. The retrieved bone cores were processed using light microscopy. Stage-two surgery was carried out after an additional 5 months.

Onlay graft preparation

FFB allograft preparation was performed in accordance with the following bone banking procedures.[12] All donors gave informed consent and were screened by a questionnaire in relation to their medical, social, and sexual histories and subsequently were interviewed by a medical doctor. Then, a thorough physical and routine blood examination were performed. Immediately after resection, the donor bone was stored at –80°C for at least 6 months, following the protocol of Egli et al.[15] Microbiologic and serologic tests were performed to minimize the risk for transmission of disease. Tests for hepatitis B and C, human immunodeficiency virus, human T-lymphotropic virus, cytomegalovirus, toxoplasma, and syphilis were performed.[12] In cases of active disease or increased titres, the donor bone was excluded from implantation. The donor bone graft was either rejected or approved for donation by the bone bank coordinator after 6 months of storage.

Specimen processing for light microscopy

The maxillary onlay graft biopsies were fixed immediately in 10% formalin, dehydrated in an ascending series of alcohol rinses, and embedded in a London resin (LR White Resin, London Resin). After polymerization, specimens were sectioned with a high-precision diamond disk and ground down to approximately 40 µm with a specially designed grinding machine (Micromet, Remet). Slides were stained with acid fuchsin and toluidine blue or with acid fuchsin and a mixture of methylene blue and Azzurro II (Merck). The slides were observed under a light microscope (Leitz Laborlux, Leica Microsystems) connected to a high-resolution video camera (3CCD JVC KY-F55B, JVC) and interfaced to a monitor and personal computer (Intel Pentium III 1200 MMX, Intel). This optical system was associated with a digitizing pad (Matrix Vision) and a histometry software package with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics; Immagini & Computer).

Statistical analysis

Baseline and posttreatment measurements and data on newly formed bone were presented as means ± standard deviation and were analyzed using a computerized statistical package (Primer 4.02, McGraw Hill).

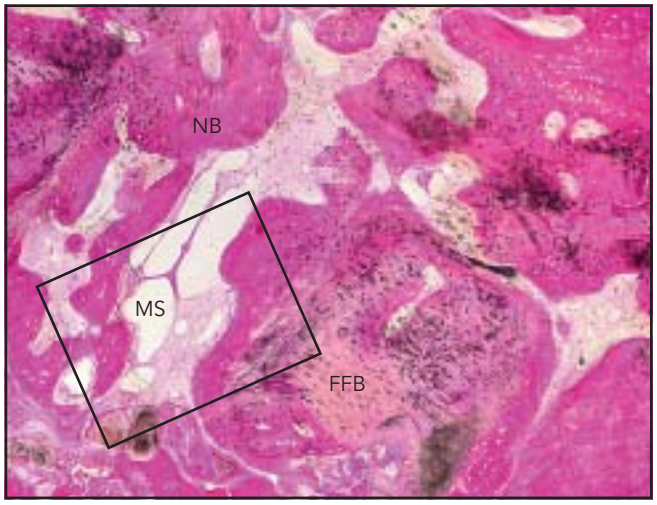


Fig 3a *Histologic specimen characterized by trabecular bone with large marrow spaces (*MS). *Newly formed bone* (NB) *surrounded the FFB graft, which showed a low affinity for acid fuchsin. FFB = fresh frozen bone (toluidine blue and acid fuchsin; original magnification ×10).*

