Extraction Socket Preservation Prior to Implant Placement

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Learning Objectives: After reading this article, the individual will learn: (1) histologic, clinical, radiographic, and histomorphometric assessments regarding the efficacy of a demineralized bone matrix material and a bioresorbable membrane for extraction socket preservation; and (2) a clinical technique and materials for socket preservation prior to implant placement.

About the Authors

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Bone preservation is a central tenet of any reconstructive protocol. Observational1–4 as well as volumetric1,2,5 studies of bone remodeling subsequent to tooth extraction have clearly demonstrated the inevitable reduction in alveolar bone volume that occurs in the absence of clinical interventions to prevent it. The clinical consequences of this process often severely compromise restorative outcomes and/or reconstructive treatment planning.

In the case of extraction sockets, immediate implant placement has yielded success in preserving bone (in the aesthetic zone6,7) but has still been shown to perform suboptimally.8,9 While bone grafting of fresh extraction sockets and other ridge preservation procedures using autogenous bone, allografts, xenografts—or various combinations of these and other materials—have, during the past 2 decades, been amassing and improving the clinical track record for alveolar ridge preservation, the need for more prospective studies and uniformity of data remains.1,10–14 Hence, bone reconstruction is often a requirement before or during implant therapy to achieve optimal physiologic architecture, function, and aesthetics.

The number of bone grafting procedures has exceeded 2.2 million per year worldwide.15 Expanded use of implants in partially and fully edentulous patients has been a primary driver of socket preservation bone grafts. The evolving scenario that surrounds treatment planning of extractions with a view to placing implants is becoming increasingly common. Since the surgical phase of implant placement is becoming a more routine component of treatment planning, socket preservation is also a treatment modality that clinicians need to consider to help optimize the site at the time of extraction.16–21 In addition, a wider array of easy-to-use allograft materials, such as demineralized bone matrix (DBM) products, is enabling greater opportunities for patients undergoing socket preservation as an increasingly predictable method of ridge preservation.

The use of bone grafts in the repair of skeletal defects has a long history of success. In 1881, Sir William MacEwen22 of Rothesay, Scotland, published the first case report of successful interhuman transfer of bone grafts. Bone grafting began gaining more widespread acceptance in orthopedic practice after publication of foundational work by Putti in 1912,23 and the first definitive text by Albee24 in 1915,25 While this success was originally and primarily with the use of autologous bone, allograft materials have been used in periodontal therapy for more than 3 decades.26,27 Continued advances have increased the use of xenografts such as Bio-Oss (Geistlich Pharma North America), and other synthetic substitutes such as bioactive glass,28,29 tricalcium phosphate,30 and hydroxyapatite.30

DBM allografts and tissue-engineering signaling molecules such as recombinant human bone morphogenetic protein-2 (rhBMP-2) have demonstrated success in a variety of dental and craniofacial indications,13,37–35 including socket preservation31,32,36,37 and alveolar ridge augmentation.33,38–42

The use of DBM allografts in fresh extraction sockets with the objective of preserving bone volume has been studied in parallel with the evolution of the predictability of dental implants.36,43–46 However, more clinical evidence is needed to support their use in this specific scenario. Bioresorbable membranes have been used in conjunction with DBM products for socket preservation procedures with demonstrated clinical success.47

Graft excipients are natural or synthetic substances formul-
lated alongside the active ingredient of a medication and are included for the purpose of serving as a “bulking agent or filler” in order to confer a therapeutic enhancement of the active ingredient. They also can contribute to scaffold formation, as well as functioning as effective carriers for bioactive molecules, helping to maximize bone growth after placement of the grafting material, and thus can be critical components of successful outcomes. One excipient currently in use, poloxamer 407, is an inert, biocompatible reverse-phase medium compound that thickens at body temperature, resists irrigation, and allows for better graft containment. It also facilitates handling, packing, and molding when used in bone-grafting procedures, especially in areas with difficult access, and in defects of various sizes and shapes. Clokie and Urist reported in 2000 significantly better performance of poloxamer 407 as a carrier of bone morphogenetic proteins (BMPs) compared with other carriers, based on histomorphometric analyses in rats. In 2003, Babbush reported favorable clinical outcomes in 10 cases with the use of a poloxamer-containing DBM putty for socket grafting prior to successful implant placement.

