Fresh-Frozen Bone Blocks for Horizontal Ridge Augmentation in the Upper Maxilla: 6-Month Outcomes of a Randomized Controlled Trial

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ABSTRACT

Purpose: This randomized controlled trial compared fresh-frozen versus autologous bone blocks for maxillary horizontal ridge augmentation in patients with Cawood and Howell class IV atrophies.

Materials and Methods: Twenty-four patients were allocated to the autologous and fresh-frozen groups in a 1:1 ratio. Patients underwent computed tomography scans 1 week and 6 months after surgery for graft volume and density analysis. Doxycycline was administered at day 120 and day 150 to label new bone formation. Biopsy for histologic and histomorphometric analyses was performed at reentry for implant insertion, 6 months after grafting.

Results: Fresh-frozen grafts had lower density than autologous bone. Autologous and fresh-frozen grafts lost, respectively, 25% and 52% of their initial volume ($p = .0041$). Histology revealed the presence of newly formed bone within both graft types, but clear signs of inflammation were present in fresh-frozen blocks.

Conclusions: According to these 6-month results, autologous bone blocks are preferable to fresh-frozen bone grafts.

KEY WORDS: allograft, alveolar ridge augmentation, autografting, homograft, randomized controlled trial

INTRODUCTION

When horizontal ridge augmentation is required, autologous bone (AB) block grafts are often employed. However, their availability is limited and harvesting requires a second surgery, which increases morbidity. These drawbacks prompt to seek alternative graft materials. Homologous bone is available either as fresh-frozen bone (FFB), freeze-dried bone, or freeze-dried-demineralized bone allografts. Strict guidelines for tissue harvesting and storing make the risk of antigenicity and primary infections acceptably low.[1]

Case reports and case series showed the feasibility of alveolar atrophy correction employing FFB blocks.[2–11] The use of FFB has been advocated also for sinus lift, either employing blocks[8,12–14] or granules.[8,15,16] Nevertheless, the effectiveness and predictability of these allografts remain unclear, and no randomized controlled trial (RCT) on FFB block grafting for ridge augmentation has been published.

This RCT compares the behavior of FFB to AB grafts for horizontal ridge augmentation in Cawood and Howell class IV atrophy in the upper maxilla,[17] using blocks and lag-screw fixation. We report on the results at 6 months.

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MATERIALS AND METHODS

General Design

Outcomes of this RCT were volume and density changes of AB and FFB measured 1 week (T1) and 6 months (T2) after grafting on computed tomography (CT) scans and histologic and histomorphometric patterns of bone biopsies taken at 6 months at the moment of implant insertion. Additionally, clinical outcomes were recorded. The protocol encompasses also the analysis of implant-related outcomes at subsequent time points. The present report will not address time points later than T2.

Twenty-four patients with Cawood and Howell class IV atrophy requiring one or multiple implants in the upper maxilla were enrolled in the study between May 2008 and August 2009 at University of Bologna, Modena e Reggio Emilia and Parma. A pilot study evaluating graft resorption at CT scans was used to estimate the sample size. Written and verbal information was given to the patients before enrollment, and written informed consent was obtained. The study was conducted in full accordance with the World Medical Association Declaration of Helsinki and it was approved by the ethics committee of the participating institutions.

Inclusion criteria were the following: at least 18 years of age; adequate oral hygiene, that is, plaque index score and full mouth bleeding score ≤25%. *Exclusion criteria* were the following: previous radiotherapy to head and neck region; smoking >10 cigarettes a day; history of leucocyte dysfunction, bleeding disorders, renal failure, metabolic bone disorders, and uncontrolled endocrine disorders; human immunodeficiency virus infection; chronic use of antibiotics; and use of steroids or alcohol, or drug abuse.

Before surgery, all patients underwent clinical and radiographic examinations. Impressions and bite registrations were taken to provide an ideal prosthetic set-up for teeth restoration.

Plaque index score and full mouth bleeding score were maintained ≤25% throughout the study. Smoking was maintained lower than 10 cigarettes a day.

