Volumetric Stability of Fresh Frozen Bone Blocks in Atrophic Posterior Mandible Augmentation

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Fresh frozen bone allografts (FFB) have become an alternative for bone augmentation in the past decades, especially because of the absence of recent reports of disease transmission or immunologic reactions when it is used. The aim of this prospective controlled study is to evaluate volumetric changes of newly created bone following reconstruction of the atrophic posterior mandible. Twenty consecutive patients presenting for reconstruction of posterior mandibular alveolar bone ridge width ≤ 6.0 mm and/or height ≤ 6.0 who met all inclusion and exclusion criteria were included. FFB blocks were used. The main outcome variable investigated was bone volume dynamics. Vertical, horizontal, and 3-dimensional bone gain data were measured from computerized tomography scans. The main predictor variable was time evaluated at 3 points: immediately after surgery (T1), at implant placement (T2), and 1 year after functional loading (T3). Secondary outcome parameters evaluated were implant survival, histologic findings, and microtomographic morphometry. The study included 28 hemi-mandibles, 50 FFB bone blocks, and 15 female and 5 male patients (mean age, 51.8 years). Block and implant survival rates were 100% and 96%, respectively, after 31.75 months of follow-up. Vertical and horizontal bone gain at T2 was 5.15 and 6.42 mm, respectively. Volumetric resorption was 31% at T2, followed by an additional 10% reduction at T3. Histologic evaluation showed newly formed vital bone in intimate contact with the remaining FFB. Microtomography revealed 31.8% newly formed bone, 14.5% remaining grafted bone, and 53.7% connective tissue and bone marrow. Thus, FFB blocks may lead to new bone formation and consolidation, with satisfactory volumetric bone maintenance, allowing implant-supported rehabilitation with high success rates.

Key Words: fresh frozen bone allograft, atrophic mandible, histologic analysis, tomographic analysis, dental implants.

INTRODUCTION

The increasing predictability of modern dental implants has created an opportunity for their installation in compromised sites.1 However, the presence of an adequate bone volume is necessary to allow correct implant positioning and provide long-term stable biomechanical results.2,3 Therefore, implants should be placed in accordance with both prosthetic planning and bone availability.4,5 Several surgical procedures for bone augmentation are currently being used. Autogenous bone graft, harvested from either intra-6 or extraoral7 donor sites, is considered the gold standard8,9 because of osteoconductive, osteoinductive, and osteogenic properties.10 However, the use of autogenous bone might result in donor site morbidity as postsurgical pain, paresthesia, and increased operative time and costs.11-16 Furthermore, graft resorption rates of up to 30% have been reported in the first year.17,18 However, it has been suggested that the addition of bone bovine mineral to the graft surface reduces resorption.19,20 In the past few years, fresh frozen bone allograft (FFB) has become an interesting alternative to overcome the disadvantages of autogenous bone.21,22 The use of FFB has been widely reported for a variety of clinical situations as sinus augmentation23,24 and onlay graft.25,26 The development of strict guidelines of tissue harvesting, processing, storing, and record keeping has considerably decreased the risk of primary infection and antigenicity.27-30 Nevertheless, only a few studies assessed the use of FFB in the posterior mandible.25,27,31 The purpose of the present study was to evaluate volumetric changes of newly created bone following reconstruction of the atrophic posterior mandible using FFB. The null hypothesis was that newly created bone will undergo dimensional changes time. The specific aims of the study are to measure (1) linear (width and height) and (2) 3-dimensional bone changes at 3 time points: immediately after bone grafting (T1), 6 months postoperatively at implant placement (T2), and 1 year after functional loading (T3). Clinical results were assessed at T2 and T3 and microtomographic and histologic findings at T2.
MATERIALS AND METHODS

Study design

This was a prospective controlled study. This study protocol was approved by the Ethical Committee for Human Studies (Committee CAAE 01473512.4.0000.5419), School of Dentistry of Ribeirão Preto, University of São Paulo, Brazil. Twenty consecutive patients with posterior mandibular residual alveolar bone ridge width ≤6.0 mm and/or height ≤6.0 were included in this study. Inclusion criteria were age ≥18 years, good general and mental health, nonsmoking, and good oral health and dental conditions with no active periodontal disease or occlusal problems. Exclusion criteria were compromised oral and/or general health (American Society of Anesthesiologists III and IV patients), pregnancy, smoking, acute or chronic alcohol abuse, radiotherapy, and use of bisphosphonates.