Despite DBMs’ history of reliability, they are understudied at the molecular level, particularly regarding allograft resorption kinetics and their influence on the healing cascade. The ideal bone graft is one that is osteoinductive as well as osteoconductive and stable in volume. These criteria are best analyzed by clinical re-entry measurements, or by 3-dimensional analyses (such as CBCT), as well as through histology and histomorphometry (which is an estimate of the proportional volume and surfaces occupied by the different components of the bone biopsy specimen).

However, only a small number of randomized controlled clinical trials have been conducted to evaluate various grafting materials using histologic techniques. The most commonly assessed histomorphometric parameters comprise percentages of vital new bone, connective tissue, and residual graft material, all of which were assessed in the exploratory prospective case series presented here.

DBM consists of the organic portion of bone, including osteoinductive factors such as BMPs. The removal of the mineral portion of bone exposes the BMP signal to the surrounding tis-

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### Table 1. Patient Characteristics

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<th>Patient ID</th>
<th>Sex</th>
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*See case report presented.

KEY: F = female, GERD = gastroesophageal reflux disorder, M = male
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...sue, encouraging the induction of bone formation.

These materials are currently being used in orthopedic procedures such as nonunion fractures and lumbar spinal fusion, and the material used in the case series and histologic case report presented in this article strictly adheres to tissue banking standards as provided by the US Food and Drug Administration, American Association of Tissue Banks, and Clinical Laboratory Improvement Amendments.

**MULTIPLE ASSESSMENTS OF SOCKET PRESERVATION**
In the case series and histologic case report presented here, clinical, radiographic (including CBCT imaging), histologic, and histomorphometric assessments were made in regard to the efficacy of a 100% DBM material (Accell Connexus [Keystone Dental]) in socket preservation procedures, when used in conjunction with a bioresorbable membrane (DynaMatrix [Keystone Dental]), for the purpose of bone volume preservation prior to placement of dental implants.

The DBM material used here differs from other currently available DBMs in that it contains a variety of growth factors, transforming growth factor-beta, and several types of BMPs, which are involved in human tissue growth, as discussed in a 2002 review by Lieberman et al. This material contains substantially greater amounts of BMPs than are found in previously de-

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<th>Table 2. Histomorphometric Data Summary By Patient, Four-Month Post-Graft Core Biopsies</th>
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*See case report presented.
KEY: M = mesial, D = distal, SD = standard deviation
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veloped DBMs,69 and has also demonstrated effective bone regeneration in a mature sheep model.70

The series reported here describes the case of a 59-year-old white female who underwent this socket-preservation procedure; 2 core biopsies were obtained of the grafted socket (one core each from mesial and distal graft borders, with care to minimize the incorporation of host bone) just prior to implant placement at 4 months post-grafting. Further, data are summarized from 10 additional patients who received the same treatment, with one core biopsy obtained from each grafted site, also at 4 months (time of implant placement in all patients).

This is the first clinical case report to evaluate a 100% demineralized, putty-like DBM bone grafting material containing BMPs, growth factors, and poloxamer 407, in fresh extraction sockets for ridge preservation, with focus on percentage of vital bone formation. To the authors’ knowledge, no study to date has made qualitative volumetric assessments of ridge preservation by socket grafting using CBCT.

**CLINICAL ASSESSMENTS AND PROCEDURES**

This was an exploratory, observational, descriptive, private practice-based, prospective case series to assess proof of principle of a 100% DBM material used with a biodegradable membrane for ridge preservation in preparation for dental implant placement.