Randomization

A locked computer software (Minitab 1.5, Minitab, State College, PA, USA) was used to randomly allocate patients to receive either autogenous bone block graft (AB group) or FFB block grafting (FFB group) in a 1:1 ratio. Blocked randomization (block size = 3) was performed to maintain equal group size. The allocation result was concealed in a closed envelope and disclosed to the surgeon only on the day of surgery. CT and histology examiners were blinded to the allocation. As a result, 12 patients were randomly assigned to the FFB group and 12 patients to the AB group.

Surgical Procedure and CT Scans

FFB grafts were provided by Banca del Tessuto Muscolo-Scheletrico (Istituti Ortopedici Rizzoli, Bologna, Italy). FFB grafts were harvested from tibial hemiplateau of cadaver donors under sterile conditions, disinfected for 72 hours at 4°C in a solution of vancomycine, polymyxine, glazidine, and lincomycine, and frozen at <80°C. Samples were harvested according to the Guidelines of the National Authority for Tissue Transplantation and preserved frozen under sterility for a maximum of 5 years.

All patients received antibiotics prophylaxis employing 2 g of amoxicillin 1 hour before surgery to minimize the risk of infection.[18]

Homologous grafts were thawed in a 600 mg/L rifampicin and saline solution at 37°C. Graft adaptation and fixation for FFB and AB consisted in full-thickness flap elevation, shaping of blocks with burs, in order to fit target bone defects, and graft fixation by lag screws (Cizeta Surgical, Bologna, Italy). Gaps were filled with ground bone particles of the same type of the block used. Collagen membranes were employed to cover the grafts (Osseoguard, Biomet 3i, Warsaw, IN, USA), as suggested by previous studies.[19,20] Two-layer sutures were placed using monofilament sutures (Prolene 3-0 and 5-0, Ethicon, Johnson & Johnson, Amersfoort, The Netherlands) for primary wound closure after releasing periostium. Antibiotics (amoxicillin 3 g/day for 6 days) and chlorexidine rinses (twice a day for 10 days) were prescribed. Postoperative pain control was individualized employing ibuprofen 600 mg tablets. Patients underwent standardized CT scans 7 days (T1) and 6 months (T2) after graft surgery. Sutures were removed at 10 days, and follow-up visits were carried out monthly.

One hundred milligram doxycycline hyclate was administered twice a day for 4 days, 120 and 150 days after surgery for subsequent histologic epifluorescence analysis.

Six months after graft surgery, a second surgical procedure was performed for implant insertion and bone

biopsies of grafts. Bone biopsies were harvested perpendicularly to the vestibular side of the graft through the mesiodistal midline using a trephine bur (Komet, Lemgo, Germany) with 2.5-mm internal diameter.

CT Scan Analysis

All the patients received CT scans (Siemens CT4350, Siemens, Munich, Germany) 7 days and 6 months after grafting.

CT settings were as follows: gantry: 0, resolution: 512×512 pixel, window level: 400, window width: 4,000, 130.00 kV, 47 mA, exposure time: 800 ms, slice thickness: 1.25 mm, and slice reconstruction: 0.5 mm.

Acrylic radiographic templates with three circular reference points (2 mm–diameter) and two extraoral circular supports connected with a spirit level were positioned to allow the realignment of different CT scans.

CT scans (Siemens Somatom Emotion 6, Erlangen, Germany) were analyzed in accordance to previously published methods[21,22] using an image processing software (OsiriX Imaging Software, Pixmeo, Geneva, Switzerland).

Briefly, cross-sectional images perpendicular to the panoramic arch were constructed in the grafted area at intervals of 0.5 mm. The graft area was traced as a region of interest (ROI) freehand on the axial cross-sectional image. The total graft volume (GV) and its minimum, maximum, and mean density were obtained by stacking the calculated ROIs.

Histology

Samples were fixed in 4% paraformaldehyde (all reagents came from Fluka, Sigma-Aldrich Schweiz, Buchs SG, Switzerland) in 0.1 M phosphate buffer pH 7.2 for 4 hours at room temperature. Specimens were dehydrated through ethanol series at 4°C, then embedded in polymethyl methacrylate (PMMA) using a water bath at 4°C as described elsewhere.[23] The PMMA blocks were serially sectioned to obtain two sets of sections: thick (100-µm-thick) and thin (5-µm-thick) sections.

