Alveolar Ridge Preservation Using Tridimensional Collagen Matrix and Deproteinized Bovine Bone Mineral in the Esthetic Area: A CBCT and Histologic Human Pilot Study

Daniele Cardaropoli, DDS¹
Lorenzo Tamagnone, DDS²
Alessandro Roffredo, DDS²
Andrea De Maria, DDS²
Lorena Gaveglio, DDS, MS²

Bone remodeling following tooth extraction is associated with horizontal (up to 63%) and vertical (up to 22%) bone loss.¹ This process can negatively affect the esthetic outcome and increase the need for additional bone and soft tissue augmentation if implant placement is planned. To this end, ridge preservation is recommended to maintain the three-dimensional ridge volume of the alveolar ridge and to support subsequent implant treatment procedures.²⁻⁸

Application of a xenogeneic collagen matrix (CM) together with deproteinized bovine bone mineral blended with 10% collagen (DBBM-C) into a fresh extraction socket seems to be a promising technique for ridge preservation, as the matrix may be applied in a circular shape for the sealing of extraction sockets in combination with DBBM-C.⁷ However, while there is much evidence that DBBM-C is efficient in ridge preservation,⁵⁻⁸ clinical data on the healing process using CM are still limited.⁹ The present study aims to gain more insights into the healing and bone dynamics of intact extraction sockets in the esthetic area on application of CM together with DBBM-C.
Materials and Methods

Study Design

A total of 12 consecutive adult patients (aged 51.08 ± 8.62 years, 5 women and 7 men) requiring extraction of a single tooth in the esthetic area of the upper arch (from first premolar to first premolar) and subsequent implant placement were enrolled in the study. The study was performed during a period of 18 months in a private practice in Torino, Italy. Only systemically healthy subjects with an intact buccal bone wall of the extraction socket were included. Heavy smokers (≥ 10 cigarettes per day), diabetics, and individuals who received oral bisphosphonate drugs were excluded. The study protocol was approved by the PRO-ED ethical committee (Torino, Italy). The study was conducted in accordance with Helsinki Declaration of 1975, as revised in 2008. All participants signed the written informed consent form and concluded the study. All measurements were performed by a single clinician (A.R.) different from the surgeon (D.C.). A blinded examiner (L.T.) evaluated all measurements.

Surgical Procedure

One representative clinical case from the 12 treated patients is shown in Fig 1. After atraumatic tooth extraction, using a minimally invasive flapless approach leaving the soft tissues intact, the socket was filled with DBBM-C (Bio-Oss Collagen, Geistlich) to the marginal/coronal portion of the alveolar bone. DBBM-C was covered with CM (Mucograft Seal, Geistlich). CM was secured with six single interrupted nonresorbable sutures (5/0 PTFE, Omnia) as follows: buccal, buccomesial, linguomesial, lingual, distolingual, and buccodistal. CM was left intentionally exposed for an open-healing process to avoid the closure of a flap over the grafted socket and the shifting of the mucogingival junction. Patients received antibiotics for 6 days (1 g amoxicillin plus clavulanic acid, every 12 hours), and rinsed with 0.2% chlorhexidine digluconate for 14 days. At day 14, the sutures were removed. If the soft tissues over the graft were not completely closed, patients continued to rinse with chlorhexidine digluconate until complete re-epithelization closure was achieved. In all cases, complete soft tissue closure was achieved before the end of the fourth week after extraction.

Data Collection and Radiographic Examination

At baseline, immediately after tooth extraction and socket grafting (T₀), and after 4 months of healing (T₁), a cone beam computed tomography (CBCT) evaluation was performed using a 5 × 5-cm FOV and 90-µm cuts (CS 9300, Carestream), and the following radiologic measurements were performed: (1) height of the alveolus at the buccal (BH) and lingual (LH) aspect at the level of the graft and of the crest; (2) horizontal ridge width (HW) at three levels (1, 3, and 5 mm below the most coronal aspect of the crest); and (3) thickness of the buccal bone plate (BBP) at three levels (1, 3, and 5 mm below the buccal bone crest) (Fig 2). BBP measurements were only performed at baseline. To perform radiographic measurements and comparisons, CBCT scans at baseline and at 4 months were converted to 3D volumetric label maps (segmentations) using an open source software package (ITK-SNAP version 3.4.0). Through segmentation, the anatomical regions of interest were defined. The corresponding voxels registration was performed, and 3D surface mesh models were obtained for quantitative and qualitative evaluations (3D Slicer version 4.6.2). Volumetric measurements were performed using a specific 3D software (Cloud-Compare, version 2.8).

