Histologic Evaluation of Soft and Hard Tissue Healing Following Alveolar Ridge Preservation with Deproteinized Bovine Bone Mineral Covered with Xenogenic Collagen Matrix

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The purpose of this study was to histologically evaluate new bone formation and dimensional soft tissue changes of two different healing protocols (16 weeks and 32 weeks) using deproteinized bovine bone mineral (DBBM) covered with collagen matrix (CM) for alveolar ridge preservation in the anterior esthetic zone prior to dental implant placement. Compared to baseline, both treatments yielded statistically significant differences in several clinical parameters and in the microarchitecture of the native bone and in the newly formed bone in the augmented sites. However, the protocol at 32 weeks determined greater new vital bone formation and fewer dimensional tissue changes. Int J Periodontics Restorative Dent 2018;38:737–745. doi: 10.11607/prd.3565

Loss or extraction of single or multiple teeth results in marked qualitative and quantitative alterations of the alveolar ridge process.¹ This condition appears to be progressive and irreversible, and the alveolar socket will commonly decrease in volume and show morphologic changes.² During the first year after tooth loss, there is a 25% decrease in the volume of the ridge, and its width reduces by 40% to 60% during the first 3 years.³,⁴ Furthermore, ridge atrophy in most cases becomes more pronounced from a buccal than from a palatal aspect,⁵,⁶ and this may be due to the loss of bundle bone that results in the loss of a portion of the buccal plate. Previous studies have shown that an average of 3.7 mm or 45% of horizontal ridge width is lost within a 4- to 6-month period after a tooth extraction.⁷,⁸ Ridge height, on the other hand, is less affected, and on average only 1.6 mm is lost.⁹,¹⁰

A number of techniques have been proposed to counteract postextraction ridge diminution, including the use of implants and bone grafts and various bone substitutes placed in the fresh extraction socket, often in combination with barrier membranes to ensure that adequate ridge width is preserved to allow implant placement.⁹,¹¹,¹² However, no protocol for alveolar ridge preservation has been proven superior to others.¹³
Over the past two decades, multiple studies evaluating the efficacy of different socket-filling approaches have been conducted. In these studies, many biomaterials have been studied, including autologous bone, bone substitutes (allografts, xenografts, and alloplasts), autologous blood-derived products and bioactive agents. Many reports indicate that autogenous bone still represents the gold standard; however, morbidity related to the donor region is a great disadvantage of this biomaterial. As an alternative to autogenous bone, encouraging results have been reported using xenograft bone for alveolar ridge preservation, which has been demonstrated to be useful as a scaffold and to promote bone growth, primarily through its osteoconductive activity. Of these, deproteinized bovine bone mineral (DBBM) has been successfully used in several studies to preserve ridge dimensions following tooth extraction. Other studies, however, have indicated an incomplete integration and incomplete reabsorption when DBBM was used. Additionally, a xenogenic collagen matrix (CM), a resorbable three-dimensional matrix designed specifically for soft tissue regeneration, showed promising results in regeneration of keratinized gingiva and as a graft for socket seal in ridge preservation procedures. With its two functional layers, CM has shown that it favors the stabilization of a blood clot, promotes cell ingrowth and early vascularization with one porous layer, and accelerates soft tissue healing with the other layers.

In light of these findings, the aim of the present case series was to further evaluate the influence of a surgical protocol with the use of DBBM and CM in the histologic healing outcomes of fresh extraction socket in the anterior esthetic zone prior to dental implant placement.

Materials and Methods

A total of 16 patients (7 men and 9 women; aged 37 to 62 years, mean age: 48.2 years), requiring a single rooted tooth extraction in the anterior area, were recruited for the study between January 2013 and December 2014. Each patient was informed about the possible risks of the study, and each provided informed written consent. The institutional ethical committee of the University of Messina approved the study protocol (#24/14 and #35/17).

The inclusion criteria were as follows: (1) aged > 18 years, (2) no history of systemic diseases that would contraindicate oral surgical treatment, (3) absence of active periodontal disease with good plaque control, and (4) scheduled for a subsequent implant-supported restoration.

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) currently taking anti-inflammatory or immunosuppressive drugs, (5) previous history of excessive drinking, (6) smoking, or (7) lack of opposite occluding dentition in the area intended for extraction and subsequent implant placement.