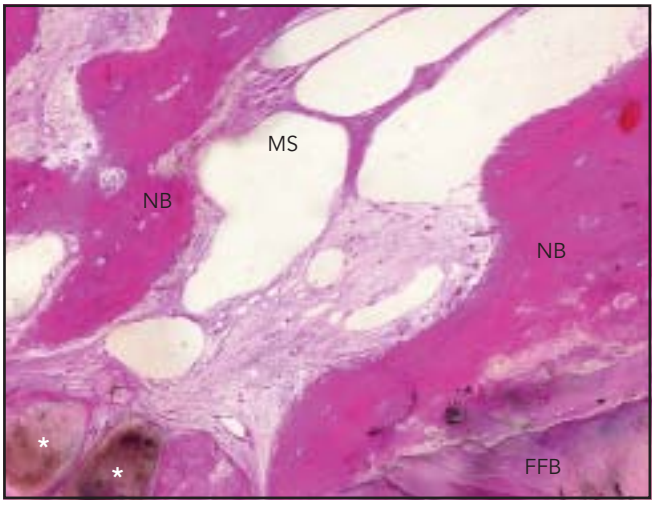


Fig 3b *High-magnification view of the boxed area of Fig 3a. Osteocytes were colonizing the newly formed bone* (NB), *which was strongly stained by acid fuchsin. The grafted bone* (FFB) *was in close continuity with NB, and marrow spaces* (MS) *contained small newly formed vessels, indicating intense angiogenesis. Small nonstained brown-gray patches, probably FFB chips or nonvital remnants, were observed* (white asterisks) *(toluidine blue and acid fuchsin, original magnification ×40).*

Results

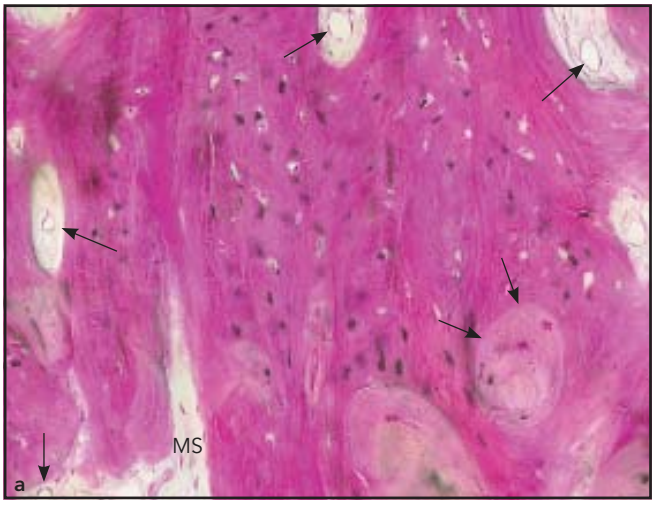
Clinical observations

No dropouts were observed during the entire observation period. Nine of 10 patients showed successful horizontal reconstruction; in 1 patient, exposure of the graft occurred after 1 month and the graft was removed. No additional postoperative complications were present during augmentation procedures or implant surgeries.

At baseline, CT scan measurements of the planned implant sites showed edentulous ridge thicknesses ranging from 1.5 to 2.8 mm, with a mean of 2.3 ± 0.4 mm. CT scan measurements performed after onlay integration and before implant placement revealed thickness differences from 6.2 to 7.6 mm (mean, 6.8 ± 0.5 mm; mean bone thickness gain, 4.6 ± 0.5 mm). Fourteen implants (4.0 mm in diameter) were placed, and after 5 months of healing, at clinical and radiographic examinations, all implants appeared osseointegrated. Table 1 shows the treated patient characteristics, pre- and postoperative measurements of maxillary grafted sites, implant dimensions, and insertion sites. All patients received provisional fixed acrylic resin prostheses after abutment connection and underwent definitive prosthetic rehabilitation with cemented metal-ceramic crowns. At 24 months, all implants were successful.

Light microscopy

FFB onlays were well integrated in the grafted regions and surrounded by newly formed bone, which was strongly stained by acid fuchsin; onlay grafts showed lower affinity for staining (Figs 3a and 3b). A



