Socket Preservation Therapy with Acellular Dermal Matrix and Mineralized Bone Allograft After Tooth Extraction in Humans: A Clinical and Histomorphometric Study

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The aim of this study was to analyze through clinical and histomorphometric parameters the use of acellular dermal matrix (ADM) with or without mineralized bone allograft (AB) on bone formation in human alveoli after a 6- to 8-month healing period. A total of 19 patients in need of extraction of the maxillary anterior teeth were selected and randomly assigned to the test group (ADM plus AB) or to the control group (ADM only). Clinical and histomorphometric measurements and histologic analysis were recorded 6 to 8 months after ridge preservation procedures. Clinical parameters and amount of mineralized and nonmineralized tissue were measured and analyzed. In the clinical measurements, the test group showed reduced bone loss in the buccopalatal dimension after 6 to 8 months (intragroup analysis P < .01). Histologic findings showed higher percentages of mineralized tissue and lower percentages of nonmineralized tissue in the test group when compared with the control group (P < .05). In this randomized controlled clinical and histomorphometric study in humans, acellular dermal matrix in association with mineralized bone allograft reduced alveolar bone loss in the anterior maxillae both in height and width after a follow-up period of 6 to 8 months. Int J Periodontics Restorative Dent 2016;36:e16–e25. doi: 10.11607/prd.2307

Preventing ridge collapse following the extraction of maxillary anterior teeth is vital to a good esthetic restorative outcome. The maintenance of the alveolar bone volume following tooth removal facilitates subsequent placement of dental implants and improves the esthetic and functional prosthetic result.1 Alveolar bone resorption after tooth extraction is an inherent condition of the healing process; it is accelerated in the first 6 months after extraction and followed by a gradual remodeling that includes changes in size and shape.2–5

Early extraction socket healing is expected to decrease the alveolar ridge by 2 to 4 mm horizontally and 1 mm vertically.6–8 A number of recently published studies carried out in animals proved that after dental extraction, healing and maturation of the alveoli occurred with dimensional changes after 3 months of healing.8,10

Ridge preservation using the guided bone regeneration (GBR) technique has been shown to improve ridge height and width dimensions when compared with
tooth extraction alone.\textsuperscript{6-8,11} It was reported in these clinical studies that implants were placed into the augmented ridges successfully, without additional bone grafting.

The general understanding is that bone graft placement in the extraction socket should offset the catabolic processes observed within the crestal ridge. Therefore, several procedures, such as the use of bone autografts, bone replacement materials, and regenerative techniques, have been proposed to prevent and correct alveolar bone resorption.

GBR is based on the principle of selecting cells using membranes to prevent epithelial proliferation.\textsuperscript{12} Some studies have shown the possibility of using acellular dermal matrix (ADM) as a biologic membrane in GBR.\textsuperscript{13-16} ADM is processed by removing the epidermis and all dermal cells; however, the complex basement membrane and the structure of collagen and elastin are preserved.\textsuperscript{17} Thereby, ADM provides structural biocompatibility when used as barrier in GBR. It functions as a scaffold for the incorporation and migration of epithelial cells, keratinocytes, and fibroblasts.\textsuperscript{12,17} The use of ADM for ridge preservation after tooth extraction has been demonstrated by successful clinical reports\textsuperscript{14,16,18,19} and histologic results.\textsuperscript{16,20}

Several biomaterials, such as hydroxyapatite, calcium sulfate, bioactive glass, and xenograft bone substitutes, have demonstrated good results when associated with membranes or ADM.\textsuperscript{20-26} Beck and Mealey\textsuperscript{27} performed a histologic evaluation of bone formation using a single bone allograft material at two different time points after tooth extraction and socket grafting and noticed that all sites examined displayed evidence of new bone formation. Other studies\textsuperscript{28-31} suggest that ridge preservation techniques using mineralized human bone allograft may promote new bone formation in the healing extraction socket.

