A Histologic and Histomorphometric Retrospective Analysis of the Outcomes of Ridge Preservation Using Anorganic Bovine Bone Minerals and a Nonresorbable Membrane

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The objective of this study was to examine wound healing in extraction sockets following ridge preservation and the outcome of implants placed in those sites. Extraction sockets (N = 31) were grafted with anorganic bovine bone mineral (ABBM) and covered with dense polytetrafluoroethylene membrane. Bone cores obtained during implant placement were examined histologically and histomorphometrically. Percentages of vital bone and residual graft were 37.5% ± 21.3% and 12.5% ± 8.9%, respectively. New vital bone showed a negative correlation with patient age. Percentage of vital bone formation in sockets was correlated with neither postgraft wound healing time nor peri-implant marginal bone level. Int J Periodontics Restorative Dent 2018;38:637–644. doi: 10.11607/prd.3313

Atrophy of the alveolar ridge may cause esthetic and functional problems, such as inadequate bone for the placement of dental implants. A previous study reported that without additional therapy following tooth extraction, the rate of horizontal alveolar bone loss was approximately 0.25 mm per week for the first 3 months following extraction and unassisted healing in a canine model.1 In a nonhuman primate model and in a clinical study, severe alveolar ridge contour collapse occurred in the crestal 6 mm of the alveolar ridge.2–4 Ridge preservation has been proposed as a strategy to reduce postextraction bone resorption.5–8 A systematic review concluded that ridge preservation techniques can significantly reduce alveolar bone resorption by 1.84 mm in width and 1.47 mm in height.9 Various techniques, materials, and barriers have been used in ridge preservation procedures1–4,10–15; however, a recent systematic review16 has concluded that there is no evidence for significant differences among the techniques and materials used for ridge preservation. Studies that directly compare various materials and techniques are lacking. While variability in the healing of extraction sockets and associated dimensional changes has been documented,17 patient- or site-specific factors responsible for this variability have not been defined.
The inclusion criteria for patient selection were flapless tooth extraction followed by ridge preservation with the use of ABBM and a nonresorbable dense polytetrafluoroethylene (dPTFE) membrane, and the availability of bone core samples collected at the time of implant placement. Exclusion criteria were lack of sufficient clinical and radiographic data at the time of ridge preservation or implant placement and lack of at least 6 months of follow-up.

The 24 consecutive patients (18 women, 6 men; mean age 56.6 ± 9.4 years, range 43 to 78 years) contributed 31 extraction sockets that were included in this analysis (Table 1). Patients were treated in a private periodontal practice by the same periodontist (H.H.Z.). All patients received prophylactic antibiotic treatment (amoxicillin 2 g or clindamycin 600 mg) and postoperative treatment for 1 week following surgery (amoxicillin 500 g three times a day or clindamycin 300 mg four times a day). Teeth were extracted using a flapless minimally traumatic technique, including sectioning roots. Piezosurgery (Mectron) was used to facilitate tooth extraction by insertion of a special tip (EX1) into the periodontal ligament space. Extraction sockets were debrided to remove all granulation tissue (Fig 1a). The sockets were grafted using large-size ABBM particles (1.0 to 2.0 mm Bio-Oss, Geistlich) hydrated with sterile saline. A nonporous dPTFE membrane (Cytoplast GBR-200, Osteogenics) was trimmed to cover the extraction socket orifice and extend approximately 3 mm inside the socket, without deliberate attempts to achieve primary closure (Fig 1b). The membrane was sutured with 4-0 nonresorbable PTFE monofilament suture (Cytoplast Suture, Osteogenics) (Fig 1c). After surgery, patients were instructed to brush the area with an ultrasonic toothbrush and rinse with 0.12% chlorhexidine gluconate. The membranes were removed after 4 weeks.

Implant placement was performed at least 3 months after ridge preservation in maxillary sites and 2 months after ridge preservation in mandibular sites. At the time of implant placement, bone cores were obtained using a trephine drill with an outer diameter of 3.3 mm, followed by placement of an implant with an outer diameter (Figs 1d and 1e) that was slightly wider than the trephine drill. Core samples were stored in 10% neutral buffered formalin until processing. The implant was restored 3 months after placement (Fig 1f). Periapical radiographs were used to evaluate the marginal bone changes (Fig 1g). Specimens were placed in 4% (v/v) paraformaldehyde and dehydrated in graded ethanol (70%, 95%, and 100%). Specimens were then decalcified using decalcifying solution and ethylenediaminetetraacetic acid, embedded in paraffin, and sectioned longitudinally at 5 μm. Hematoxylin-eosin (h&e) staining was performed using standard protocols, and sections were also stained with Masson trichrome. The stained specimens were viewed and imaged using a microscope (Leica DM4000 M, Leica Microsystems). All bone core samples were evaluated by histology and histomorphometry.