Each PMMA block was serially sectioned along the longitudinal axis of the cylindrical bone sample to its center using a diamond saw microtome (SP1600, Leica Microsystem, Nußloch, Germany). A thick section (200 µm) was obtained from the center of cylindrical sample using the diamond saw microtome. The section was reduced to 100 µm by grinding, perfectly polished with emery paper and alumina, then x-ray microradiographed (3 K5, Italstructures, Riva del Garda TN, Italy) at 15 kV and 10 mA on high-resolution film (SO 343, Eastman Kodak Co, Rochester, NY, USA). A set of 5-µm-thick sections was obtained starting from the level at which the thick section was performed, using a tungsten carbide knife (Profile D) on a bone microtome (Autocut 1150, Reichert-Jung GmbH, Nußloch, Germany). Thin sections were stained with toluidine blue or trichrome Gomori stain. The microradiographs and the sections were analyzed and photographed using a microscope (Axiophot, Carl Zeiss AG, Oberkochen, Germany) under ordinary and fluorescent light.

Histomorphometry was performed by a single trained operator using a suitable program for image analyzer and software (AnalySIS®, Soft Imaging System GmbH, Münster, Germany). The following parameters were calculated:[24]

- bone volume (BV)/total volume (TV)
- GV/TV
- viable bone (VB)/TV
- newly formed bone (NB)/TV
- mineral apposition rate (MAR)

The presence of inflammation was evaluated using the scoring system proposed by Panzarini and colleagues[25] and the examination was statistically compared using the Mann-Whitney *U* test. Two blinded examiners, calibrated prior to reading, scored the grafts for the presence of inflammatory infiltrate.

Statistical Analysis

The significance of differences in volume and density changes between AB and FFB was assessed by means of Student's *t*-test or Mann-Whitney *U* test for nonparametric data.[26,27] Data are expressed as mean ± standard deviation. The null hypothesis H_0 was rejected for a critical significance level of $p < .05$.

RESULTS

Clinical and CT Outcomes

Population characteristics are summarized in Table 1. At T1, FFB graft density was significantly lower (619 ± 277 HU vs 935 ± 250 HU; $p = .007$). The initial volume of FFB and AB blocks was not significantly different (1.5 ± 0.91 cm^3 vs 0.44 ± 1.04 cm^3; $p = .15$).

At T2, both AB and FFB grafts underwent extensive remodeling as evidenced by volume change at CT scans, but FFB showed significantly more resorption. AB lost

TABLE 1 Table Summarizing Baseline Characteristics of the Study Population and Main Findings

	AB (Control Group)	FFB (Test Group)	Total
n enrolled	12	12	38
Sex (M/F)	3 M	5 M	8 M
	9 F	7 F	16 F
Mean age	54	49	55.4
Age range	24 to 76	24 to 73	24 to 76
Smoking	<10 cigarettes/day	<10 cigarettes/day	
Failed	0	0	0
n completed protocol	12	12	24
			p
Volume T1	0.44 ± 1.04 cm^3	1.5 ± 0.91 cm^3	.17
Volume T2	0.67 ± 0.68	0.79 ± 0.62	.66
Volume change	−25% ± 12.73	−52% ± 25.87	.004
Density T1	935 ± 250 HU	619 ± 277 HU	.007
Density T2	1086 ± 202.2	685 ± 385.1	.004
Density change	18% ± 33.42	9% ± 32.44	.50

Values as reported as mean ± standard deviation.
AB = autologous bone; F = female; FBB = fresh-frozen bone; M = male.

an average of 25% of the initial volume, whereas FFB decreased by 52% ($p = .004$; Figure 1A). Interestingly, in one case, an FFB graft was completely resorbed and could not be observed at the second CT scan. Density changes were comparable ($p = .50$; see Figure 1B).

