Use of corticocancellous allogeneic bone blocks impregnated with bone marrow aspirate: A clinical, tomographic, and histomorphometric study

Carlos Eduardo Sorgi da Costa, PhD • Andre Antonio Pelegrine, PhD • Djalma Jose Fagundes, PhD • Manuel de Jesus Simoes, PhD • Murched Omar Taha, PhD

The aim of this study was to evaluate the efficacy of allogeneic block grafts impregnated with autologous bone marrow in horizontal ridge augmentation therapy. Ten patients with severe ridge volume deficiency in the anterior maxilla were treated with horizontal ridge augmentation. The patients were randomized into two groups. Five patients, using two allogeneic block grafts, were in the control group, and five patients, using two allogeneic block grafts impregnated with autologous bone marrow, were in the test group. Hematologists collected 4 mL of bone marrow from the iliac crest of the patients in the test group immediately prior to the surgeries. The blocks were fixed using titanium screws to obtain rigid fixation and to standardize the reference points for measurement purposes. CT scans were obtained both preoperatively and six months postoperatively to allow evaluation of horizontal bone gain. After a healing period of six months, the sites were reopened and the screws were removed. Before implant placement, bone cores were harvested and prepared for histologic and histomorphometric evaluation. Tomographic and histomorphometric measurements were recorded.

The test group demonstrated better tomographic results (P < 0.05) in augmenting alveolar thickness, with a mean value of 4.60 ± 1.43 mm (118.23 ± 56.93%), while the control group had bone gain of 2.15 ± 0.47 mm (49.91 ± 20.24%). Despite the different results in alveolar thickness gained between groups, all sites received dental implants. The histomorphometric analysis also showed better results (P < 0.05) in the amount of vital mineralized bone in the test group as compared to the control group. The findings of this study suggest that an autologous bone marrow aspirate can increase the regenerative potential of corticocancellous allogeneic bone grafts.

Received: August 27, 2010
Accepted: November 19, 2010

Resorption of the edentulous alveolar ridge makes implant placement in an ideal position challenging, especially in the anterior maxilla, where more than 30% of alveolar ridge reduction in thickness occurs in a six-month period after tooth extraction. Therefore, gaining an adequate bone contour by bone graft techniques is frequently indicated prior to dental implant placement. Autogenous bone grafts are considered the gold standard in bone regeneration procedures; however, harvesting autogenous bone grafts carries the risk of complications and postoperative morbidity. To avoid harvesting a fresh autogenous graft, the use of bone substitute materials has been proposed.

Some studies have shown that a corticocancellous allogeneic bone block, whether fresh-frozen or freeze-dried, can be used for alveolar bone reconstruction. Despite the recognized osteoconductive and possible osteoinductive potential of an allogeneic graft, no osteogenic potential can occur, because this bone is acellular. Some studies have investigated the use of bone marrow stromal stem cells to create an osteogenic environment in vivo. This was justified by the bone marrow stem cell possibly differentiating into osteoblasts in a bone site.

Despite the major aim in the field of tissue engineering toward working with a bone substitute material that has osteoconductive, osteoinductive, and osteogenic potential, there are only a few studies regarding the use of osteogenic principles associated with allogeneic bone grafts in humans. These osteogenic principles were utilized by adding bone marrow in natura or cultivated bone marrow stem cells in vitro to the allogeneic bone graft. However, these studies are clinical case reports or animal model studies; therefore, the aim of the present study was to perform a human randomized controlled clinical, tomographic, and histomorphometric...
study of alveolar bone reconstruction by using corticocancellous allogeneic bone blocks that were or were not impregnated with an autologous bone marrow aspirate.

Materials and methods

Subjects

All subjects were patients referred to the Sao Paulo Hospital. Ten patients were selected (two males and eight females), ranging in age from 40–55 and requiring bone augmentation in the anterior maxilla. All patients were missing their maxillary incisors. The patients were randomized into two groups of five patients each. Inclusion criteria were that the patients needed bone augmentation prior to implant placement in the anterior maxilla, were in generally good health, and had no contraindications to treatment. Exclusion criteria included pregnancy, immunocompromised or diabetic status, smoking, and use of medicine(s) that could interact with those prescribed in the study surgical protocol. All patients selected for the study signed an informed consent.