### Materials and Methods

The protocol for the present retrospective study was reviewed and approved by the Institutional Review Board (IRB) of the University of Southern California. A total of 24 consecutive patients from the periodontal private practice of one of the authors (H.H.Z.) were included in this study.

### Table 1 Characteristics of Patients and Teeth Included in This Study

| Patients | 24 (18 women, 6 men) |
| Age (y) | 56.6 ± 9.4 (43–78) |
| Teeth (n) | |
| Maxilla | 14 |
| Anterior | 4 |
| Premolar | 5 |
| Molar | 5 |
| Mandible | 17 |
| Anterior | 10 |
| Premolar | 4 |
| Molar | 3 |
| Healing time (d) | 162 ± 120 (70–635) |

Therefore, the objective of the present retrospective analysis was to assess the biologic outcome of ridge preservation using anorganic bovine bone mineral (ABBM) and to elucidate factors that potentially affect wound healing by examining the histologic response of alveolar bone following ridge preservation. This comprehensive retrospective analysis of the outcome of ridge preservation included microcomputed tomography (mCT) analysis of bone cores and the outcomes of implants placed in grafted sockets, reported separately.
Histomorphometric analysis was completed by two blinded examiners (S.M. and M.F.). Actual-size digital images were made using microscopy imaging software (SPOT Basic, SPOT Imaging). Images were imported and subjected to quantitative histomorphometric analysis using digital image manipulation software (PhotoShop CC, Adobe Systems) to identify bone, residual graft, and void volumes in bone core samples obtained from the grafted extraction socket sites. Standard methods and nomenclature of the American Society for Bone and Mineral Research (ASBMR) were used to determine bone fill in the specimens. The bone parameters measured included the percentage of bone relative to total tissue volume (BV/TV), residual graft volume, and void volume. Descriptive statistics were calculated for all variables of interest. Continuous measures were summarized using means and standard deviations. Nonparametric regression modeling was performed to compare BV/TV and residual graft volume and their possible correlations with healing time, age, and marginal bone level (MBL) around the implant. All analyses were carried out using SAS version 9.3 (SAS Institute). Periapical radiographs were taken at the time of implant insertion and follow-up visits of at least 6 months and were used to evaluate MBL changes around the implants.
The radiographs were first calibrated based on a known distance (the length of the implants), and then MBL was measured as the distance between the implant platform and first bone-to-implant contact.

**Results**

The clinical characteristics of study patients and tooth sites are listed in Table 1. The mean healing time following ridge preservation was 162 ± 120 days (range 70 to 635 days).

**Histologic Observations**

Examination of representative histomicrographs stained with h&e revealed the presence of strongly eosinophilic osseous tissue containing round-to-void osteocytes in lacunae, demonstrating the viability of the tissue (Fig 2). Sections stained with Masson trichrome showed distinct reversal lines in woven bone. In areas of active apposition, osteoblast-like cells with large nuclei, occasional nucleoli, and granular cytoplasm were seen rimming the bone. Occasional osteoclast-like cells were observed within Howship lacunae or resorption pits adjacent to osteoblasts, all indicating active remodeling of the bone (Fig 3). Hematopoietic cells and erythrocytes were observed in the lumen of the blood vessels in the fibrovascular stroma that surrounded the bone.

**Histomorphometric Analysis**

Histomorphometric analysis was performed to determine the representation in histologic sections from various tissue components. This analysis revealed that the percentages of BV/TV, residual graft volume, and void volume were 37.5% ± 21.3%, 12.5% ± 8.9%, and 49.8% ± 19.0%, respectively. Moreover, there were no statistically significant differences in the percentages of BV/TV according to the location of the extraction socket (ie, maxilla vs mandible, anterior vs molar, anterior vs premolar, premolar vs molar) (Fig 4). Similarly, there were no statistically significant differences in the percentages of void or of residual graft according to the socket location.

**Implant Outcome**

A total of 26 implants were placed in sites treated by ridge preservation. Implant survival rate was 100%.
Mean MBL change on the mesial and distal aspects of the implant was $-0.01 \pm 0.16$ mm and $0.02 \pm 0.15$ mm, respectively.

Correlations

Nonparametric regression modeling was performed and adjusted for correlations among repeated measurements taken from the same patient. The model examined the relationship between BV/TV and healing time after adjusting for age (left as a continuous variable). The percentage of BV/TV was significantly correlated with age ($P = .04$), such that increasing age led to decreasing BV/TV (Fig 5). Figure 6 shows that the percentage of BV/TV was not significantly correlated with healing time ($P = .31$). A similar analysis found that age and healing time were not significantly correlated with the residual graft volume (Figs 5 and 6).