Specimen Processing for Light Microscopy Analysis

At the 4-month visit (corresponding to implant placement), a biopsy specimen was harvested for each augmented extraction socket in the site of implant placement previously grafted. The bone core biopsies of the regenerated areas were fixed in 10% formalin and processed for histologic analyses. The specimens were dehydrated in an ascending series of alcohols and embedded in London White resin (LR White Resin). After polymerization, the specimens were sectioned along their longitudinal axis with a high-precision diamond disk at 150 µm and ground to approximately 40 µm
with a grinding machine (Micromet, Remet). The undecalcified ground sections were stained with acid fuchsin and toluidine blue and analyzed with a light microscope (Nikon Eclipse, Nikon). Histomorphometric data were measured as percentages of the total sample area and included proportion of new bone, proportion of residual graft material, and nonmineralized fraction (connective tissue plus bone marrow).

Statistical Analysis

Metric parameters were analyzed for arithmetic mean and SD. Data analysis was performed via Wilcoxon signed rank test using SPSS version
Spearman rank correlation coefficient was used to analyze a correlation between the thickness of BBP at baseline and horizontal remodeling of the ridge height (HW at T0 - HW at T1) at three different levels (1, 3, and 5 mm) 4 months postsurgery. A P value of .05 was regarded as significant.

Results

Healing was uneventful in all patients that completed the study without any complication according to the protocol. Complete soft tissue closure was observed in all patients no later than 4 weeks after extraction, confirming the advantages of CM when using the open-healing approach to avoid flap mobilization.

Radiographic Examination of Ridge Dimensions

Ridge alterations, examined using CBCT at baseline (immediately after tooth extraction and socket grafting) and after 4 months, are reported in Table 1. At the level of the graft, the mean difference between T0 and T1 was 1.35 ± 1.34 mm (12.14% ± 10.01%) on the buccal side and 1.42 ± 1.47 mm (13.08% ± 11.29%) on the lingual side. At the level of the crest, the mean height at the buccal site was 8.87 ± 2.64 mm at T0 and 8.75 ± 2.69 mm at T1 (difference of 0.11 ± 0.07), corresponding to a 98.64% preservation of the buccal alveolar height. At the lingual site, the mean alveolar height was 8.63 ± 2.30 mm at T0 and 8.58 ± 2.33 mm at T1, resulting in 99.42% preservation of alveolar ridge height at the lingual aspect.

The horizontal ridge width at 1 mm (HW-1) was 8.32 ± 1.11 mm at baseline and 7.69 ± 1.19 mm at 4 months, with a difference of 0.62 ± 0.32 mm (7.68% ± 4.52%). HW-3 was 8.35 ± 1.20 mm at T0 and 7.94 ± 1.07 mm at T1, resulting in a difference of 0.40 ± 0.26 mm (4.76% ± 2.80%). HW-5 was 8.59 ± 1.54 mm at T0 and 8.48 ± 1.55 mm at T1, with a difference of 0.10 ± 0.09 mm (1.27% ± 1.07%). Accordingly, the preservation of the horizontal width of the alveolus after tooth extraction was 92.32% at HW-1, 95.24% at HW-3, and 98.73% at HW-5.

Table 1 Radiographic Examination of Ridge Dimensions

<table>
<thead>
<tr>
<th></th>
<th>Baseline (T0) (mm)</th>
<th>4 mo (T1) (mm)</th>
<th>T0 – T1 (mm)</th>
<th>T0 – T1 (%)</th>
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<tbody>
<tr>
<td>BH graft</td>
<td>10.36 ± 3.30</td>
<td>9.01 ± 2.66</td>
<td>1.35 ± 1.34</td>
<td>12.14 ± 10.01</td>
</tr>
<tr>
<td>LH graft</td>
<td>10.29 ± 2.70</td>
<td>8.87 ± 2.24</td>
<td>1.42 ± 1.47</td>
<td>13.08 ± 11.29</td>
</tr>
<tr>
<td>BH crest</td>
<td>8.87 ± 2.64</td>
<td>8.75 ± 2.69</td>
<td>0.11 ± 0.07</td>
<td>1.48 ± 1.18</td>
</tr>
<tr>
<td>LH crest</td>
<td>8.63 ± 2.30</td>
<td>8.58 ± 2.33</td>
<td>0.05 ± 0.09</td>
<td>0.68 ± 1.58</td>
</tr>
<tr>
<td>HW-1</td>
<td>8.32 ± 1.11</td>
<td>7.69 ± 1.19</td>
<td>0.62 ± 0.32</td>
<td>7.68 ± 4.52</td>
</tr>
<tr>
<td>HW-3</td>
<td>8.35 ± 1.20</td>
<td>7.94 ± 1.07</td>
<td>0.40 ± 0.26</td>
<td>4.76 ± 2.80</td>
</tr>
<tr>
<td>HW-5</td>
<td>8.59 ± 1.54</td>
<td>8.48 ± 1.55</td>
<td>0.10 ± 0.08</td>
<td>1.27 ± 1.07</td>
</tr>
</tbody>
</table>

At baseline and after 4 months, buccal (BH) and lingual (LH) height of the alveolus at the level of the graft and crest, as well as horizontal ridge width (HW) at three levels (1, 3, and 5 mm) below the most coronal aspect of the crest were measured.
correlation was found between the initial thickness of the buccal bone and the horizontal alveolar bone loss after 4 months at 1 mm ($r = -0.209$), at 3 mm ($r = -0.18$), or at 5 mm ($r = -0.181$) (Table 2).

