The Fence Technique: 100% Autogenous Bone Graft vs 50% Deproteinized Bovine Bone Matrix and 50% Autogenous Bone Graft. A Histologic Randomized Controlled Trial

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The aim of this histologic, single-blind, parallel, randomized clinical trial was to compare vertical bone augmentation grafting with 100% autogenous bone (group AB) vs 50% deproteinized bovine bone matrix (DBBM)/50% autogenous bone (group BOAB) using the Fence Technique in a two-stage implant placement. A biopsy was performed in the regenerated area at implant insertion 6 months after the augmentation surgery. The results reflect a sample size of four patients treated per group. At implant placement, 6 months after grafting, no significant differences were evident in the histomorphometric comparisons, even if the percentage of residual graft was obviously greater in the BOAB group (P = .0314). Int J Periodontics Restorative Dent 2020;40:181–190. doi: 10.11607/prd.4116

Bone augmentation could be required for patients lacking an adequate quantity of bone for implant treatment.1–3 In vertical bone augmentation, barrier membranes in combination with a variety of graft materials, such as autogenous bone, allografts, xenografts, and alloplastic materials, are often used.4–11 A bone substitute of bovine origin is frequently used for various augmentation procedures.12–18 Recently, a new surgical procedure (the Fence Technique) was developed in which the volume of the bone augmentation is planned in advance.19,20 The use of bone substitute reduces the entity of intraoral harvesting when compared to the use of autologous bone alone and could result in a larger bone volume for dental implant placement. In a systematic review, the mean implant survival rate for vertical and/or lateral ridge augmentation was 100% for bone substitute materials mixed with autologous bone and 98.6% for autologous bone alone, but a comparison with a meta-analysis was not possible due to missing data.21

Histomorphometric analysis is considered to be the gold-standard method for estimating the amount of newly formed bone, residual graft particles, and soft tissue components.22 A recent systematic review compared the histomorphometric effectiveness of bone grafts.23 No
statistically significant differences were found in the percentage of new bone from pairwise comparisons between any of the two bone grafts. Treatment ranking based on the evidence network indicated that autografts presented the highest percentage of newly formed bone, followed by synthetic grafts, xenografts, and allografts.\textsuperscript{23} Regardless, bone substitute materials seemed to be good alternatives to autogenous bone and could be considered grafting materials to avoid the disadvantages related to autografts, including morbidity rate, limited availability, and high volumetric change.\textsuperscript{24} At present, no data are available on the comparison of 100% autogenous bone or 50% deproteinized bovine bone matrix (DBBM)/50% autogenous bone using the Fence Technique.

The aim of this randomized clinical trial (RCT) is to compare vertical bone augmentation grafting with 100% autogenous bone or 50% DBBM/50% autogenous bone using the Fence Technique.

The study took place at a private center in Rimini, Italy. The dental office obtained the approval of the local authorities to conduct clinical studies (protocol number 221722/P).

The investigators explained the nature of the trial, the aim, and the methods to the patients, as well as anticipated benefits, potential risks, and any form of discomfort that participation might entail. The patients read and asked questions inherent to the study prior to signing the informed consent.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. An independent Ethics Committee (Ethical Committee IRST-IRCCS – Area Vasta Romagna) approved this clinical study (protocol 962/2015 I.5/95, date 20-02-2015). The principal investigator has 30 years of experience in dental implant surgery and dental implant prosthesis rehabilitation.

Materials and Methods

Trial Design

This was a monocenter, single-blind clinical trial, with balanced randomization and parallel two-group design. Grafting material used distinguished the two groups: 100% autologous bone graft (group AB); and 50% DBBM (Bio-Oss, Geistlich) plus 50% autogenous bone graft (group BOAB). Titanium osteosynthesis plates supporting biodegradable collagen porcine membranes (Bio-Gide, Geistlich) stabilized the grafting material.