Histology

Histology could be performed in 10 FFB samples and in nine AB ones, as in the other cases either the harvested material proved inadequate for analysis or the biopsy was impossible. Samples were analyzed under polarized light to identify woven bone, typical of newly formed tissue (Figure 2, insets). Gomori trichrome and toluidine blue staining was used to detect viable osteocytes. Osteocyte lacunae were mostly empty and scarce in both AB and FFB grafts (see Figure 2, A and E, white arrowheads), whereas the new-formed bone contained numerous viable osteocytes (see Figure 2, B and F, white arrowheads). Bone forming osteoblasts (see Figure 2, C and G, white arrows) and fluorescent labeling (see Figure 2, D and H, white arrows) were detected on both autologous and homologous grafts. Dense connective tissue with the presence of inflammatory cells and numerous eroded areas were observed in FFB samples (see Figure 2E, red arrow). The weighted mean inflammation score of AB group was 1.0, while that of FFB group was 1.67, statistically significant ($p = .016$) after Mann-Whitney *U* test.

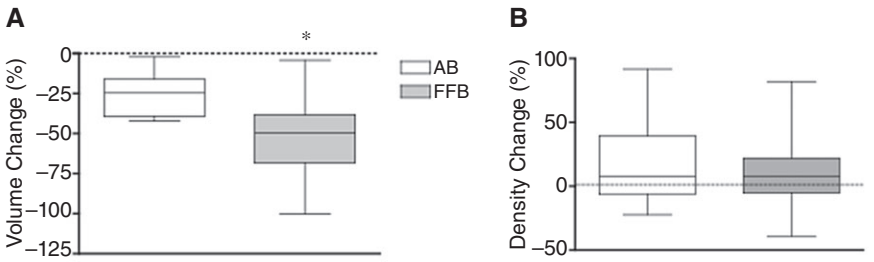

Figure 1 Graphs depicting (A) volume change and (B) density change of AB and FFB grafts after 6 months as determined by CT analysis. Median, minimum, maximum, and standard deviation; *$p = .004$. (AB = autologous bone; CT = computed tomography; FFB = fresh-frozen bone.)