All surgeries were performed by the same experienced surgeon, according to the technique described by Dias et al.25 Patients were instructed to maintain strict oral hygiene in the 2 weeks that preceded both grafting and implant placement surgical procedures. Prophylactic amoxicillin (1 g) was prescribed in all cases. For the grafting surgery, corticocancellous bone blocks from a musculoskeletal tissue bank (UNIOSS, Marilia, Brazil) were obtained from distal femoral epiphysis with approximate dimensions of 20 × 10 × 6 mm. Under local anesthesia (2% mepivacaine with 1:100 000 epinephrine), a full-thickness flap was performed for gaining access to the alveolar ridge bone defect. To improve vascular graft nutrition, the native bed bone was perforated on its cortical buccal aspect. FFB bone blocks were sculpted and shaped tridimensionally according to the bone defect using diamond burs and a micro saw. After passive adaptation of the shaped block over the alveolar defect, it was fixed in the proper position with 1.5-× 10-mm titanium screws (Synthes, Oberdorf, Switzerland). All blocks were perforated on the cortical aspect. The cortical sharp edges of the graft were removed with the same diamond burs, and 0.25- to 1.0-mm bovine bone mineral granules (Bio-Oss, Geistlich Pharma, Basel, Switzerland) were used to cover the block and proximal areas. The surgical site was completely covered with a resorbable collagenous membrane (Bio-Gide, Geistlich Pharma) before wound closure (Figure 1a through f). Tension-free closure and passive sutures were achieved through internal periosteal release of the buccal flap and partial detachment of the mylohyoid muscle. The graft-healing time was 6 months. None of the patients were allowed to use any kind of prosthesis during this period. At 6 months after grafting, a single biopsy was collected from each patient for histologic analysis from the middle of the graft. In the same surgical procedure, conventional cone morse implants were installed (TitamaxCM Cortical, Neodent, Curitiba, Brazil). Six months after implant installation, the patients were rehabilitated prosthetically with cemented implant-supported single-tooth restorations and followed for 1 year after functional loading.

Tomography

Cone beam computerized tomography (CBCT) scans were taken immediately after the grafting surgery (T1), 6 months postoperatively at implant placement (T2), and 1 year after functional loading (T3). A CBCT scan was also performed before grafting procedure for surgical planning purposes, such as to investigate bone atrophy and inferior alveolar bundle position. This CBCT was not used for linear and volumetric measurements. The CBCT model iCat Classic (Imaging Science International, Hatfield, EUA) with exposure factors of 120 kV and 36.12 mAs with a 0.25-mm reconstruction interval and slice thickness was used. The DICOM files were processed by Mimics version 8.13 (Materialise, Leuven, Belgium) to assess linear and volumetric changes at the different times of the study (Figure 2). For linear measurements, fixation screws heads were used as reference points. The values found between the crest of the residual alveolar ridge and the superior and lateral border of the graft were used for evaluation of height and thickness, respectively. To determine the volume of the grafts, the area of the graft in all of the CT slices was measured. All measurements were performed on sagittal slices (cross sections). The contour of the graft was manually traced on each CT slice. The contrast/exposure of the images was adjusted for better assessment of the delineation of structures. The graft area was calculated automatically with Mimics software. The graft volume was also automatically calculated, corresponding to the sum of all sagittal areas slices. This methodology has been widely used, and this measurement protocol has become the most acceptable for bone graft evaluation.32–34 An expert technician performed both linear and volumetric measurements.

Micro CT

Bone biopsies were taken during implant surgery. Samples were passively removed from the trephines and kept in 10% buffered formalin at pH 7.4 for 48 hours and transferred to a solution of 70% ethanol for 72 hours. Micro-CT analysis was performed using the SkyScan 1172 system (Bruker-SkyScan, Kontich, Belgium). Morphometric analysis was used to quantify the percentage of the histologic elements.