Eligibility Criteria for Participants

Thirty partially edentulous patients in need of esthetic implant treatment with extensive vertical osseous defects were included in the study; the present report examines the findings from the first 8 patients.

Eligible participants were adults aged 18 or older, partially dentate, and could benefit from extensive vertical osseous augmentation for placement of a single or multiple implants. Patients should have extremely atrophic localized alveolar crest in the mandible or maxilla (Class V of Cawood and Howell\textsuperscript{26}).

Exclusion criteria were: general contraindications to implant surgery, patients irradiated in the head and neck area, patients treated with intravenous aminobisphosphonates, patients with poor oral hygiene (full-mouth plaque score and full-mouth bleeding score \( \geq 20\% \)) and lack of motivation, uncontrolled diabetes (reported levels of glycaemia over the normal threshold), pregnancy and lactating period, allergy to collagen, substance abusers, and smoking habit with more than 20 cigarettes per day or equivalent.

Setting, Locations, and Ethics

A new, two-stage procedure for bone regeneration in vertical defects, the Fence Technique, was applied.\textsuperscript{19} One surgeon (M. Merli) performed all the surgical interventions.
A preliminary cone beam computerized tomography scan was performed, confined to the atrophied arch, to evaluate the three-dimensional morphology of the hard tissue. The data were used to create a stereolithographic model using specific epoxy, thermosetting, and light-sensitive resins (3DM-Flex, 3DM). This anatomic resin model served as a framework for presurgical planning of the bone reconstruction procedure. An osteosynthesis titanium plate was adapted to the model to determine the volume for the bone regeneration.

The osteosynthesis plate used in the reconstructive stage was shaped in order to constitute a physical barrier (the “fence”) that contained the biomaterial necessary for the regeneration, permitting a vertical augmentation.

Surgery

The patients underwent prophylactic antibiotic protocol with 2 g of amoxicillin taken orally 1 hour prior to surgery. Patients were subjected to intravenous sedation. They received fractioned administration of 0.5 to 1 mg midazolam with 0.5 mg atropine. The following analgesics were administered intravenously: tramadol 100 mg, ketorolac 30 mg, and betamethasone 4 mg. Articaine with 1:100,000 adrenaline was used as local anesthetic.

The Fence Technique for localized three-dimensional bone augmentation was performed: in brief, full-thickness flaps were raised to expose the area to be regenerated. The titanium osteosynthesis plate was fixed using titanium screws. When the plate was fixed and stable, the bone grafting phase began. At this stage, a sealed opaque envelope indicating the type of graft to use according to the pre-trial randomization allocation plan was opened.

Autologous bone was harvested from intraoral sites, preferably the mandibular angular region, at the base of the ramus. The harvested bone was then mashed using a manual milling machine (Hu-Friedy), and in the BOAB group it was mixed with DBBM (Bio-Oss), which consists of cancellous bovine bone granules. The biomaterial was then positioned to fill the entire space between the bone wall and the titanium plate. The vertical defect was filled with autogenous bone alone (AB group, n = 4 in the present report) or autogenous bone with DBBM (BOAB group, n = 4 in the present report) as grafting material and covered with bioresorbable collagen membranes (Bio-Gide) fixed by titanium tacks inserted on the palatal/lingual and vestibular aspect of the surgical area.

The creation of a perfect seal along the primary horizontal incision line is fundamental to avoid the risk of dehiscence, possible exposure of the membrane surface, and infection of the grafted bone. The flap extension technique (muscular dissection and/or perioplasty) fixed with a double suture was followed in order to avoid this type of complication.

Amoxicillin-clavulanic acid (1 g, twice a day for 6 days) and ibuprofen (600 mg, twice a day for 2 to 3 days and as needed thereafter) were prescribed to all patients. Ice packs were given to the patients for the first few postoperative hours.