The influence of tissue response to ridge preservation on the outcome of implants placed in treated sites was examined. To that end, the correlations between histomorphometric parameters and peri-implant MBL of implants placed in grafted sites were assessed. The results demonstrated that the peri-implant MBL changes on the mesial and distal aspects of implants placed in grafted sites were not significantly correlated with BV/TV ($P = .96$ and $P = .84$, respectively) (Fig 7) or residual graft volume ($P = .41$ and $P = .29$, respectively) (data not shown).

Discussion

Multiple studies have reported significant horizontal and vertical bone loss following tooth extraction$^{1,2,4,19-21}$ A number of studies have demonstrated that ridge preservation can reduce dimensional changes of the alveolar ridge following tooth extraction$^{7,8,22,23}$ The outcomes of implants placed in sites following ridge preservation have also been investigated in a few studies$^{24}$; however, few studies have examined the correlations between the histologic tissue response of grafted sockets and implant outcomes. Moreover, because numerous protocols for ridge preservation exist$^{16}$, it is important to provide documentation of the outcomes for each of these protocols.

This study sought to examine wound healing in sockets grafted with ABBM and covered by a nonresorbable membrane that were allowed to heal without intentional primary coverage. The present retrospective analysis was...
undertaken to better understand the clinical and biologic outcomes of a ridge preservation procedure. In addition to selection of materials for ridge preservation, it is critical to understand how the wound healing of the extraction socket may be affected by local factors that can lead to erratic healing, including age, systemic disease, and the site itself.25 Scant data is available on the association between the composition of bone formed in grafted extraction sockets and patient- and site-specific factors such as age, tooth location, and wound healing time. The present study is unique in reporting some of these parameters. Since this was a retrospective analysis, there was heterogeneity with regard to patient age, postextraction healing time, and anatomical location of the teeth in the oral cavity. This study allowed examination of such parameters.

**Fig 5** (left) The percentage of bone relative to total tissue volume was significantly correlated with age, such that increasing age led to decreasing bone volume (parameter estimate −1.08; 95% confidence interval (CI): −2.01 to −0.14; P = .04). The percentage of residual graft volume was not significantly correlated with age (parameter estimate 0.01; 95% CI: −0.39 to 0.41; P = .96).

**Fig 6** (below) The percentage of bone relative to total tissue volume was not significantly correlated with healing time (parameter estimate −0.12; 95% CI: −0.36 to 0.12; P = .31). The percentage of residual graft volume was not significantly correlated with healing time (parameter estimate 0.05; 95% CI: −0.06 to 0.15; P = .41).
Thus, age of the patient and wound healing time were evaluated using histology and μCT analysis (reported separately). The present data revealed that age was negatively correlated with BV/TV volume (P = .04). On the other hand, postextraction healing time was not correlated with BV/TV volume (P = .31). Moreover, neither patient age nor wound healing time was associated with residual graft volume (P = .96 and P = .41, respectively). In general, increased age is frequently associated with reduced immune response, bone metabolism, and regenerative potential.26,27 The results of the present study are in agreement with this generality in that older subjects appeared to have a lower percentage of BV/TV in the grafted extraction socket. Furthermore, peri-implant MBL was relatively stable and not correlated with the composition of tissues found in grafted extraction sockets. Therefore, there was no evidence that a certain percentage of BV/TV was required for the proper function of implants placed in those sites. This finding may be attributed to some of the observations showing that regardless of the composition of the surrounding tissues, bone-to-implant contact ensues, perhaps as a result of de novo bone formation (ie, contact osteogenesis).28,29 It may be posited that regardless of the composition of the bone following healing of the ridge preservation, placement of the implant may once again alter the dynamics of the tissues in the site and drive osteogenesis. Experimental evidence to investigate this hypothesis is needed.

As with any retrospective study, this report has several limitations, including its retrospective design; variability in healing time following extraction, ridge preservation and implant placement; and limited sample size.

Conclusions

The present investigation was undertaken as part of a comprehensive effort to examine wound healing in grafted extraction sockets. Within the limitations of this study, ABBM used in conjunction with a nonresorbable membrane for ridge preservation lead to an amount of vital bone that was comparable to that reported in previous studies. Increasing age of the patient was found to be a possible negative predictor of vital bone formation in grafted extraction sockets. Notwithstanding these histologic observations, the present data found no evidence to indicate that the histologic composition of tissue formed following ridge preservation can influence the outcome of implants placed in sockets treated by ridge preservation.
References