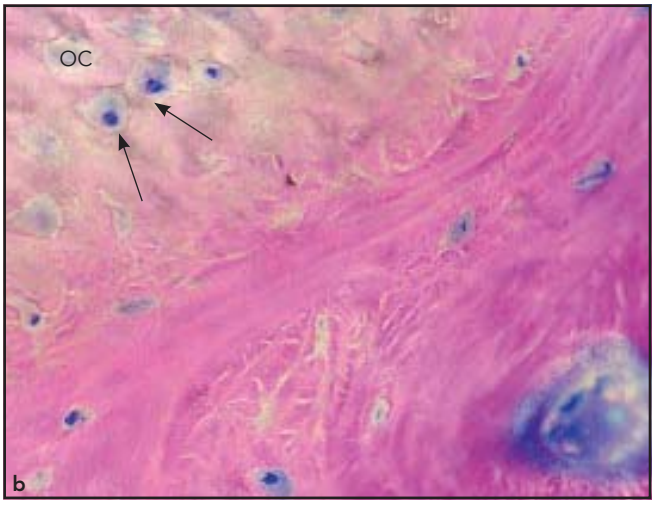
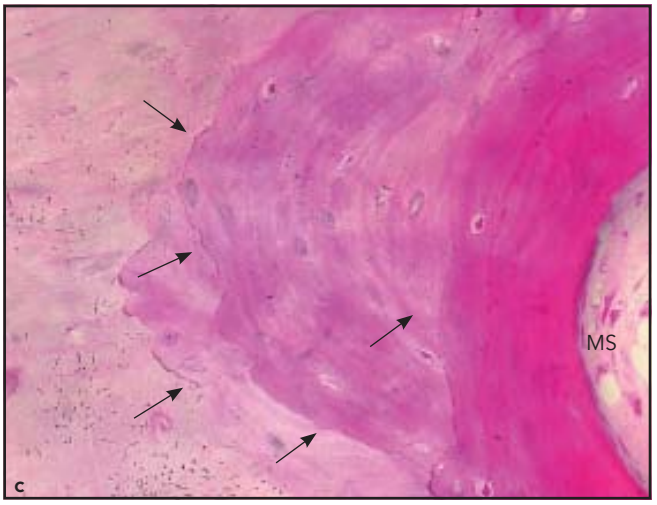


Fig 4a *The regenerated bone region presented features of mature bone with well-organized lamellae, extensive angiogenesis, and formation of a few osteonic structures* (arrows). *MS = marrow space (toluidine blue and acid fuchsin, original magnification ×20).*

Fig 4b *Numerous osteocytes* (OC, arrows) *were evident trapped in their mineralized matrix (acid fuchsin and Azzurro II, original magnification ×40).*

Fig 4c *At high magnification, it was possible to identify that the bone lamellae formed at different times were delimited by cement lines* (arrows). *MS = marrow space (toluidine blue and acid fuchsin, original magnification ×40).*



few nonstained brown-gray patches were observed sporadically, possibly ascribed as FFB chips or nonvital FFB remnants, and were well incorporated and surrounded by new bone (Fig 3b). The newly formed osseous tissue presented features of mainly trabecular bone with large marrow spaces (Fig 3b). Numerous osteocytes were trapped within the mineralized matrix; extensive angiogenesis and few osteonic structures were observed (Fig 4a). Several regions presented mature bone characterized by the presence of numerous small lacunae hosting osteocytes (Fig 4b). All bone formation phases were visible: regions in which newly formed nonorganized bone was present (characterized by a high affinity for acid fuchsin staining) and regions where organized bone showed mature lamellae with a slightly different affinity for fuchsin staining and delimited by cement lines (Fig 4c). No acute inflammatory infiltrate and no evidence of aberrant tissue reactions were present.

Histomorphometry showed that the percentage of bone was 57.5% ± 24.7%, while the remaining part of the specimen was occupied by marrow spaces.

Discussion

The present study shows that FFB block, if used alone as an onlay graft, promotes bone formation for horizontal maxillary reconstruction, promoting bone regeneration and implant osseointegration. The greater amount of high-quality bone after ridge augmentation allows the clinician to place implants in sufficient bone volume and in the proper position. Indeed, FFB onlays prepare the alveolar ridge with adequate width of bone, thus both facial and lingual/palatal implant surfaces can be osseointegrated circumferentially.

Revascularization is the key factor for successful incorporation and remodeling of the bone graft.[16] Close adaptation of the graft to the recipient site, together with firm stabilization of the block, are pivotal to obtaining rapid integration of the graft.[16–19] Furthermore, the bone healing potential following piezoelectric osseous surgery seems to be more favorable compared with traditional bone surgery performed using burs or saws.[20] Optimal integration of FFB onlay grafts in host sites may depend on the fact that the banked FFB blocks, following FFB preparation standards, contained living cells with growth capacity.[11,21] In fact, previous studies reported that the vital cells contained in human allografts, such as FFB, positively influence osteoconduction because they stimulate the release of chemotactic factors, which contribute to increased vascularization, followed by resorption of the grafted bone by osteoclasts and formation of new woven bone by osteoblasts.[7,22]

