Use of Autogenous Tooth-Derived Mineralized Dentin Matrix in the Alveolar Ridge Preservation Technique: Clinical and Histologic Evaluation

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This study clinically and histologically evaluated the new bone formation and soft tissue changes when an autogenous tooth-derived mineralized dentin matrix (DDM) graft covered with a free gingival graft (FGG) was used for alveolar ridge preservation, as compared to spontaneous healing. Using a split-mouth protocol, 14 consecutive patients who required two extractions of a single-rooted tooth in the maxillary arch were enrolled. In each patient, one extraction site was treated with DDM and FGG (test group), while the other extraction site was covered with FGG and healed spontaneously (control group). In both test and control sites, implant placement was performed after a 16-week healing period. Compared to baseline (immediately after tooth extraction), both treatments yielded statistically significant differences in some clinical parameters and in the bone micro-architecture within the augmented sites. However, the use of DDM with the FGG created greater new vital bone formation, more newly formed bone, and fewer dimensional tissue changes than spontaneous healing with FGG.


After tooth loss, there is a 25% decrease in ridge volume in the first year, which becomes a 40% to 60% loss during the first 3 years.¹⁻³ In most cases, this is from a buccal aspect rather than a palatal aspect at 4 to 6 months after tooth extraction.⁵

In the last few decades, different techniques have been proposed to counteract postextraction ridge resorption, often combined with barrier membranes.⁶⁻⁸ However, no specific protocol for alveolar ridge preservation has been demonstrated to be superior to others.⁹ A number of studies have evaluated the efficacy of different socket grafts using bone substitute biomaterials, including autologous bone and bone substitutes (such as allografts, alloplasts, and xenografts).¹⁰,¹¹

In the last few years, encouraging results have been reported with the use of an extracted tooth as an alternative to autogenous bone, as it is a biomaterial that can provide autogenous graft while eliminating the need for a secondary bone-harvest site. Tooth-derived mineralized dentin matrix (DDM) graft has been studied to learn whether teeth represent a viable alternative. More specifically, DDM showed similar composition to bone¹²,¹³ and was a viable option for alveolar bone augmentation following tooth extraction.¹⁴,¹⁵

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Submitted January 10, 2022; accepted February 19, 2022.
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Although the mineral content of bone grafts is believed to provide osteoconductivity and space-maintenance properties, dentin contains several growth factors (including transforming growth factor-beta, insulin-like growth factor-II, and bone morphogenetic protein-216), which could be pivotal for healing the alveolar socket’s soft and hard tissues, especially when covered with a free gingival graft (FGG). In light of these findings, the present study aimed to evaluate histologic and clinical healing outcomes of a surgical protocol that combined DDM and FGG for alveolar ridge preservation prior to implant placement.

Materials and Methods

Study Design

Fourteen patients (6 men, 8 women) aged 37 to 62 years (mean age: 48.2 years) who required two extractions of a single-rooted tooth in the maxillary arch were recruited for the study between June 2019 and July 2021. Each patient was informed about the study’s possible risks, and each one provided informed written consent.

The exclusion criteria were as follows: (1) Any systemic condition that might affect the study; (2) pregnancy; (3) previous or current radiation or immunosuppressive therapy; (4) medication by anti-inflammatory and immunosuppressive drugs; (5) previous history of excessive drinking; (6) smoking; and (7) lack of opposite occluding dentition in the area intended for extraction and subsequent implant placement. Moreover, participants were excluded if more than 50% of the buccal bone plate was not present after tooth extraction.

The inclusion criteria were as follows: (1) 18+ years of age; (2) no history of systemic diseases that would contraindicate oral surgical treatment; (3) absence of active periodontal disease with good plaque control; and (4) having been scheduled for a subsequent implant-supported restoration. After admission to the study, each patient was given supplemental oral hygiene instructions and underwent full-mouth supragingival scaling by ultrasound and/or hand instrumentation.

Surgical Protocol

Tooth extractions were performed, taking great care to preserve the buccal bone plate and surrounding soft tissues. Before surgery, standardized periapical radiographs of the extraction sites were taken, study casts were made, and clinical photographs were taken. At each surgical site, after local anesthesia using mepivacaine with adrenaline (1:100,000), a mucoperiosteal envelope flap including the adjacent teeth was reflected no more than 2 mm beyond the bone crest, and the tooth was extracted using a minimally invasive technique (Fig 1).

After surgery, the patient’s two extraction sites were randomized into the different treatment groups: DDM covered with FGG (test) or spontaneous healing covered with FGG (control). Each patient had one test site and one control site. Before any evaluation, an investigator (S.T.) who was not involved in...
recording and processing the data assigned the sealed envelopes by marking the patient’s initials and date of birth and the designated group of each tooth. The allocation concealment was performed through serially numbered sealed envelopes, and the details of the sequence were not identified to the clinician who performed the test group treatment. Subsequently, another clinician who performed the implant placement and surgical procedures opened the envelope to view the patient’s assigned group. At the appropriate randomized time, the patient returned for implant surgery.