Patients were instructed to refrain from mechanical plaque removal in the surgical area for 2 weeks, to use 0.12% chlorhexidine mouth rinse twice a day from the first postoperative day and to apply chlorhexidine gel on the wound area after the sutures removal. Patients were advised to avoid smoking during the prescribed recovery period.

After a healing period of 6 months, implants were placed during second-stage surgery. A representative case of the AB group is shown in Fig 1.

Histologic Analysis

During the second surgical phase for implant placement, bone core biopsy samples of the augmented tissue were retrieved from the regenerated areas of all eight patients using a trephine bur, immediately fixed in 10% buffered formalin, and processed for histologic and histomorphometric analysis (A.M.). The specimens were dehydrated in an ascending series of alcohols and embedded in London White resin (LR White Resin, Agar Scientific). After polymerization, the specimens were sectioned along their longitudinal axis with a high-precision diamond disk at 150 μm and grinded to approximately 40 μm with a specially designed grinding machine (Micromet, Remet). The undecalciﬁed ground sections were stained
with acid fuchsine and toluidine blue staining. The specimens were observed under normal transmitted light with a light microscope (Nikon Eclipse, Nikon). The histomorphometric analysis was carried out using the light microscope connected to a high-resolution video camera and the images were elaborated using Image J software (National Institutes of Health). The tested variables in the histomorphometric analysis were: newly formed bone, mineralized bone (native bone), residual graft particles, marrow spaces, and soft tissue (connective tissue). Presence of the mentioned factors was expressed in percentage.

Figs 1a to 1f  AB group. (a) Preoperative view. (b) Preoperative cone beam computerized tomography scan. (c) Lateral and (d) occlusal views of the osteosynthesis titanium plate fixed at surgery. (e) Cone beam computerized tomography; (f) osteosynthesis titanium plate with the regenerated tissue.
Outcome Measures

In this report, only histologic data of the first eight patients included in this RCT were described. The clinical variables on the full sample size will be reported in a forthcoming publication.

Percentages of new bone, mineralized native tissue, residual graft, marrow space, and soft tissue were calculated. An assessor blinded to the treatment administered registered the outcome measurements.

Sample Size

To detect the difference of 0.4 cm$^3$ in osseous volume differences between groups (standard deviation: 0.34 cm$^3$) with a two-side 5% significance level and a power of 80%, a sample size of 30 patients (15 patients per group) will be necessary, given an anticipated dropout rate of 20%. The present histologic report comprises data from 8 sample patients who underwent biopsy.

Randomization

Participants were randomly assigned to either the AB group or the BOAB group with a 1:1 allocation ratio. A computer-generated list of random numbers was used, and 15 patients were included in each treatment group. A blocked randomization was used: the first 8 patients were divided in 4 patients per treatment method, and therefore the biopsies described herein were performed in an equal number of cases for each treatment.

Figs 1g to 1j  AB group. (g) Regenerated tissue at 6 months postsurgery. (h) Two implants were inserted, and one bone core biopsy sample was taken in the regenerated region at 6 months postsurgery. (i) Lateral view and (j) periapical radiographs at 30 months postsurgery.
Allocation Concealment

The allocation sequence was concealed from the researcher (M.N.) enrolling and assessing participants in sequentially numbered, opaque, sealed envelopes. The surgeon opened the envelopes only during the surgical procedure.

Blinding

Whereas the surgeon was aware of the allocation arm, patients and the outcome assessor were kept blinded to the allocation, even if Bio-Oss grafting material can be easily detected during the histomorphometric analysis.

Statistical Method

Descriptive statistics were performed using mean and standard deviation for quantitative data and frequency and percentage for qualitative data. Percentages of new bone, mineralized native tissue, residual graft, marrow space, and soft tissue were calculated. The statistical unit was the patient; when more than one biopsy was executed, the mean per patient was used. Intention-to-treat analysis was performed. Three t-test analyses were conducted using the treatment (AB vs BOAB) as explicative variable and the percentage of new bone, the percentage of the mineralized native tissue, and the percentage of the residual graft as outcome variables.