After admission to the study, the patients were given supplemental oral hygiene instructions and underwent full-mouth supragingival scaling with ultrasound and/or hand instrumentation. Before surgery, a standardized periapical radiograph of the extraction site, study casts and clinical photographs were carried out in each patient.

Surgical Protocol

Tooth extraction was performed with great care to preserve the buccal bone plate and the surrounding soft tissues. 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 (Figs 1 and 2). Participants were excluded if more than 50% of the buccal bone plate was not present.

The extraction socket was thoroughly curetted and irrigated with sterile saline solution. Subsequently, in each patient the socket was filled with DBBM with 10% collagen (BioOss, Geistlich). A xenogenic resorbable CM (Mucograft, Geistlich) was adapted to the marginal soft tissue and placed to cover the xenograft to promote primary healing. A buccolingual/palatal resorbable suture (4/0 Vicryl Plus, Ethicon) was placed over the wound to stabilize the CM and allow a tension-free flap closure. All patients received oral hygiene instructions and were instructed to continue antimicrobial
therapy consisting of chlorhexidine mouthrinse twice a day for 14 days and amoxicillin 1 g twice a day for 3 days after surgery. Postoperative pain and edema was controlled with 400 mg ibuprofen taken orally every 12 hours for the first 2 days.

After tooth extraction, the vertical distance from the center of the buccal and the palatal/lingual alveolar crest (AC) to a reference periodontal probe that connected the cementoenamel junction (CEJ) of the adjacent teeth was measured using a second periodontal probe. The buccopalatal/lingual alveolar width was measured intrasurgically at the center of both buccal and lingual walls, 1 mm apically from the crest, using a manual caliper.

Implant Placement

After the surgical procedure was completed, each patient was randomized into the short-term (16 weeks of healing) or the long-term evaluation (32 weeks of healing). The allocation concealment was performed through serially numbered sealed envelopes, and the details of the sequence were unidentified to the clinicians participating in the study. Before evaluation, an investigator not involved in the recording and processing of data (F.C.) performed the assignment of the sealed envelopes marked with the patient’s initials and date of birth and containing a short- or long-term evaluation. Subsequently, another clinician (G.I.) opened the envelope with the assigned number for one of the two evaluations. At the appropriate randomized time point, the patient returned for implant surgery.

After flap elevation, 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. Then, an implant of at least 4.0 mm in diameter was placed into the grafted alveolus and a tension-free flap was replaced to obtain primary soft tissue closure.

Patients received the same drug regimen as prescribed after the extraction surgery. The temporary prosthetic restoration used after the first surgical step was applied again. Final prosthetic restorations were initiated after 3 months from implant placement in both groups (Fig 3). Patients were recalled for follow-up every 6 months. Implant success was evaluated at the follow-up examinations for tissue integration by recording any biologic complications. The peri-implant marginal bone levels were evaluated on intraoral radiographs 1 year after final prosthetic restoration.

Fig 1 (a) Site filled with osseus xenograft after tooth extraction in the in the short-term group (16 weeks). (b) The collagen membrane that completely covered the socket and extended a minimum of 3 mm on intact alveolar bone. (c) Surgical reentry at 16 weeks. (d) Prosthetic rehabilitation of the case.
Histologic Analysis

The bone core biopsy samples were placed in sample holders filled with 4% formaldehyde solution in 0.1-M phosphate-buffered saline (PBS), pH 7.3, and stored at 4°C. For the histomorphometric analysis, the two most central sections were obtained from each specimen. 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 the qualitative and morphologic analysis of the modeling process, the stained preparations were examined under a light microscope (Zeiss Axioplan) at a minimum ×20 magnification and the entire section was evaluated. Ten digital images of each section were acquired and were used to trace the areas identified as vital bone, biomaterial particles, and connective tissue.

Statistical Analysis

A two-sample Student t test was performed for the analysis of the clinical ridge dimensional changes and the histologic parameter changes between the two treatment groups. A P < .05 value was set as statistically significant.

Results

All the enrolled patients in both groups successfully completed the study. After the alveolar ridge preservation procedure, the clinical healing was uneventful and without infection in all patients from both groups.

Eight patients in the short-term and eight patients in the long-term group were included to obtain clinical and histologic data.