For test group sites, a clinician (S.D.T.) cleaned the whole extracted tooth using a diamond drill (no. 6855, Dentsply Maillefer) with abundant water irrigation. The extraction socket was thoroughly curetted and irrigated with a sterile saline solution. In each patient, the test-group socket was subsequently filled with DDM. Any filling materials (gutta-percha, composite, etc) were carefully removed from the tooth. After, the tooth was cut into small pieces (10 × 10 mm), a milling device (Tooth Transformer, Biomax) was inserted. According to the manufacturer’s instructions, a single-use box containing a disposable liquid solution was inserted in the device to guarantee graft decontamination (Figs 1b and 1c). After 25 minutes, particle graft bio-materials were obtained, placed in the alveolar postextraction socket sites, and finally covered with an FGG harvested from the patient’s hard palate (Fig 2). A buccal-palatal resorbable suture (5-0 Vicryl Plus, Ethicon, Johnson & Johnson) was placed over the wound to stabilize the FGG and to allow tension-free flap closure (Fig 2b).

Patients in the control group had the extraction sites covered with FGG, and the sites were left to heal spontaneously.

All patients received oral hygiene instructions and were told to continue antimicrobial therapy consisting of chlorhexidine mouthwash twice a day for 14 days and amoxicillin (1 g) twice a day for 3 days.
postsurgery. Postoperative pain and edema were controlled with ibuprofen (200 mg) taken orally every 12 hours for the first 2 days.

Implant Placement

After 16 weeks of healing (Fig 3), implants were placed. After flap elevation and before implant placement, a core biopsy sample was obtained using a trephine with an external diameter of 3.5 mm and an internal diameter of 2.5 mm. The implant placement procedure was standardized. An implant at least 4.0 mm in diameter was placed into the grafted alveolus in the middle of the socket, and a tension-free flap was replaced to obtain primary soft tissue closure (Figs 4a and 4b).

In both groups, patients received final prosthetic restorations 3 months after implant placement (Fig 4c). Patients were recalled for follow-up every 6 months. The peri-implant marginal bone levels were evaluated on intraoral radiographs 1 year after final prosthetic restoration.

Measurements

The buccopalatal alveolar width was intrasurgically measured at the center of both buccal and palatal walls, 1 mm apical from the crest, using a manual caliper. After tooth extraction, the vertical distance from the center of the buccal and the palatal alveolar crest (AC) to a reference periodontal probe that connected the cementoenamel junction (CEJ) of the adjacent teeth was recorded by a periodontal probe. Measurements were taken at baseline (immediately after tooth extraction) and at 16 weeks after tooth extraction, when the bone core biopsy samples were harvested.

Histologic Analysis

The bone core biopsy samples were placed in sample holders filled with 4% formaldehyde solution in 0.1 M phosphate-buffered saline (pH 7.3) and were stored at 4°C. Bone core biopsy material was fixed in 10% buffered formaldehyde solution. Following dehydration, the biopsy material was embedded in paraffin, and 6-mm sections were prepared. The sections were stained with routine hematoxylin-eosin stain. For qualitative and morphologic analysis of the modeling process, the stained preparations were examined under a light microscope (Axioplan, Zeiss) at ×50 magnification, and the entire section was evaluated. Ten digital images of each section were acquired and used to trace the areas identified as vital bone, biomaterial particles, and connective tissue. The two central-most sections were obtained from each specimen for histomorphometric analysis.

Statistical Analysis

Two-sample Student t test was performed to analyze the clinical ridge dimensional changes and the histologic parameter changes between the two treatment groups. Moreover, Mann-Whitney U test was performed to confirm findings from Student t test, while Spearman rank
and Pearson correlation tests between clinical and histologic parameters were also used. *P* < .05 was considered statistically significant.

**Results**

All enrolled patients completed the study successfully, with uneventful clinical healing and without infection. Both the control and test sites in each patient were included to obtain clinical and histologic data.

**Clinical Results**

With regard to the clinical parameters study sites, the groups were similar at baseline. Further baseline comparison between groups showed that there were no significant differences in the mean buccopalatal thickness in the control group (1.12 ± 0.24 mm) compared to the test group (1.16 ± 0.14 mm) (*P* = .13).

For the ridge dimensional changes after surgical reentry at 16 weeks, no significant difference was found between groups (*P* = .23) in the change of the vertical buccal distance (CEJ–AC; control: 2.32 ± 0.17 mm; test: 2.44 ± 0.24 mm) and in the vertical palatal distance (CEJ–AC; control: 2.39 ± 0.23 mm; test: 2.34 ± 0.21 mm) (Table 1). Moreover, there was no significant difference (*P* = .16) in the buccopalatal alveolar width (control: 1.11 ± 0.19 mm; test: 1.15 ± 0.17 mm) between groups (Table 1).