Histology

After micro-CT analyses, samples were submitted to histologic assessment using hematoxylin and eosin staining, according to Xavier et al.24 Histologic evaluation aims to assess the presence/absence of newly formed vital bone, remaining grafted bone, and connective tissue in the specimens.

Statistical analysis

For statistical analyses, first a Shapiro-Wilk normality test showed a nonnormal distribution of the data, which were expressed as mean ± standard deviation. Statistical significance was determined by one-way analysis of variance and the Mann-Whitney U test. The Tukey multiple comparison posttest was also used to isolate the group per groups that differed from the others. Both graft and implant survival were estimated using Kaplan-Meier survival curves. SigmaPlot software version 11 (Systat Software, Witzhenhausen, Germany) was used for all analyses. A value of $P < .05$ was considered statistically significant.
RESULTS

Clinical outcomes

The Table summarizes the demographic data. A total of 20 patients (15 women and 5 men) with a mean age of 51.8 ± 7.5 years (range, 37–64 years) were included in the study. Twenty-eight hemi-mandibles were reconstructed with 50 FFB bone blocks. Thirty-two blocks were used for the molar region, and 18 were used for the premolar region. Eight percent of the grafts were used to gain height, 48% were used to gain width, and 44% were used to gain both height and width.

All surgeries were well tolerated by the patients. No bone block was lost. Small expositions of the graft were observed between 15 and 45 postoperative days in 6 patients, and all of them were treated with 2% chlorhexidine gel until spontaneous closure occurred within 10 days.
Tomography

Vertical bone gain at T1 was 5.15 ± 1.04 mm, decreasing (24%) to 3.91 ± 0.94 mm at T2 and an additional 25% to 3.91 ± 0.94 mm at T3 (Figure 3). Horizontal bone gain was 6.42 ± 1.20 mm at T1, decreasing (28%) to 4.64 ± 1.32 mm at T2 and an additional 13% to 4.02 ± 0.71 mm at T3 (Figure 4). Bone gain in the horizontal dimension showed a tendency to exceed bone gain in the vertical dimension over the entire evaluation period, although it was not statistically different (eg, analysis of variance, \( P > 0.05 \)). Volumetric bone gain was \( T_1 = 1176.62 \pm 358.08 \text{ mm}^3 \), \( T_2 = 785.78 \pm 201.16 \text{ mm}^3 \), and \( T_3 = 689.72 \pm 187.45 \text{ mm}^3 \) (Figure 5). Thus, general volumetric bone resorption of 31% ± 15% at T2, followed by an additional 10% ± 14% reduction was noted at T3 (Figure 6).

Six months after the grafting procedures (T2), 50 implants were installed with a torque of insertion of 46 ± 4.9 N/cm. Implant length ranged from 9.0 mm to 11.0 mm. The implant diameter was similar (3.75 mm). The implant survival rate was 96% after 31.75 ± 6.99 months of follow-up. Two implants failed at second-stage surgery. The clinical view of the bone around the implants was healthy, and the reason for failure was probably lack of osseointegration.

Micro CT

Micro-CT images showed an intimate contact between newly formed bone and remaining grafted bone in all samples, resembling the histologic findings (Figure 7). Micro-CT morphometric analysis revealed newly formed bone = 31.8% ± 0.5%, remaining grafted bone = 14.5% ± 0.2%, and connective tissue and bone marrow = 53.7% ± 0.5%.

Histology

The histologic analysis revealed newly formed vital bone, remaining grafted bone, and connective tissue in all specimens. Empty osteocyte lacunae were used for identification of the remaining grafted bone. Newly formed vital bone with viable osteocyte was found in intimate contact with remaining grafted bone. Osteoblasts were seen at the margins of the calcified regions. Blood vessels within the connective tissue were also found. No evidence of acute or chronic inflammation infiltrate was observed (Figure 8).

DISCUSSION

This study was designed to evaluate the dimensional changes of new gained bone following augmentation of atrophic posterior mandibles with corticocancellous FFB blocks. The grafting procedures enabled the adequate reconstruction of alveolar ridges for further implant placement. A similar grafting technique has been previously reported by Nissan et al.\(^3\)

However, in the present study, corticocancellous FFB blocks were used instead of cancellous freeze-dried bone allograft.