The clinical and histologic analysis of the use of mineralized bone allograft (AB) with ADM for the treatment of ridge defects after tooth extraction has not been reported in the literature. Therefore, this study aims to analyze through clinical and histomorphometric parameters the use of both biomaterials to reduce alveolar bone loss after tooth extraction over a 6- to 8-month period.

Materials and methods

The present study was performed at the University of São Paulo School of Dentistry of Ribeirão Preto, São Paulo, Brazil, between June 2010 and June 2013. It was performed in agreement with the World Medical Association, which developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

Patients

A total of 19 patients (7 men and 12 women; age range: 25 to 68 years) were selected. The sample size provided 83% statistical power to detect differences buccopalatally, between groups, of 1.5 mm in all parameters.
evaluated. To be included in the study, the patient had to present ≥ 2 hopeless, single-rooted, nonadjacent teeth in the maxilla (Fig 1). This was intended to avoid the interference of the bone plate of one group with the healing process of the other group, since the bone plates could be in intimate contact. All patients received detailed written information about the treatment and signed an informed consent form.

Exclusion criteria were antibiotic therapy in the last 6 months, systemic involvement, smoking habits, and pregnant or lactating patients. At baseline and after 6 months the following parameters were analyzed: (1) distance from the membrane-fixating titanium pins to the palatal bone margin (external vertical palatal measurement [EVPM]), (2) distance from the pins to the buccal bone margin (external vertical buccal measurement [EVBM]), and (3) distance from the buccal alveolar bone margin to the palatal alveolar bone margin (alveolar horizontal measurement [AHM]).

For the external vertical measurements, the top of the fixation titanium pin was used as a reference point.

One week before the extractions and regeneration procedures, scaling and root planing of all teeth in the mouth was performed using ultrasonic instruments and Gracey curettes, and all patients received oral hygiene instructions.

**Surgical procedures**

In this split-mouth study, the test group had 19 sockets treated with AB (MinerOss, BioHorizons) associated with ADM (AlloDerm, BioHorizons) and the control group (blood clot) had 19 sockets treated only with ADM. The randomized allocation to the sites for the test and control groups was selected using a software program (SPSS, IBM) by a computer-generated randomly permuted block. The study design was performed as previously described in the literature.11 Surgical procedures were performed under local anesthesia, and a periotome was used for the extractions to reduce trauma to the bone. Intrasecular incisions were performed, and a full-thickness mucoperiosteal flap was elevated to expose the labial and palatal aspects of the alveolar ridge. After tooth removal, the granulation tissue was curetted. The two sockets selected for the study were treated with GBR, using ADM as membrane. Randomly, one alveoli was left filled with a blood clot (control group) and the other one was filled with AB (test group), both under the ADM (Fig 2). Furthermore, four holes were made to insert the pins, which served as a fixed point for the clinical measurements and also to fix the ADM on the buccal and palatal bone (Fig 3). Full thickness buccal and lingual flaps were repositioned and sutured with 5.0 nonresorbable sutures (Fig 4). When possible, ADM was intentionally left exposed in its central portion (1 to 2 mm) to induce an increase in the width of the keratinized tissue.
All patients received antibiotic therapy (875 mg amoxicillin and 125 mg clavulanic acid) every 12 hours for 10 days and analgesics (750 mg acetaminophen) every 8 hours for 2 days. Patients were instructed to rinse twice daily with a 0.12% chlorhexidine gluconate solution for 15 days, and the sutures were removed 2 weeks later. Temporary removable partial dentures were fabricated and installed in all patients 3 weeks after the first surgery to avoid pressure on the gingival tissues immediately after the surgery. Every 15 days during the first 2 months, and monthly, until the sixth to eighth month, patients were called for reevaluation that included prophylaxis and oral hygiene instructions.

Of the 19 patients who had taken part at baseline, 16 remained in the second phase. At 6 to 8 months after the first surgical procedure, a reentry surgery (Fig 5) was performed using the same approach previously described to repeat the measurements of the clinical parameters, collect data, and remove the titanium pins. Only 10 patients were selected for implant placement, and in these cases biopsies were harvested to realize histomorphometric analysis. After implant placement (second surgical phase), oral rehabilitation with fixed prosthesis was performed for all patients.