Histologic Results

In the short-term group (16 weeks), the resorption of DBBM was slow and residual particles in bulked distribution embedded in coarse connective tissue without apposition of bone on the biomaterial were detected in bone tissue. Few remainders of the covering native CM were observed in the mucosal connective tissue, with no sign of inflammatory reactions. The histomorphometric analysis of the soft tissue specimen showed the presence of salivary glands and remnants of membrane material. Intergranular tissue showed no signs of inflammatory reaction (Fig 4).
In the long-term group (32 weeks), vital bone was identified as areas of bone with osteocytes occupying lacunae and was most often woven bone. Residual graft particles were identified as areas of primarily lamellar bone, and there were no osteocytes occupying the lacunae. Apposition of new vital bone on residual graft particles was often noted, which comprised loose fibrovascular connective tissue. The histomorphometric analysis of the soft tissues showed that salivary glands and remnants of membrane material were present, and intergranular tissue showed no signs of inflammatory reactions (Fig 5).

The percentage of new vital bone was significantly different between the short-term and the long-term group ($P = .01$). The short-term group had a mean of 35.58% vital bone compared to 47.76% in the long-term group (Table 1). With regard to percentage of residual graft, there was no significant difference between groups (short-term 34.23%; long-term 25.43%).

**Clinical Results**

With regard to the study sites, at baseline both groups were similar. A further comparison between

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**Fig 3** Histomorphometric analysis of specimens in the (a) short- (16 weeks) and (b) long-term (32 weeks) groups. (a) Details from soft tissue in a short-term group specimen show salivary glands, membrane remnants, and leucocytes. (b) Orthokeratinized epithelium and connective tissue with coarse collagen fiber bundles were seen in the long-term group. Newly formed bone and Bio-Oss are stained dark magenta, original bone light magenta, and soft tissue blue (undecalcified ground sections stained with azure II and pararosaniline).

**Fig 4** Details of histomorphometric analysis in the short-term group (16 weeks). Partially preserved biopsies; Bio-Oss (BO) particles embedded and partially encapsulated in connective tissue with coarse collagen fibers; no apposition of newly formed bone; no inflammatory reactions. (a) Details from coronal bone. (b, c) Details from apical bone.
groups showed that, at baseline, there were no significant differences in the mean thickness of the buccal plate in the short-term group (1.09 ± 0.26 mm) compared to the long-term group (1.15 ± 0.31 mm) (P = .18).

With regard to ridge dimension changes, no significant difference was found (P = .14) between groups after surgical reentry in change of the vertical buccal distance (CEJ–AC) (short-term 2.38 ± 0.22 mm vs long-term 2.49 ± 0.26 mm) and in the vertical palatal/lingual distance (CEJ–AC) (short-term 2.41 ± 0.31 mm vs long-term 2.37 ± 0.24 mm). Moreover, there was no significant difference in the buccolingual alveolar width (P = .12) between groups (Table 2).

Discussion

This case series compared the histologic effects of healing time on new bone formation after alveolar ridge preservation with DBBM and a CM over the short (16 weeks) and long term (32 weeks) following single tooth extraction in the anterior esthetic zone. Compared to the short-term healing group, the long-term group showed significantly more new vital bone before implant placement. There were no significant differences in ridge dimension changes between groups.

Although several studies have shown that implants may be placed in grafted sites successfully and without additional bone grafting, some authors have reported a horizontal loss of up to 1.7 mm and that original ridge contours were not completely preserved.9,23

The design of this case series, which has been used with previous studies,24,25 was chosen to allow a direct comparison of new vital bone formation between the two groups with few confounding variables, such as the source of bone graft material for both experimental groups and the inclusion of only sites in the anterior area.

Buccal bone was shown to be one of the most important features when satisfactory esthetic results

Table 1  Histologic Results (Mean ± SD) for the Short- and Long-Term Healing Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Vital bone (%)</th>
<th>Residual graft (%)</th>
<th>Connective tissue (%)</th>
</tr>
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<tbody>
<tr>
<td>Short-term (16 wk)</td>
<td>35.58 ± 15.56</td>
<td>34.23 ± 11.14</td>
<td>30.19 ± 9.56</td>
</tr>
<tr>
<td>Long-term (32 wk)</td>
<td>47.76 ± 12.31*</td>
<td>25.43 ± 12.87*</td>
<td>26.81 ± 8.67</td>
</tr>
</tbody>
</table>

*P < .05 short-term vs long-term.