An initial concern about the possibility of introducing infectious diseases with the donor bone and inducing unfavorable immune responses was present. However, recent studies have shown that the method of FFB processing and storing respects high standards for screening and collecting procedures; thus, it is safe and useful for osseous reconstructive surgeries.[23,24] Moreover, cryopreservation of FFB using dimethyl sulfoxide and glycerol may preserve up to 80% of viable cells by removing water during the freezing process, and osteoblast-related cells can be grown from FFB and have been morphologically indistinguishable from those grown from freshly harvested trabecular bone.[21,25–27]

A major possible negative consequence of the presence of viable cells is acute allograft rejection responses.[26] However, no acute allograft rejection responses after impaction grafting were seen in the present clinical study. After 24 months of follow-up, no infective complications were noted. In fact, using clinical and radiographic evaluations, good osseointegration of the restored dental implants was recorded. Although postoperative radiographic examinations at the time of implant-abutment connection and at 24 months showed no relevant change of bone height around the implants, a potential limitation of this study is that patients were not submitted to a third CT scan at this

latter time period. Thus, it was not possible to discern exactly whether, and of which account, resorption of the grafted FFB was present. Published data on resorption rates and speed related to autologous augmentation material vary. Most studies describe reduction of approximately 30% after 1 year.[28] Resorption usually stagnates after 1 year,[29] and long-term evaluations show favorable data on clinical outcomes of implants placed in the reconstructed areas.[30–32] Calvarium has been suggested as a reliable source of autogenous bone to limit resorption of the grafts because of its dense cortical bone.[4,33] However, the drawback of postoperative morbidity is always present, even if it has been reported to be less painful than harvesting bone from the iliac crest.[4] Therefore, the absence of postoperative complications, the human origin, the reported safety of the FFB graft, and its biocompatibility and osteoconductive properties render this material a possible good alternative for reconstruction of the alveolar ridge. Qualitative and quantitative evaluations by means of light microscopy and histomorphometry demonstrated that the regenerated region shows features similar to pre-existing osseous tissue, and FFB acts as an osteoconductive conduit on which host bone is laid down, presenting all the phases of bone formation starting from highly woven fuchsin-stained osseous tissue surrounding the residual FFB and leading to more mature trabecular bone with numerous osteocytes at the periphery of the graft.

The results of this study could increase scientific knowledge in understanding the events occurring after FFB implantation and have confirmed pre-existing literature in which regenerative procedures were performed using either autogenous bone or different allografts. Indeed, both clinical and biologic responses to FFB onlay grafts were favorable and allowed implant placement in regions previously reported as having poor bone quantity.[13,30–33] Indeed, although recent scientific literature revealed accepted biocompatibility of FFB grafts by means of several in vitro studies[11,15,21,25] and a clinical report in orthopedic surgery,[34] very few clinical studies have been performed on oral regenerative procedures,[35–39] and, to the authors' knowledge, there is only one in vivo study supported by histologic data that has proven the utility and safety of human FFB for maxillary sinus augmentation procedures.[12] The fact that FFB grafts were perfectly incorporated in the recipient site and formed a well-structured and consolidated osseous tissue in which dental implants were inserted successfully warrant future studies on performing immediate loading protocols, since the survival rates of immediately loaded implants in grafted sites are consistent with those of implants placed in native nonreconstructed bone.[4] Moreover, even if it has been reported that horizontal reconstructions are generally more stable than vertical augmentations,[32] to test the stability of FFB over time, future prospective studies will be directed toward the resorption rate analysis of this biomaterial.

Conclusions

This in vivo study represents the first attempt to reveal a case series of clinical, histologic, and histomorphometric data on FFB apposition grafts used for horizontal maxillary reconstruction in humans. Despite the limited number of patients, it may represent an important start for forthcoming clinical trials and additional long-term analyses. The use of FFB in oral and maxillofacial surgery could become very important, especially in the reconstruction of severe atrophies and important posttraumatic defects where large amounts of bone graft are needed and intraoral and extraoral sources can be difficult to find.

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