Descriptive summaries from clinical, radiographic, histologic, and histomorphometric study data were used to compare bone healing, and in particular, degrees of new vital bone formation after socket grafting, with other published allograft data62,64,71-80 as well as to evaluate morphological changes as observed by CBCT imaging at different time points (24 hours post surgery, one-month post-surgery, and 4 months post-surgery).

A total of 11 patients received the DBM material, which was covered in all cases with a biodegradable membrane after indicated extraction of a single tooth (excluding third molars and lower incisors). All patients had autogenous bone (harvested from the external oblique ridge or basal bone in the surgical site) incorporated in their DBM socket grafts.

Performing all extractions with an open-flap technique inherently increased the potential for greater socket resorption.81 Since this case series focused on preservation rather than reconstruction, case selection also aimed to graft only those sites in which the post-extraction buccal wall anatomy was intact. Supplementation with local autogenous bone was done in an effort to minimize socket resorption and further enhance overall bone...
growth results for each individual patient.

Case Selection
Healthy patients who therapeutically required extraction of at least one natural tooth (with the exception of third molars and mandibular incisors), and for whom a socket preservation procedure was desired and feasible prior to implant placement, voluntarily chose to undergo these procedures after receiving detailed explanations of what they entailed, the potential risks, and the potential for variability in individual clinical outcome. All patients gave written, signed informed consent to undergo the procedures with this understanding.

All individuals who received grafts were patients of record in a private periodontal surgical practice, and all had had recent dental exams, head and neck exams, prophylaxes, and update interviews for medical history, in addition to full-mouth radiographic series prior to evaluation for extraction and grafting, as part of a comprehensive and best-practice diagnostic standard of care.

The graft/implant site(s) could not have a history of a failed implant. Alveolar ridge dimensions had to be sufficient to accommodate and sustain proper implant placement without augmentation beyond intrasocket grafting.

Women who were pregnant, as well as patients with history of serious or chronic disease—including diabetes; uncontrolled hypertension; malignancy; severe coronary heart disease; collagen or bone disease; local oral, respiratory or systemic infection; or any immunocompromising disease, or were receiving long-term corticosteroids or current bisphosphonate therapy—could not undergo the procedures. Table 1 summarizes the characteristics of all patients who received socket grafts and subsequent implants.

All extractions were performed under local anesthesia and intravenous conscious sedation by the lead author (Dr. Mandleliris). Surgery was performed as an open-flap design. Following atraumatic delivery, all sockets were thoroughly degranulated under copious sterile saline irrigation. Appropriate antibiotics and analgesics were prescribed for a minimum of 10 days (amoxicillin 250 mg every 8 hours, or clindamycin 150 mg 4 times daily if the patient was penicillin-allergic; ibuprofen 600 mg every 6 hours). Patients were instructed not to brush or floss at the surgical site for 2 to 3 weeks, and to rinse with chlorhexidine (0.12%) daily for 2 to 3 weeks after the first postoperative visit.

The DBM putty (Figure 1) was then injected immediately into each fresh extraction socket, followed by placement of a single layer of bioresorbable barrier membrane (DynaMatrix), which was custom fitted to the extraction site. Primary closure was attempted in all cases and achieved with 5-0 poliglecaprone 25 (MONOCRYL [Ethicon Endo-Surgery]) by buccal and lingual (when possible) periosteal releasing incisions or blunt dissection. Healing was evaluated at one to 2 weeks and 4 weeks post-surgery.

Re-entry of grafted sites was performed at 4 months post-graft in all cases. Implant placement was performed in all cases 4 months post-surgery using the standard surgical protocol for the appropriate implant system, as necessitated by each patient case. One bone core biopsy (2 in the case presented below) was secured from each healed graft site prior to implant osteotomy site preparation. A focused-field or single-arch CBCT scan was performed prior to core retrieval and at 24 hours post-extrac-
tion, as well as at one-month post-extraction, to evaluate morphological changes from cross-sectional and axial perspectives. A final surgical followup was done for all patients 12 to 16 weeks after implant placement.