A single statistician (M.N.) performed all statistical analyses. The statistical software was JMP version 13 (SAS Institute).

Results

This histologic study was performed on the first eight patients included in the RCT. All eight patients were analyzed and there were no dropouts. Four patients were assigned and treated with 100% autogenous bone (AB group), and four patients were assigned and treated with the 50% Bio-Oss/50% autogenous bone (BOAB group).

Baseline information is presented in Table 1. One protocol deviation occurred: In one patient allocated to the AB group, the surgeon decided to add 20% DBBM to the autologous bone graft because he considered the graft insufficient to fill the bone defect. During the second surgical phase for implant placement, seven biopsy samples were taken from four AB-group patients and seven biopsy samples were taken from four BOAB-group patients.

Histologic appearance of the regenerated area of the AB group revealed new bone formation in contact with mature bone, with minor marrow spaces containing a low number of osteocytes (Fig 2). Histologic appearance of the regenerated area of the BOAB group revealed new bone formation in contact with mature bone and in contact with grafting particles. Several areas of concentrated grafting particles were also noted, as well as marrow spaces and osteocytes (Fig 3).

The histologic data were analyzed using the percentage of new bone, mineralized native bone, residual graft, marrow spaces, and soft tissue as variables. The means, standard deviations, differences between treatments, 95% confidence intervals of the differences, and P values are reported in Table 2. Unfortunately, one sample from the BOAB group could not be histomorphometrically processed because the biopsy sample involved only soft tissue. The comparisons were all statistically insignificant with the exception of the percentage of the residual graft that remained greater in the BOAB group (P = .0314). The percentage of new bone and of native bone (mineralized tissue) is similar in the two groups. The difference was –1.0% (P = .9241) for the new bone and –4.5% (P = .5132) for the native bone.

Table 1 Baseline Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>AB group (n = 4)</th>
<th>BOAB group (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (SD)</td>
<td>44.3 (10.7)</td>
<td>68.0 (4.5)</td>
</tr>
<tr>
<td>Female patients, n (%)</td>
<td>2 (50%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Treatment location, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxilla</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>Mandible</td>
<td>2 (50%)</td>
<td>3 (75%)</td>
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</tbody>
</table>

AB group = patients receiving 100% autogenous bone graft; BOAB group = patients receiving 50% DBBM/50% autogenous bone graft; SD = standard deviation.
Discussion

The aim of this RCT was to perform a histomorphometric comparison of vertical bone augmentation grafting with 100% autogenous bone or 50% DBBM/50% autogenous bone using the Fence Technique in a two-stage procedure in patients with extensive vertical osseous defects. This article exclusively reports the histologic results.

The comparisons were all similar and statistically insignificant with the exception of the percentage of the residual graft, which remained greater in the BOAB group.

The xenograft (Bio-Oss) used in this study is derived from bovine bone without the organic substances, opportunely removed. The complete resorbability of Bio-Oss is questionable. A 10-year case report showed that resorption of Bio-Oss is a slow process. Recently, a study reported histologic and histomorphometric data in 12 human biopsy samples harvested 14 to 80 months after bone augmentation procedures with autologous bone and Bio-Oss. No signs of inflammation were visible, and no tendency toward a decreased volume of DBBM over time was observed.