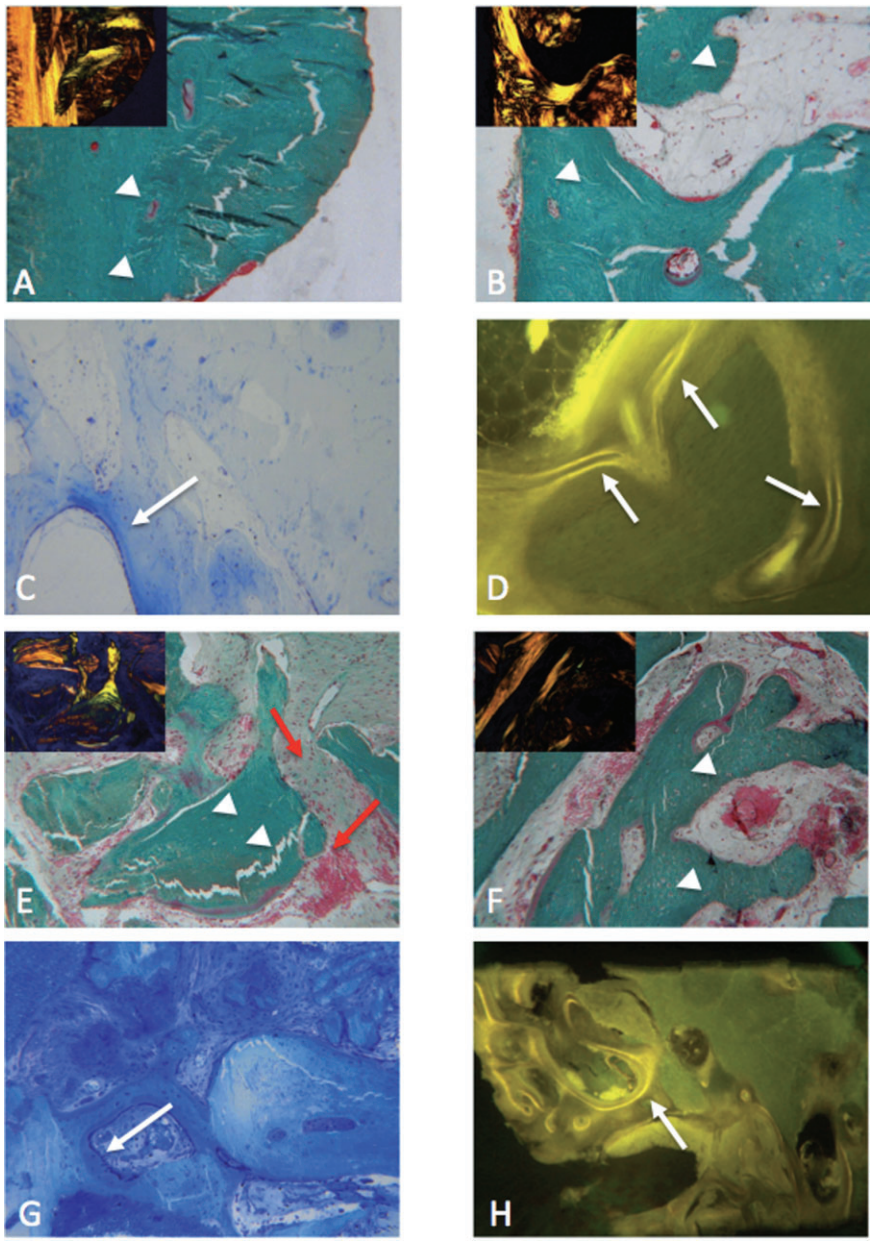

Figure 2 Histologic analysis of AB (A–D) and FFB (E–H) grafts. *A–B*, *E–F*, Toluidine blue and Gomori trichrome staining for viable osteocytes (*white arrowheads*) and polarized light microscopy for newly formed tissue (insets). New bone is easily identified by a woven, irregular distribution of collagen fibers, whereas the grafted bone appeared lamellar structured, with ordered arranged collagen fibers. *C* and *G*, Toluidine blue staining for osteoblasts, osteocytes, and newly formed bone. New bone and osteoid (*white arrows*) appear stain deepened than older or grafted bone. *D* and *H*, Transmitted fluorescence microscopy of doxycycline labels (*white arrows*). Doxycycline was administered at days 120 and 150 and was incorporated into new bone, thus labeling bone apposition lines. Dense connective tissue with the presence of inflammatory cells and numerous eroded areas were observed in FFB samples (E, *red arrow*). (AB = autologous bone; FFB = fresh-frozen bone.)

Histomorphometry is summarized in Figure 3. No significant differences were observed in the percentage amounts of bone (BV/TV), soft tissue (ST)/TV, GV/BV, VB/BV, or NB/BV. Although the MAR for FFB was lower (1.12 ± 1.94 mm/day) than for AB (9.16 ± 14.37 mm/day), the difference was not statistically significant ($p = .20$).

DISCUSSION

Graft remodeling strongly affects the possibility of implant placement and the support given to the surrounding STs, with both functional and esthetic consequences.

The present RCT compared dimensional changes at CT and histology of AB versus FFB in upper maxilla of