Bone Biopsies

At 4 months post-extraction (time of implant placement), a 2-mm trephine bur (Salvin Dental) (Figure 2) was used to obtain core biopsies in all patients, to evaluate the histology of the hard tissue at the grafted site. Although incorporation of some native bone into the specimen was a possibility, every effort was made to procure only grafted tissue. Each bone core biopsy sample was left in the trephine and placed in formalin in a tissue container, to be shipped for histologic analysis.

Sections 5 µm in thickness were prepared and evaluated using light microscopy. Histomorphometric measurements of the tissue fractions (percentages of vital bone formation, remaining soft connective tissue, residual graft material) were performed for each biopsy sample of each grafted area.

Histologic Analysis

The bone-core specimens were stored in 4% paraformaldehyde and sent to the Philip Boyne Bone Lab at Loma Linda University, School of Dentistry (Loma Linda, Calif) for histologic and histomorphometric analysis. All histologic and histomorphometric analysis was performed by the second author (Dr. Lu).

The specimens were placed in 70% ethanol and sequentially dehydrated in 95% and 100% ethanols. Samples were embedded for 4 to 5 hours in an aqueous encapsulating gel, placed into a mega cassette, and embedded in celloidin-paraffin. A microtome was used to obtain the 5-µm sections, which were stained with hematoxylin and eosin (HE).

Whole-slide photomicrographs were captured using a whole-slide scanning microscope (Olympus VS120 [Olympus Corporation]). Histomorphometry was performed in each specimen under original, 4x, and 2x magnifications. The analysis was based on the entire specimen using Image-Pro Plus quantitative analysis software (Media Cybernetics).

Five slides were selected from each specimen for histomorphometry. The mean of these 5 slides for each specimen was reported for the percentages of new bone, soft connective tissue (bone marrow and cells), and residual graft material.

CASE REPORT

After receiving infiltration local anesthesia with 36 mg lidocaine with 0.018 mg epinephrine per 1.8 mL, and under intravenous conscious sedation (induced via midazolam and fentanyl titration; 0.2 mg glycopyrrolate was also administered as an antiallogogue) a 50-year-old white female in good general health was treated. Her daily medications included sumatriptan once daily and Excedrin PM (as needed) for management of migraine headaches. She underwent extraction of nonrestorable tooth No. 31 via an open flap technique (Figures 3 and 4). DBM (Figure 5) and bioresorbable membrane (DynaMatrix II [Key-
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Stone Dental) were placed (Figure 6) following atraumatic extraction and thorough degranulation, and primary closure achieved, as described above (Figure 7). Other medications administered intravenously included 30 mg Toradol for analgesia, and 8 mg dexamethasone to combat swelling and nausea.

After 4 months of healing (Figures 8 and 9), re-entry was performed, the width of the newly grafted ridge assessed (Figure 10), and 2 core biopsies obtained, one each from the mesial and distal sockets previously grafted (Figure 11). Opposite-border biopsies were performed to avoid procuring any native bone (eg, from the furcation area or mesial/distal socket wall borders) that might influence the percentage of vital bone formation assessed by histomorphometry.

After the biopsies were obtained, osteotomy site preparation was performed (bone harvested during osteotomy was placed into the biopsy sites) and a Keystone Genesis 5-mm implant (Keystone Dental) was placed, with an initial mean resonance frequency implant stability quotient (ISQ) of 72. Figures 12 and 13 show implant placement and postoperative radiograph, respectively. Figure 14 shows the final post-restoration periapical radiograph (30 months post-implant placement), demonstrating osseointegration and uniform bone growth within the graft site, and Figure 15 shows the final photo of the full-cast gold implant crown (30 months post-implant placement). Of note, both images also show effective restoration of tooth No. 30 with a PFM crown.

The surgical postoperative course was uneventful in both extraction/grafting and re-entry/biopsy/implant placement in all cases. Other than expected slight to moderate discomfort and swelling at the operative sites postoperatively, no adverse events were reported by any patient at either surgical phase.