### Table

<table>
<thead>
<tr>
<th>Gender</th>
<th>Bone Blocks</th>
<th>Implants</th>
<th>Survival Rate, %</th>
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<tbody>
<tr>
<td>Patient No.</td>
<td>Male</td>
<td>Female</td>
<td>Age, y*</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>15</td>
<td>51.8 ± 7.51</td>
</tr>
</tbody>
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*Data expressed as mean ± standard deviation.
blocks. Corticocancellous bone blocks present a thin external cortical layer, allowing easier locking of fixations screws and numerous internal cancellous sites for increasing graft perfusion.\textsuperscript{34-36} Furthermore, FFB blocks were covered with bovine bone mineral and a collagenous membrane once to assist in volumetric maintenance of the new gained bone.\textsuperscript{20,25,37,38}

![Figures 3-6](images/figures.png)

\textbf{Figures 3-6.} \textbf{Figure 3.} Box plot of the vertical bone gain. T1: immediately after bone grafting; T2: 6 months postoperatively at implant placement; T3: 1 year after functional loading. Median, minimum, maximum, and standard deviation (*eg, analysis of variance [ANOVA], H = 42.905, df = 2, P < .05). \textbf{Figure 4.} Box plot of horizontal bone gain. T1: immediately after bone grafting; T2: 6 months postoperatively at implant placement; T3: 1 year after functional loading. Median, minimum, maximum, and standard deviation (*eg, ANOVA, H = 68.843, df = 2, P < .05). \textbf{Figure 5.} Box plot of the volumetric bone gain. T1: immediately after bone grafting; T2: 6 months postoperatively at implant placement; T3: 1 year after functional loading. Median, minimum, maximum, and standard deviation (*eg, ANOVA, H = 66.139, df = 2, P < .05). \textbf{Figure 6.} Box plot of the volumetric bone resorption. T2: 6 months postoperatively at implant placement; T3: 1 year after functional loading. Median, minimum, maximum, and standard deviation (*eg, Mann-Whitney U test, U = 712.500, P < .05).

It has been suggested that mandibular grafting is less predictable than grafting in the maxilla.\textsuperscript{39} In this study, there...
were no complications related to the graft incorporation process, as graft block detachment of the recipient bone bed. All of the blocks were fully attached to the recipient sites during all time points (survival rate = 100%). This is comparable to previous reports.32,40

In the present study, bone gain in the vertical and horizontal dimensions resembled values reported by Nissan and colleagues.31 The newly gained bone allowed adequate implant placement. All of the implants showed proper primary stability (insertion torque = 46 ± 5 N/cm). Only 2 of 50 implants failed and had to be replaced (implant survival rate = 96%). The results of the present study are consistent with previously published data.25,27,31,41

For any kind of graft, a major drawback is the volume reduction that occurs over time.1 Most of the available studies evaluating the dimensional changes of the grafts have drawbacks.42–44 One of the limitations is the performance of the linear measurements. Linear measurement techniques depend on a reference point, and they can easily under- or overestimate the results. Bone dynamics occur in a nonuniform volumetric pattern.1 For this reason, 3-dimensional measurements are more accurate.

It can be speculated that implant placement at T2 contributed to the decrease in the resorption rate of the new gained bone between T2 and T3.45

The microtomographic and histologic analysis of the grafts

Figure 7. Microtomography views and 3-dimensional reconstruction of a bone sample at 6 months postoperatively. Newly formed bone (blue arrows) and remaining grafted bone (yellow arrows).
after a follow-up period of 6 months showed newly formed vital bone in contact with the remaining grafted bone. In this study, the morphometric findings were similar to those reported by Nissan et al.27 Dias et al.25 and Spin-Neto and colleagues.46 The residual grafted bone did not influence the implant osseointegration outcome, as demonstrated by the results.

CONCLUSION
Within the limits of the present study, FFB blocks lead to new bone formation and consolidation, with satisfactory volumetric bone maintenance, allowing implant-supported rehabilitation with high success rates.

ABBREVIATIONS
CBCT: cone beam computerized tomography
FFB: fresh frozen bone allografts

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