**Histologic preparation**

At the reentry surgery, biopsies measuring $2 \times 5$ mm were taken from the center of the preexisting extraction socket areas with a 2.75-mm trephine drill (outer diameter) in both groups. Some patients were not included in this phase of the study because the remaining bone was not sufficient for implant placement, and others did not want to continue participation. So, 10 of 16 patients were selected for the biopsies and implant placement. The biopsies were fixed using 4% formalin at pH 7 for 10 days and transferred to a 70% ethanol solution to wait for processing. They were embedded in LR White resin (London Resin); subsequently, two sections from the center of the tissue blocks were made using a microtome. One group of sections were stained with Stevenel’s blue and alizarin red S, and the other with toluidine blue for optical microscopy. With this last stain, it was possible to identify the bone tissue that was in formation during the healing process and the bone that was being deposited at the time of the biopsy.

**Histomorphometric analysis**

The bone area was determined within a rectangle that comprised the central region of the socket (frame area: 17.84 mm²), measured starting at the most cervical point of the socket. These measurements evaluated the percentage of the region occupied by mineralized bone in relation to the percentage occupied by marrow spaces.
Histologic sections from each biopsy were captured through a video camera (Leica DC300F; Leica Microsystems) joined to a stereomicroscope (Leica MZFL III). The images were analyzed using the ImageJ program to determine the area measurements (in mm²) of the mineralized tissue and the nonmineralized tissues. In the test group, the amount of residual graft particles was not measured due to its similarity to the newly formed bone. The particles were detected by the examiner due to differences in structure, but due to similarities in tone they could not be identified by the software used.

Statistical analysis

Data were recorded as mean ± SD for clinical parameters, and the experimental unit was the individual. The split-mouth design was used, and statistical analysis was performed by applying nonparametric paired tests. To compare the results obtained in the control and test groups before and after treatment, Wilcoxon signed rank test was applied. For all statistical analyses, a significance level of 5% (*P < .05) was used.

Results

Clinical findings

The surgical procedures were well tolerated by all the patients with no postoperative complications. No sockets presented exfoliation of the bone graft, indicating that the use of ADM was appropriate for graft retention at the healing phase.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>6 mo</th>
<th>Intragroup</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVBM</td>
<td>7.18 ± 1.44</td>
<td>7.32 ± 1.14</td>
<td>.4391</td>
<td>5.97 ± 0.86</td>
</tr>
<tr>
<td>EVPM</td>
<td>2.53 ± 0.55</td>
<td>2.29 ± 0.62</td>
<td>.4927</td>
<td>2.50 ± 0.88</td>
</tr>
<tr>
<td>AHM</td>
<td>7.34 ± 0.96</td>
<td>7.42 ± 1.12</td>
<td>9651</td>
<td>4.44 ± 0.78</td>
</tr>
</tbody>
</table>

*Wilcoxon signed rank test; statistically significant difference between the groups (*P < .01).

EVBM = external vertical buccal measurement; EVPM = external vertical palatal measurement; AHM = alveolar horizontal measurement.

After the clinical parameters were analyzed, the data showed no statistically significant differences between test and control groups in the initial (baseline) and the 6- to 8-month postoperative evaluation. For the baseline, test and control values were, respectively (in mm): EVBM = 7.18 ± 1.44 and 7.32 ± 1.14; EVPM = 2.53 ± 0.55 and 2.29 ± 0.62; AHM = 7.34 ± 0.96 and 7.42 ± 1.12; after 6 months, test and control values were, respectively: EVBM = 5.97 ± 0.86 and 5.44 ± 0.95; EVPM = 2.50 ± 0.88 and 2.44 ± 0.81; AHM = 4.44 ± 0.78 and 4.32 ± 1.01. At 6 months, the intragroup analysis showed a statistically significant reduction (*P < .01) for the parameters of EVBM and AHM for both groups (Table 1).