However, it should be noted that some sites healed with secondary intention while others, such as the case reported here, healed with primary intention wound healing. In an effort to obtain the most favorable earlier wound healing, primary wound closure was attempted. However, based on current literature, the absence of primary flap closure in extraction socket preservation healing does not appear to affect the percentage of vital bone formation.

Histologic Interpretation

Figures 16 to 19 show core biopsy histology from the mesial and distal biopsy samples of position No. 31 (pre-osteotomy). Figures 16 and 18 show histologic sections at 4x (mesial biopsy, Figure 16) and 2x magnifications (distal biopsy, Figure 18). Figures 17 and 19 identify detailed parameters of regenerative activity at 40x magnification, from mesial and distal biopsies, respectively.

The majority of the specimens showed new bone growth as woven bone. Figure 16 shows the whole-slide photomicrograph of the mesial core biopsy of the grafted position No. 31, captured using a whole-slide scanning microscope (Olympus VS120) at 4x magnification. The woven new bone was stained red in HE.
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Figure 17 shows the same section, captured at a higher magnification (40x), of a mesial biopsy section from grafted position No. 31, showing detailed new bone, residual graft material, bone marrow and cells, and blood vessels. Figure 18 shows 2 pieces of the whole-slide photomicrograph of distal core biopsy of the grafted position No. 31, captured using a whole-slide scanning microscope (Olympus VS120) at 2x magnification. Figure 19 shows a higher magnification (original magnified 40x) for the distal biopsy of grafted position No. 31, showing detailed new bone, residual graft material, bone marrow and cells, large and multinucleated osteoclasts near the residual graft material, mononucleate osteoblasts lining the surface of osteoid seam, and lacunae containing osteocytes surrounded by bone matrix.

Table 2 summarizes the histologic and histomorphometric data from the core biopsies of all patients who received the DBM and membrane. Osseointegration was radiographically verified for all implants, with an implant survival rate of 100% at last observation.

CBCT Data
Figures 20 to 27 show the graft dimensions assessed by CBCT scans immediately post-graft (within 24 hours) and at the time of biopsy and implant placement (4 months).

Figure 20 shows the full-arch field (mandible) CBCT panoramic view immediately after grafting (within 24 hours). Figure 21 shows the cross-sectional slice through the mesial-root portion of the grafted socket of position No. 31 at 24 hours post-graft. Figure 22 shows the same slice secured at 4 months, but viewed from a focused-field CBCT scan taken prior to biopsy and implant placement. Figures 23 and 24 show the same cross-sectional analysis and time points as Figures 21 and 22, but through the distal socket.

Figures 25 and 26 show CBCT axial sections at the approximate level of the former furcation area of position No. 31 (mesial and distal roots of tooth No. 30 are visible, somewhat inferior to the furcation level) at 24 hours post-graft and 4 months, respectively. Favorable, uniform bone growth can be observed in the 4-month axial section (focused-field CBCT image).

Figure 27 shows a section of the final focused-field CBCT panoramic view of position No. 31 at 4 months, also demonstrating radiographically uniform bone growth just prior to biopsy and implant placement. Focused-field imaging provides a higher level of resolution, and with less radiation exposure to the patient (in terms of total anatomic area exposed), although the total dose received at the more limited field is higher when compared to a full arch. A higher level of resolution was desired at the 4-month time point to better understand bone healing characteristics, and this is primarily why a limited field of view was chosen. This is of particular interest when evaluating the crestal bone of the extraction socket at 4 months. Almost complete new cortical bone lining/bridging can be observed at a site that was previously devoid of any superior socket lining (socket orifice).