Histomorphometric Evaluation

Histologic evaluation of biopsy specimens showed new bone formation without any signs of inflammation or fibrous encapsulation. No inflammatory signs were found in the soft tissue area of the biopsy samples, and no CM remnants were detected (Fig 3). The histomorphometric analysis revealed an average new bone formation of $44.92\% \pm 6.67\%$ and proportion of residual graft of $13.92\% \pm 6.83\%$ 4 months after tooth extraction. Thus, the mineralized fraction averaged $58.83\% \pm 10.89\%$, whereas the nonmineralized fraction was $41.17\% \pm 10.89\%$ (Fig 3).

### Discussion

The present study was designed to gain more insight into the healing of extraction sockets with intact walls in the esthetic area after application of CM together with DBBM-C.

Using a minimally invasive flapless procedure, complete soft tissue closure was achieved in all patients, demonstrating that CM may enhance wound healing with an open-healing approach.9 The use of DBBM-C confirms the osteoconductive potential of this xenograft in oral bone regeneration procedures.10–13

Three-dimensional radiographic examination of ridge dimensions at the day of tooth extraction and 4 months later revealed nearly stable horizontal ridge width (from 92% to 99%) and height (from 98% to 99%). These results confirm the outcomes obtained in a clinical trial where a similar approach with CM in conjunction with DBBM-C was used.7 For successful placement of an implant with a diameter of 4 mm, a ridge width of at least 6 mm was shown to be required.14 In the present study, the horizontal ridge width was around 8 mm. This indicates that application of CM together with DBBM-C allows maintenance of adequate ridge width necessary for implant insertion in the esthetic area.

Implant placement is particularly demanding in the esthetic area because buccal walls commonly are thin. The thickness of the buccal bone plate here measured at baseline (0.7 to 1.2 mm) was comparable with the physiologic thickness of the buccal walls reported in the literature.15,16 In the present study, no correlation between initial thickness of the buccal bone plate

<table>
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<tr>
<th>Measuring point below the buccal bone crest (mm)</th>
<th>Thickness of the buccal bone plate at baseline (mm)</th>
<th>Spearman rank correlation coefficient (BBP/HW-3 T0–T1)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.74 ± 0.23</td>
<td>-0.209</td>
<td>.515</td>
</tr>
<tr>
<td>3</td>
<td>0.97 ± 0.37</td>
<td>-0.018</td>
<td>.956</td>
</tr>
<tr>
<td>5</td>
<td>1.24 ± 0.68</td>
<td>-0.181</td>
<td>.574</td>
</tr>
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Table 2 Thickness of the Buccal Bone Plate and Spearman Rank Correlation Coefficient

Thickness of the buccal bone plate (BBP) was measured 1, 3, and 5 mm below the buccal bone crest. The correlation between the thickness of BBP at baseline and horizontal remodeling of the ridge height (HW T1 - HW T0) at three different levels 4 months postsurgery was analyzed using Spearman rank correlation coefficient. T0 = baseline; T1 = 4 months.

Fig 3  Screenshot of the volumetric comparison performed on 3D imaging software.
and alveolar bone loss was found, confirming that preservation of the alveolar ridge is independent of the initial thickness of the buccal bone in grafted sockets.\textsuperscript{17,18} In contrast, ungrafted sockets with spontaneous healing have shown a strong negative correlation between the initial thickness of the buccal bone and alveolar bone loss.\textsuperscript{17} In summary, ridge preservation using CM in conjunction with DBBM-C can compensate for alveolar bone contraction, regardless of the initial buccal wall thickness.

The histologic evaluation showed new bone formation and a low amount of residual graft material after 4 months. The soft tissue healing was uneventful, without any sign of inflammation and with no CM remnant. These data point to an efficient replacement of grafted material by new bone after the healing period, confirming the outcomes of previously published studies with similar dynamics of bone remodeling and almost complete incorporation of xenograft particles in the new bone.\textsuperscript{8,19–22} Although this study provides evidence on the efficacy of CM together with DBBM-C in ridge preservation after tooth extraction in the esthetic area, there are still some limitations due to the exploratory nature of the study in a clinical setting, the absence of a control group (eg, spontaneous healing), and the small number of patients included. Considering these limitations, the advantages of CM in conjunction with DBBM-C need to be proven by further clinical investigations.

**Conclusions**

Within the scope of the present study and considering the limited sample size, the application of CM together with DBBM-C was shown to efficiently maintain and preserve the original ridge volume of intact sockets in the esthetic area. Complete soft tissue closure points to the special suitability of the minimally invasive flapless approach for ridge preservation using CM and DBBM. In summary, synergistic effects of DBBM-C and CM can promote efficient alveolar ridge preservation.

**Acknowledgments**

The authors reported no conflicts of interest related to this study.
References