Surgical protocol

All patients were carefully evaluated by clinical and tomographic examinations. Before the bone augmentation procedure, the patients in the experimental group had 4.0 mL of bone marrow aspirated from the iliac by hematologists (Fig. 1). For bone marrow aspiration, a punch in the posterior upper iliac crest using a 40 x 12 mm needle was performed after local anesthesia with 2% lidocaine. To avoid blood coagulation, the bone marrow aspirate was maintained in heparin (1.0 mL).

In all recipient sites, after the administration of local anesthesia with 2% mepivacaine cloridrate with epinephrine 1:100,000, a crestal incision between the maxillary canines and two releasing incisions at the buccal distal line angles of the canines were performed (Fig. 2). A full-thickness buccal flap was elevated and bone was removed from the buccal bone plate using a 1.0 mm round bur (Fig. 3). In all patients, an allogeneous bone block was used that had been unfrozen for 30 minutes in a saline solution. These blocks were adapted to the bone defect and fixed with titanium screws (Kopp Dental Industry Products) (Fig. 4).

The patients in the experimental group were grafted with an allogeneic bone block embedded with an autologous bone marrow aspirate, while the patients in the control group received a pure allogeneic bone block, without bone marrow aspirate (Fig. 5). After a periosteal releasing incision, the flap was advanced and sutured with 5-0
nylon sutures (Fig. 6), which were removed after 10 days.

After six months, tomographic examinations were performed before implant placement. A full-thickness buccal flap was elevated, the screws were removed, and four bone cores of 7.0 x 2.0 mm were harvested from each patient with a trephine bur (Fig. 7) and prepared for histological evaluation. The four sites that were utilized for core removal were prepared for implant placement (Kopp Dental Industry Products) (Fig. 8).

**Bone loss evaluation**

In each patient, two sites, one on the right side and one on the left side, were measured for bone thickness using tomographic examinations, both at baseline and at six months postsurgery (Fig. 9). For each patient, the tomographic measurements were averaged and statistical comparisons between the control and experimental sites at baseline and six months postsurgery were performed using unpaired Wilcoxon tests. The intragroup comparisons between baseline and six months postsurgery were performed, also using paired Wilcoxon tests.

**Specimen preparation**

After being fixed for 72 hours in 10% formalin, the specimens were decalcified and embedded in paraffin, and 5.0 µm plane sections were prepared and stained using hematoxylin-eosin (H&E) (Fig. 10). Four specimens were obtained from each patient, and four plane sections were used for histomorphometric analysis.

**Histomorphometric analysis**

Four H&E-stained sections from each patient were subjected to histomorphometric evaluation using the AxioVision software (release 4.7.1, Carl Zeiss MicroImaging, LLC) and the AxioCam HRc adapted to the Axioskop 2 plus microscope (Carl Zeiss MicroImaging, LLC). The quantity of mineralized and non-mineralized tissue in each section was measured. For each patient, the H&E-stained section measurements were averaged and statistical comparisons between the control and
Table 1. Tomographic parameters comparison (in millimeters) between baseline and six months.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Six months post-surgery</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>4.3</td>
<td>8.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Control</td>
<td>4.8</td>
<td>6.9</td>
<td>0.05</td>
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Table 2. Alveolar thickness gain.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD (in mm)</th>
<th>Mean ± SD (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>4.6 ± 1.43</td>
<td>118.23 ± 56.93</td>
</tr>
<tr>
<td>Control</td>
<td>2.15 ± 0.47</td>
<td>49.91 ± 20.24</td>
</tr>
<tr>
<td></td>
<td>P = 0.002</td>
<td>P = 0.002</td>
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</tbody>
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Table 3. Histomorphometric analysis of mineralized vital bone (percentage).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Experimental</td>
<td>60.7 ± 16.18</td>
</tr>
<tr>
<td>Control</td>
<td>41.4 ± 12.5</td>
</tr>
<tr>
<td></td>
<td>P = 0.019</td>
</tr>
</tbody>
</table>

Results
A total of 40 dental implants were installed, with all of them becoming osseointegrated and receiving fixed prostheses. The tomographic measurements are presented in Table 1. A statistically significant difference was found comparing baseline and six-month values (intragroup comparison). The alveolar thickness gain is presented in Table 2.