Table 2  Clinical Results (Mean ± SD) for the Short- and Long-Term Healing Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Vertical buccal distance (CEJ–AC) (mm)</th>
<th>Vertical palatal/lingual distance (CEJ–AC) (mm)</th>
<th>Buccopalatal/lingual alveolar width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.25 ± 0.13</td>
<td>2.21 ± 0.15</td>
<td>11.5 ± 1.8</td>
</tr>
<tr>
<td>Short-term (16 wk)</td>
<td>2.38 ± 0.22</td>
<td>2.41 ± 0.31</td>
<td>9.5 ± 1.3</td>
</tr>
<tr>
<td>Long-term (32 wk)</td>
<td>2.49 ± 0.26</td>
<td>2.37 ± 0.24</td>
<td>9.8 ± 1.2</td>
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Fig 5  Details of histomorphometric analysis in the long-term group (32 weeks). Dense trabecular structures formed by mature lamellar bone (LB) with integrated Bio-Oss particles (BO); osteoblasts generating osteoid (O).
are required. In the case of insufficient bone at the implant site, a staged surgical approach involving bone augmentation is advantageous. A systematic review showed that there was 29% to 63% horizontal bone loss and 11% to 22% vertical bone loss after 6 months following tooth extraction and demonstrated rapid dimension reductions in the first 3 to 6 months, followed by gradual reductions. For this reason, it appears that a staged surgical approach is necessary when part of the buccal bone wall is missing at extraction of a failing tooth. Moreover, it has been suggested that extensive resorption of even intact buccal plates is a common phenomenon following tooth removal.

With regard to the ridge dimension changes, no significant difference was found in the change in CEJ–AC and in the buccolingual alveolar width between groups. However, all clinical values showed a slight improvement when compared to the baseline measurements. A recent meta-analysis demonstrated that alveolar ridge preservation is effective in limiting physiologic ridge reduction compared to tooth extraction alone. Furthermore, subgroup analyses of this study revealed that flap elevation, the use of a membrane, and the application of a xenograft or an allograft are associated with superior outcomes, particularly on midbuccal and midlingual height preservation.

Lindhe et al reported marked differences in mineralized bone formation and in the amount of bone marrow at 6 months between postextraction sockets augmented with DBBM and CM and non-grafted sites. Based on this pilot observation, the present study was designed to compare the clinical and histomorphologic effects of DBBM + CM in a short or long-term time point before implant placement after single tooth extraction.

The present study showed that an average new vital bone formation of 35.58% was found after 16 weeks (short term) of healing compared to 47.4% at 32 weeks (long term) of healing. Similar previous reports were in accordance with these results, showing 46.3% vital bone formation after 9 months of healing following ridge preservation with DBBM. The use of DBBM without a membrane in alveolar ridge preservation yielded 25% vital bone with 15% residual graft particles following only 12 weeks of healing.

Moreover, the reasons for alveolar ridge preservation include maintenance of the existing soft tissues and maintenance of a stable ridge volume to optimize the functional and esthetic outcomes. In this study, an increase in the keratinized tissue level was obtained using an alveolar ridge preservation technique. The influence of the hard tissues on the position of the peri-implant soft tissues in postextraction sites was described in previous studies. The soft tissue augmentation obtained in this study may have been due to the site characteristics (and an amount of tissue similar to the original biotype of the patient) and width of keratinized gingiva at the time of extraction, minimal mucoperiosteal flap elevation, and the absence of periodontal disease.

The primary outcome of this case series was to describe the histologic healing following two different healing protocols for alveolar ridge preservation. This study indicates that there was significantly greater new vital bone formation using a xenograft protocol for alveolar ridge preservation with DBBM plus CM at 32 weeks compared to 16 weeks prior to dental implant placement. In addition, there were differences in ridge dimension change between the different timing protocols.

The combined approach to alveolar ridge preservation with DBBM concomitant with application of a CM described in this case series offered a number of advantages, such as the use of implants with adequate diameter and limitation on the amount of alveolar resorption, and allowed optimal management of the level of keratinized and facial soft tissues.

Conclusions

This technique was demonstrated as a safe and simple approach that allowed satisfactory results to be obtained. However, this study presents some limitations, such as the absence of cone beam computed tomography or oral scanner evaluations, which could be useful to better analyze the residual anatomy of the soft and hard tissues. This case series is promising and demands further studies with a larger sample to better understand the role and potential benefits of this combined xenograft protocol in the alveolar ridge preservation technique.
Acknowledgments

This work was performed with institutional funding only. The authors reported no conflicts of interest related to this study.

References