DISCUSSION
Alveolar ridge preservation via grafting of extraction sockets has paralleled the evolution of dental implants as a standard of care during the past 3 decades. Clinically acceptable results have been obtained using hydroxyapatite, tricalcium phosphate, bioactive glass, xenografts, autologous bone, and biomimetic of DBM alone with DBM combined with rhBMP-2. Another study by Kim et al evaluated the DBM product DynaBlaster in combination with DynaMatrix membrane (similar to the one used in this case series), and found this combination effective in ridge preservation even in the absence of primary flap closure.

El-Chaar compared a DBM putty (Puros [Zimmer Spine])
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Published preclinical studies have documented the use of this DBM product in rabbit calvarium, in which it showed lower ossification and healing rates than Bio-Oss or control groups; however, in athymic rat calvarium, this DBM product was found to produce comparable levels of new bone growth to that achieved by BMP-7 + Type 1 collagen, at 4 weeks and 8 weeks.

Of note, a clinical study (in lumbar spinal fusion) comparing grafts using autologous iliac crestal bone and the DBM material used in this series found that both groups performed equally well for spinal fusion surgery bone healing.

The inherent limitations of case reports preclude controls, comparators, and statistical hypothesis testing, as can only be achieved in appropriately powered, randomized, controlled clinical studies. However, the histomorphometric data observed in this case series demonstrate that there was considerable bone growth at 4 months, when all of the core biopsies were obtained.

The variation in the percentages of new bone that was observed in this case series was considerable (25.5% to 75.5%); it is likely that some native bone was inadvertently incorporated into the biopsies, and/or individual healing rates varied considerably among patients.

In addition, all patients in this series received autologous bone as part of their DBM grafts with the intent of augmenting the clinical outcome for each individual patient. The influence of this factor on histomorphometry would require comparative evaluation, ideally in a split-mouth assessment that compares intrapatient graft response in the presence or absence of autologous bone.

Furthermore, biopsies were obtained at only one time point (4 months). In light of findings from a recent case series published by Scheyer et al., it could be hypothesized that subsequent biopsies at later time points might indicate an increasing ratio of new bone to connective tissue and residual graft material. These authors used a syringeable paste allograft (Dynablast [Keystone Dental]) which was placed in fresh extraction sockets and covered with an extracellular matrix membrane (DynaMatrix) similar to that used here. They reported new bone growth histology at 6, 12, and 24 weeks, to coincide with implant placement (as in our case series), and found progressively increasing amounts of regenerated woven bone in biopsies at later time points. Their 24-week (6-month) biopsy group most closely approximated our single observation point (4 months); degrees of healing prior to this point were not assessed in our series. Qualitative histologic results in that study showed osteoblastic activity as early as 6 weeks post-graft, and increasingly robust vital bone formation at 12 and 24 weeks. However, the value of sequential earlier biopsies must be weighed against risk associated with disturbing a graft too early and jeopardizing its survival.

These variabilities notwithstanding, the DBM putty and...
membrane materials used in this case series were associated with sufficient vital bone formation for successful placement of implants in all cases. The DBM material used in these cases was easily contained and utilized; its putty-like formulation retained a thickened consistency during injection, facilitating accurate placement via syringe and enabling it to stay in place. It also handled well in a variety of oral environments and degrees of saliva flow, or absence thereof, which afforded less potential for contamination of the graft site.

CONCLUSION

The results from this exploratory case series offer considerable evidence to support the osteoinductive activity of the DBM graft and bioresorbable membrane materials tested, their clinical acceptability in preserving sockets, and their ability to generate new bone capable of reliably supporting implants. The highest percentages of new vital bone content observed here (up to 75.5%) are, to the authors’ knowledge, among the highest reported for socket-based ridge preservation, as evaluated in an exploratory case series.

Continuing study of this DBM material and membrane is warranted and necessary, especially in controlled study designs and in direct comparison with other DBMs and alternative socket grafting materials. Randomized clinical studies with appropriately powered sample sizes, appropriately safe procurement of core biopsies at multiple time points, and inpatient comparisons could provide quantitative data of considerable clinical utility in restorative and reconstructive dental-implant and dentofacial treatment planning.

Acknowledgments

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References

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