**Histologic Results**

In the test group, vital bone was identified as areas with osteocytes occupying lacunae and was most often woven. Residual graft particles were identified as areas of primarily lamellar bone, and no osteocytes were occupying the lacunae. Apposition of new vital bone on residual graft particles was often noted, which comprised loose fibrovascular connective tissue (Fig 5a). The intergranular tissue showed no signs of inflammatory reactions.

In the control group, the bone resorption was natural, and bone particles in bulked distribution were embedded in coarse connective tissue with low apposition of vital bone on the overview sections of the core biopsy samples surrounded by newly formed connective tissue. Resorption of lacunae with large, multinucleated osteoclasts were seen, and osteoblasts adjacent to osteoid tissue was visible at the remodeling sites. Intergranular tissue showed no signs of inflammatory reaction (Fig 5b).

Table 2 shows the results of the histomorphometric analysis. There was a statistically significantly different percentage of new vital bone between the control and the test group (*P* = .047). Vital bone was 30.22% ± 14.48% in the control group compared to 34.23% ± 13.56% in the test group. Regarding connective tissue, the control group showed a mean of 29.23% ± 10.16% compared to 27.36% ± 9.65% in the test group. Furthermore, the mean percentage of residual grafts in the test group was 19.61% ± 11.49%.

**Discussion**

This study analyzed the effects of alveolar ridge preservation with DDM graft covered with FGG vs spontaneous healing covered with

<table>
<thead>
<tr>
<th>Table 1 Clinical Results of the Control and Test Groups</th>
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<tr>
<td><strong>Buccopalatal alveolar width, mm</strong></td>
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<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>Baseline</td>
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<tr>
<td>16 wk</td>
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CEJ = cementoenamel junction; AC = alveolar crest. Results are presented as mean ± SD.
FGG on new bone formation. Compared to the control group, the test group showed statistically significantly more new vital bone before implant placement. There were no significant differences in ridge dimensional changes between treatment groups.

Authors have pointed out that a 1.9-mm horizontal loss occurred regardless of treatment method, and other studies have reported similar results in addition to the finding that the original ridge contours were not completely preserved. Buccal bone was shown to be one of the most critical factors when trying to achieve satisfactory long-term results. Therefore, it appears that a staged surgical approach including bone augmentation is necessary to obtain positive results.

With regard to the ridge dimensional changes, no significant difference was found in the vertical, palatal, and buccal distances nor in the buccopalatal alveolar width. In line with the present results, some studies have reported that alveolar ridge preservation with the use of a graft is more effective at limiting physiologic ridge reduction than spontaneous healing alone.

In this regard, Kim et al demonstrated that the inorganic and organic composition of dentin-dominated by Type I collagen fibers and presenting noncollagenous proteins (such as phosphoproteins, osteocalcin, proteoglycans, and glycoproteins) in its organic matrix—could represent a valid bone substitute with good potential for successful results in terms of osteoconductive and osteoinductive properties. Based on the pivotal observations by Kim et al, the current study was designed to further analyze the clinical and histomorphologic effects of DMM covered with FGG on alveolar ridge preservation.

In agreement with previous studies, the present results showed an average new vital bone formation.

### Table 2 Histologic Results of the Control and Test Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vital bone, %</th>
<th>Residual graft, %</th>
<th>Connective tissue, %</th>
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<tbody>
<tr>
<td>Control</td>
<td>30.22 ± 14.48</td>
<td>–</td>
<td>29.23 ± 10.16</td>
</tr>
<tr>
<td>Test</td>
<td>34.23 ± 13.56*</td>
<td>19.61 ± 11.49</td>
<td>27.36 ± 9.65</td>
</tr>
</tbody>
</table>

Results are presented as mean ± SD, determined from values at 16 weeks postextraction.

*P < .05 (control vs test group).
formation of 30.22% in the control group and 34.23% in the test group. In this regard, an important role was also played by the FGG, which ensured the presence of connective and vascular tissues and guaranteed alveolar socket healing.

The soft tissue augmentation obtained in the present study may have been due to patient characteristics of keratinized gingiva at the extraction time (as most patients presented with a sufficient amount prior to extraction), a minimal mucoperiosteal flap elevation, and the absence of periodontal disease.27–31

Conclusions

Several bone substitutes have been analyzed and compared for use in alveolar ridge preservation techniques in the last few decades. The present results indicate that there was significantly greater new vital bone formation when a xenograft protocol (DMM covered with FGG) was used for alveolar ridge preservation rather than spontaneous healing with FGG alone. This combined approach offered promising results. Further studies with a larger cohort are needed to better understand the role and potential benefits of using DMM with FGG for alveolar ridge preservation.

Acknowledgments

This work was performed with institutional funding only. The authors declare no conflicts of interest.

References