The differences obtained in each group (between the initial measurements and 6 to 8 months postoperative) were compared and showed a trend for less bone loss in the ADM plus AB group, but it was not statistically significant (Table 2).

For a better representation of the results, the intergroup analysis was subdivided into two dental groups: central incisor and lateral incisor. The central and lateral incisor data were recorded as mean ± SD and are indicated in Table 3. The
Table 3  Mean ± SD (mm) of the clinical parameters at baseline and after 6 months for the central incisor and lateral incisor groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intergroup comparison</th>
<th>Baseline</th>
<th>6 months</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td></td>
<td>Lateral</td>
<td>Central</td>
<td>P</td>
</tr>
<tr>
<td>EVBM</td>
<td>6.95 ± 1.64</td>
<td>7.44 ± 1.16</td>
<td>.1739</td>
</tr>
<tr>
<td>EVPM</td>
<td>2.60 ± 0.48</td>
<td>2.44 ± 0.59</td>
<td>.2312</td>
</tr>
<tr>
<td>AHM</td>
<td>7.00 ± 0.80</td>
<td>7.72 ± 1.02</td>
<td>.1352</td>
</tr>
</tbody>
</table>

*Wilcoxon signed rank test; statistically significant difference between the groups (P < .01).

EVBM = external vertical buccal measurement; EVPM = external vertical palatal measurement; AHM = alveolar horizontal measurement.

Table 4  Mean ± SD (mm) of the differences between initial measurements and after 6 months for two dental groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lateral</th>
<th>Central</th>
<th>P Intragroup</th>
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<tr>
<td></td>
<td>Test</td>
<td>Control</td>
<td>P intergroup</td>
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<tr>
<td>EVBM</td>
<td>0.90 ± 1.40</td>
<td>1.20 ± 1.14</td>
<td>.225</td>
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<tr>
<td>EVPM</td>
<td>−0.22 ± 0.91</td>
<td>0 ± 0.80</td>
<td>.283</td>
</tr>
<tr>
<td>AHM</td>
<td>2.60 ± 1.10</td>
<td>2.75 ± 0.90</td>
<td>.455</td>
</tr>
</tbody>
</table>

EVBM = external vertical buccal measurement; EVPM = external vertical palatal measurement; AHM = alveolar horizontal measurement.

differences obtained in each group are shown in Table 4. Percentage data for the difference between initial measurements and 6 months for both groups were also calculated and are shown in Table 5. In the test group, as these results show, the central incisor group had statistically more bone loss in the AHM and EVBM measurements when compared with the lateral incisor group.

**Histologic observations**

It was possible to observe the presence of mineralized tissue and fibrous tissue plus marrow spaces (nonmineralized) in the sections from both groups (Fig 6). Histology of the test site revealed vital bone in close proximity to the residual graft particles indicating ongoing new bone formation. Due to histology staining and the use of allogeneic graft material, both residual bone (older) and recently formed bone were dyed the same color and were intertwined in the same structure (Fig 7).

The control and test groups showed an osteoid matrix that was also identified in some areas on the external surfaces of the bone. It was paved with osteocytes in the
interior of the lamellae, and osteoclasts were present on the outer bone surface (Fig 8). Interrupted/partially resorbed lamellae and incremental lines were observed, indicating regions of new bone formation in both groups.

**Histomorphometric findings**

Analysis of the outcomes based on mineralized bone (MT) showed the following values for the control and test sides respectively: 6.42 ± 1.72 mm² and 7.72 ± 1.14 mm². For non-mineralized tissues (fibrous tissue plus marrow spaces) the measurements were 6.33 ± 1.80 mm² and 6.90 ± 1.38 mm² for control and test groups, respectively. At 6 to 8 months, the histomorphometric analysis showed 35.99% of mineralized tissue and 35.51% of nonmineralized tissue in the control group, while the test group presented 43.29% of mineralized and 38.66% of nonmineralized tissue. The results showed statistically significant differences (P < .05) between the test and control groups, as shown in Table 6.