A study on maxillary floor elevation in which a 50/50 mixture...
of autologous bone and DBBM was used reported percentages of 19.4% and 36% of residual graft and vital bone, respectively. A randomized controlled trial on lateral ridge augmentation used an autologous bone/DBBM mixture (40/60), and the residual graft constituted 28.3% of the augmented tissue and total

### Table 2 Histologic Data Analyzed Using the Percentage of New Bone, Mineralized Native Bone, Residual Graft, Marrow Spaces, and Soft Tissue

<table>
<thead>
<tr>
<th>Variable</th>
<th>AB group (n = 4)</th>
<th>BOAB group (n = 3)</th>
<th>Difference</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>New bone, % (SD)</td>
<td>20.4 (10.4)</td>
<td>19.4 (15.9)</td>
<td>–1.0</td>
<td>–26.3, 24.3</td>
<td>.9241</td>
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<td>Mineralized tissue, % (SD)</td>
<td>40.9 (8.3)</td>
<td>36.3 (8.7)</td>
<td>–4.5</td>
<td>–21.1, 12.1</td>
<td>.5132</td>
</tr>
<tr>
<td>Residual graft, % (SD)</td>
<td>0.9 (1.7)</td>
<td>13.9 (8.8)</td>
<td>13.0</td>
<td>1.7, 24.3</td>
<td>.0314</td>
</tr>
<tr>
<td>Marrow spaces, % (SD)</td>
<td>13.2 (7.1)</td>
<td>10.1 (6.2)</td>
<td>–3.1</td>
<td>–16.4, 10.1</td>
<td>.5685</td>
</tr>
<tr>
<td>Soft tissue, % (SD)</td>
<td>25.0 (8.2)</td>
<td>20.8 (12.2)</td>
<td>–4.2</td>
<td>–23.8, 15.4</td>
<td>.6039</td>
</tr>
</tbody>
</table>

AB group = patients receiving 100% autogenous bone graft; BOAB group = patients receiving 50% DBBM/50% autogenous bone graft; CI = confidence interval; SD = standard deviation.

### Fig 3 BOAB group.
(a) Histologic appearance (toluidine-blue and acid fuchsine; original magnification ×2,000) of the regenerated area revealed new bone formation (NB) in contact with mature bone and in contact with grafting particles. An area of concentrated grafting particles is also noted, as well as marrow spaces (MS) and osteocytes (Oc). (b) Higher magnification (×10,000) showing new bone formations in contact with mature bone, with large marrow spaces and a large number of osteocytes. (c) The histological appearance in an even higher magnification (×20,000) revealed a majority of grafting particles and scarce mature bone areas. Minor marrow spaces containing osteocytes are also present.
bone was 20.0%. These values are not very different from those of the present study: In the BOAB group, the DBBM residual graft constituted 13.9% of the augmented tissue, new bone formation was 19.4%, and the mineralized tissue was 36.3%.

Regarding the 100% autologous bone, the percentage of total bone was 41.1% in a randomized controlled trial on maxillary floor elevation and was 40.1% in another randomized trial. Even these values are not so different from those of the present study: In the AB group, there was 20.4% new bone and 40.9% mineralized tissue. These values are also similar in the BOAB group, where there was 19.4% new bone and 36.3% mineralized tissue.

In previous publications on the Fence Technique, only qualitative histologic analyses were performed. The present study is the first randomized controlled trial on the Fence Technique. The results of the histomorphometric analysis show that the values of new bone and mineralized tissue obtained in the AB and BOAB groups using the Fence Technique are similar to those obtained with other less-demanding surgical techniques such as the maxillary sinus augmentation and horizontal bone augmentation.

A limit of this histologic study is the small sample size, which is related to a low statistical power. In addition, an expert surgeon with more than 30 years of experience in implant surgery performed all the interventions. This should be taken into consideration when extrapolating the results from this trial to other settings. In some cases, clinical measurements are not consistent with histologic findings. The clinical investigation with an appropriate sample size could verify these observations.

Conclusions

The comparisons between bone augmentation grafting with 100% autogenous bone or 50% DBBM/50% autogenous bone using the Fence Technique in a two-stage procedure were all statistically insignificant with the exception of the percentage of residual graft, which remained greater in the BOAB group. The percentage of new bone and of native bone (mineralized tissue) is similar in the two groups. From a histologic point of view, the two techniques can be indifferently used.

Acknowledgments

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References