The experimental group demonstrated a statistically significant increase in thickness as evaluated by the tomographic exams (P = 0.005), with a bone gain of 4.6 ± 1.43 mm. This represented a bone gain of 118.23 ± 56.93%. The alveolar thickness gain in the control group was lower, 2.15 ± 0.47 mm, or 49.91 ± 20.24%. A statistical difference between the groups was observed by intergroup comparison (P = 0.0002).

The histomorphometric analysis showed statistically different amounts of mineralized vital bone between groups (P = 0.019%). In the experimental group, the percentage of mineralized vital bone was 60.7 ± 16.18, while the control group had a percentage of 41.44 ± 12.5 (Table 3).

Discussion
The loss of alveolar thickness is a common finding in the edentulous anterior maxilla.1,2,4,25 Therefore, alveolar bone reconstruction frequently is required in this area to allow appropriate implant installation.6,26–28

The autogenous bone graft is considered the gold standard for bone reconstruction because it has osteoconductuve, osteoinductive, and osteogenic properties; however, the harvest of autogenous bone is associated with some degree of morbidity.29,30

To avoid the need for a donor site, the allogeneic bone graft has been used in both particulated and block form.4,6,12,13 The allogeneic graft has been used in either freeze-dried or fresh-frozen forms, with the latter recognized as a better choice in terms of bone regeneration.14,35,31 However, the fresh-frozen allogeneic bone graft has no osteogenic potential because the bone is acellular.16

The goal of alveolar tissue engineering is to obtain a material similar to that of autogenous bone, with osteogenic, osteoinductive, and osteoconductive properties but without the need for a donor site. It has been speculated that bone marrow stromal stem cells can be incorporated into bone regenerative therapy to promote osteogenesis.1,6,11,32,33

In the present study, the tomographic analysis illustrated a statistically significant increase in alveolar thickness gain when the autologous bone marrow aspirate was incorporated into the fresh-frozen allogeneic bone block. The histomorphometric analysis also showed a statistically significant increase when the aspirated bone marrow was used. A previous animal study also showed better bone reconstructive results in onlay grafts when adding autologous bone marrow to the allogeneic bone; however, those results were not statistically significant, perhaps due to the low number of specimens.25

Other studies in both animals and humans have indicated that the use of pure autologous bone marrow and cultivated bone marrow stromal stem cells in inlay defects also resulted in more bone gain when compared to a control group.1,18,34–40 These results led to the speculation that the stromal stem cells in the marrow had differentiated into osteoblasts, which allowed an osteogenic potential in the experimental group. That said, it must be remembered that bone marrow can contain a number of other factors.
that could contribute to bone regeneration, such as other cell types and several growth factors.41

There have been only a few studies regarding the materials used in the present study, so further investigation is required. The long-term survival pattern of the implants, the outcome of the newly formed bone, and the potential risks of the proposed technique need further evaluation as well.

Conclusion

The only grafting technique, using an allogeneic bone block associated with autologous bone marrow, promoted more bone formation than an allogeneic bone block used alone.

Acknowledgements

The authors would like to thank Kopp Dental Industry Products for their support of this study.

Disclaimer

The authors have no relationship with any of the manufacturers listed in this article.

Author information

Dr. Costa is a postgraduate student, School of Medicine, UNIFESP, Sao Paulo, Brazil, where Drs. Fagundes, Simoes, and Taha are professors. Dr. Pelegrine is a professor, Sao Leopoldo Mandic Dental School, Campinas, Brazil.

References


Manufacturers
Carl Zeiss MicroImaging, LLC, Thornwood, NY
800.233.2343, www.zeiss.com/micro
Kopp Dental Industry Products, Curitiba, PR, Brazil
55.41.3296.6159, www.implantkopp.com