After the rehabilitation phase, all patients were included in a maintenance program and were recalled every 6 months for a period of 2 to 3 years (Figs 9 and 10) following implant placement.

**Discussion**

This study describes clinical and histologic outcomes for two commercially available biomaterials for ridge preservation, when used as membrane and socket regenerative materials, for posterior implant...
placement. GBR has been used successfully to prevent alveolar ridge deformities. A number of materials, nonresorbable and resorbable, have been used as membranes, with similar results in terms of bone formation. The ideal barrier should be made of material that is less susceptible to membrane exposure or that cannot be significantly colonized by periodontopathogenic bacteria when exposed to the oral cavity. The present study shows that the technique of GBR with ADM was able to reduce initial bone resorption, since there was resorption of 2.94 mm for the test group and 3.18 mm for the control group on the horizontal aspect, and 1.41 mm and 1.97 mm for the test and control groups, respectively, of the buccal plate.

In a recent animal model study, Blanco et al showed that remodeling after extraction was also more pronounced on the buccal bone plate than that on the palatal aspect. Thus, the authors demonstrated that in cases treated with flap surgery, the average bone resorption obtained on the buccal bone plate was 1.33 mm, compared with 0.33 mm on the palatal.

The presence of tooth and the functional supporting tissues (cementum, periodontal ligament, and bone) play a crucial role in maintaining the dimensions of the alveolar process. Alveolar deformities resulting from tooth loss can cause esthetic and functional problems, especially in the anterior maxilla, which could impair and compromise the prosthetic rehabilitation with implants or conventional prostheses.

In the present study, mineralized bone allograft was used in association with ADM. The data show that the graft (MinerOss) efficiently
reduced bone loss of the buccopalatal dimension, since the width of the ridge (AHM) went from a reduction of 2.94 ± 1.01 mm for the test group and 3.18 ± 1.11 mm for the control group to 2.53 mm for the test group and 3.40 mm for the control group. It was also observed that there was less bone loss in the vertical alveolar ridge (EVBM) in the test group when compared with the control group (1.41 ± 1.33 mm and 1.97 ± 1.33 mm, respectively) showing that the technique of GBR with ADM was able to reduce initial bone resorption.

The use of ADM plus AB in postextraction alveoli to reduce ridge deformities and to induce bone formation within the alveoli was also evaluated. The histomorphometric analysis showed 43.29% of mineralized tissue for the grafted sockets and 35.99% for the control group, and 38.66% of nonmineralized tissue for the test and 35.51% for the control group. It is important to emphasize that the lower percentage of the area of total bone formation is due to socket healing beginning with bone formation from the lateral walls and moving to the center of the alveoli with time; in addition, it forms from the apical region upward. This pattern and the time of analysis together explain in part the results obtained, since biopsies were taken from the center of alveoli, the last area to heal.

The basic principle of GBR is the isolation of epithelial and connective tissue cells from the bone defects. ADM, which has a tendency to collapse into larger bone defects. To maintain space and to act as a scaffold for cell migration, proliferation, and differentiation, an association of bone grafts and GBR is suggested.

Gapski et al suggest that the same bone allograft used in this study can be successfully used in sinus-lifting procedures, since histology reported from bone biopsy core samples revealed newly formed bone with a well-organized lamellar bone structure with remaining particles observed in contact with surrounding newly formed bone. An allograft paste composed of osteoinductive demineralized bone matrix was used in another study and was able to prevent ridge resorption and promoted bone maturation compared with extraction sites alone. The histologic analyses reported a mean of new bone formation of 37.4% for the test sites and 35.5% for the control sites. Thus, although the data in this study showed no statistically significant difference for the group with the presence of AB, the association of the bone graft and the ADM provided a clinical perception of denser bone during implantation.

Conclusions

In this randomized controlled clinical and histomorphometric study in humans, ADM in association with mineralized bone allograft reduced alveolar bone loss in the anterior maxillae both in height and width after a follow-up period of 6 to 8 months.

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References