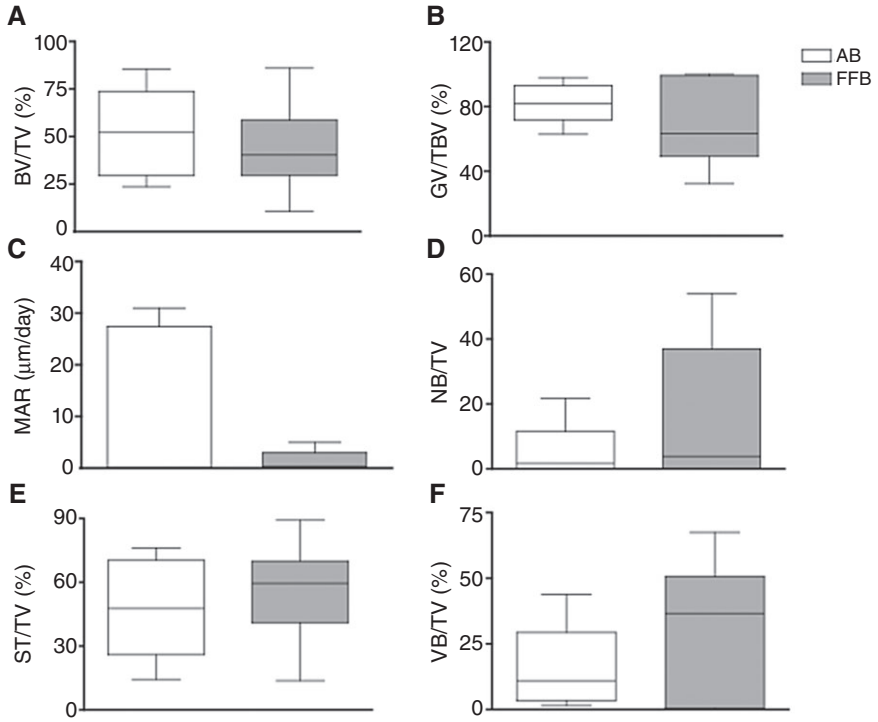

Figure 3 *A–E*, Architectural and dynamic histomorphometry of AB and FFB grafts after 6 months. (AB = autologous bone; BV/TV = bone volume; FFB = fresh-frozen bone; GV/TV = graft volume; MAR = mineral apposition rate; NB/TV = newly formed bone; ST/TV = soft tissue; VB/TV = vital bone.)

patients requiring horizontal ridge augmentation prior to implant insertion.

CT scans revealed that both AB and FFB grafts underwent extensive resorption at 6 months, and FFB grafts lost significantly more volume (−25% vs −52%, $p = .004$). FFB resorption showed, however, high variability, with wide differences from case to case, ranging from complete resorption to almost unmodified graft. In this regard, it must be noted that FFB had lower density than AB grafts, which may, at least in part, explain its higher resorption.

The high variability in FFB performance in our study may depend on their microarchitecture. Our study analyzed allografts from tibia, a long bone that possesses a large epiphysis that tapers down into a narrower, denser diaphysis, mainly composed of thicker cortical bone. The FFB blocks used in the present study were harvested from the tibial hemiplateau, in a region mainly composed of cancellous bone with a low average density. Other variables, however, like donor's age and gender, could affect graft performance, but no data are hitherto available in the literature to this regard.

Histologic and histomorphometric analyses allow to investigate FFB safety, as well as biological aspects of grafts incorporation and their influence on clinical outcomes. Our histologic analysis provided insight into the biological behavior of FFB. It must however be considered that biopsies were representative of a limited area, which may have different remodeling patterns from the adjacent ones.

We observed no viable osteocytes inside the grafted tissue at T2. In the AB case, this suggests that osteocytes are unable to survive the procedure, in agreement with previous observations.[28,29] It has been reported that living cells can be detected in nonprocessed deep-frozen bone allografts.[30] Even if we cannot exclude the presence of living cells at the moment of the graft, there was no trace of viable osteocytes from the donor at T2. These findings indicate that both autologous and homologous grafts act as scaffolds, although with different biological responses.

It is important to underline the increased presence of dense connective tissue and inflammatory cells in FFB samples, in contrast to AB ones where no inflammatory infiltrate was observed, consistently with the literature data.[28,29] We may ascribe the inflammatory reaction to the presence of residual donor marrow in FFB cancellous compartment (see Figure 3, E and F). This finding

may suggest that cortical FFB grafts may be preferable. However, Spin-Neto and colleagues,[11] using cortical FFB blocks, observed the lack of direct contact between graft and native bone and the presence of necrotic areas in the graft, even if they did not observe immune or inflammatory infiltrate. In addition, they found only sparse areas of NB in cortical FFB samples, corresponding to their clinical finding of a nearly unmodified graft at reentry.

Interestingly, we observed comparable amounts of NB in FFB and AB, although the former displayed high variability. The presence of NB in FFB samples is consistent with reports by Contar and colleagues,[4,5] who used tibia homologous grafts, and Orsini and colleagues[9] (origin not specified), who also reported a good integration between FFB blocks and grafted areas.[4,5,9] The different biological behavior of AB and FFB is also confirmed by MAR, which shows a tendency to a slower bone apposition on FFB during the limited time frame of doxycycline labeling, between day 120 and day 150.

Based on the present data, it can be concluded that AB is preferable to FFB from tibial hemiplateau for maxillary grafts. Further studies however are required to investigate the performance and resorption of FFB grafts with higher density.

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