Histomorphometric and 3D Cone-Beam Computerized Tomographic Evaluation of Socket Preservation in Molar Extraction Sites Using Human Particulate Mineralized Cancellous Allograft Bone With a Porcine Collagen Xenograft Barrier: A Case Series

Stephen Wallace, DDS, MHS

The purpose of this study was to evaluate the results of socket preservation after extraction using human particulate mineralized cancellous allograft bone (MCAB) and type I porcine collagen membranes (PCM) as a guided bone regeneration barrier. Fourteen patients, 12 women and 2 men, were selected who had a diagnosis of one or more unsalvageable teeth with a treatment plan to replace them with implant-supported single crown restorations. Extractions were performed atraumatically by sectioning teeth for removal to avoid damaging the socket walls and by immediately placing MCAB graft to fill the sockets. The sockets were occluded with a new PCM. The membranes were cut to overlap the facial and lingual (or palatal) socket rim by at least 5 mm (or more if necessary) to cover bony wall fenestration or dehiscence defects. Implants were then placed 16 weeks after the extractions and augmentation. The results were evaluated clinically, histomorphometrically, and with cone-beam computerized tomographic scanning. The formation of new bone in the treated sites averaged 11.2%, with a range of 1.8% to 43%, in bone biopsies trephined from the center of the grafted socket sites. Density, calculated with proprietary software and measured in Hounsfield units (HUs), was 543 HU with a range of 420 to 822 HU. The resulting new bone regeneration varied widely, but the barrier membranes showed potential for promoting significant bone regeneration. A larger sample of treated cases is needed. Wall defects did not appear to influence the histologic results, but the number of sites was too small to determine their significance.

Key Words: allograft bone, extraction site, porcine collagen, guided bone regeneration

INTRODUCTION

The goal of socket preservation is to prevent the inevitable bone remodeling and resorption that takes place after extractions. Studies have documented that the width of the alveolar ridge decreased by 50% 12 months after extraction, and that two-thirds of this resorption took place during the first 3 months. The horizontal width decrease can be critical, as a 2-mm width of bone adjacent to the facial aspect of maxillary implants has been shown to be necessary to prevent fenestration and dehiscence defects. Maintaining 3-dimensional alveolar bone volume allows for ideal implant positioning, which is required for esthetic and functional restorations.

Autogenous bone is the first choice for augmentation because of the inherent osteogenic, osteoconductive and osteoinductive properties. Limited quantities of autogenous bone and a potential second surgery involving the ramus, chin, tibia, or iliac crest add significant morbidity and make finding a substitute material highly desirable.

Guided bone regeneration with barrier membranes only showed effectiveness compared with nonmembrane socket healing in a split-mouth study. Nonmembrane extraction sites lost 4.6 mm in width and 1.5 mm of vertical dimension, whereas sites treated with a resorbable membrane alone showed 1.32 mm loss in width and 0.38 mm loss in vertical dimension. Significant differences in bone loss were seen in a study in which socket grafting was combined with covering the graft with a resorbable collagen membrane. Nongrafted sites allowed to heal naturally showed a decrease in width of 2.7 mm, whereas sites treated with demineralized freeze-dried allograft bone covered with a collagen resorbable membrane showed a width decrease of 1.2 mm. Subsequent studies and database searches demonstrated that nontreated extraction sites showed less vital bone formed and more resorption...
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vertically and horizontally, compared with sites augmented and covered with a barrier membrane. Treated sites required less augmentation, and larger diameter implants could be placed. 7–10

A recent review identified 144 bone substitutes: 93 were allografts, 30 were alloplasts, and 21 were xenografts. 11 Vital, vascular bone provides the initial mechanical support for implants and is required for sustained long-term osseointegration, 12,13 so human allograft bone is desirable as it generates a higher percent of vital vascular bone earlier than alloplasts and xenografts in socket grafts. 14

Biologic growth factors can be used to promote bone fill in extraction sites. In a comparison study, platelet-derived growth factor (PDGF) added to cancellous allograft in extraction sites with an acellular dermis barrier membrane showed 41.8% vital bone compared with 32.5% in sites without PDGF after 4 months. 15 A recently published case series reported that adding recombinant human bone morphogenetic protein 2 on a collagen sponge and placing it over socket sites produced 46.8% vital bone at 4 months with no filler material of any type placed into the sockets. 16

The purpose of this consecutive case series was to obtain densitometric, histologic, and histomorphometric data on socket site preservation in sockets that were intact and in those with buccal wall defects using mineralized cancellous allograft under porcine barrier membranes after 4 months.

METHODS AND MATERIALS
This case series protocol was carried out with patient informed consent following guidelines according to the Helsinki Declaration of 1975, as revised in 2000. Subjects were between the ages of 25 and 70. Subjects excluded were those with active periodontal disease, evident periapical radiolucencies or abscesses, or autoimmune disorders; those taking bisphosphonate medications for osteoporosis; those with congenital or metabolic bone disorders or uncontrolled diabetes; smokers; and pregnant women. Two male patients and 12 female patients participated in this study. Surgeries were carried out with monitored intravenous sedation using an automatic pulse oximeter displaying heart rate, electrocardiogram readings, oxygen saturation, and blood pressure. Sedation was initiated with intravenous injection of medications that were titrated to oxygen saturation, and blood pressure. Sedation was initiated with intravenous injection of medications that were titrated to oxygen saturation, and blood pressure. Sedation was initiated with intravenous injection of medications that were titrated to oxygen saturation, and blood pressure. Sedation was initiated with intravenous injection of medications that were titrated to oxygen saturation, and blood pressure. Sedation was initiated with intravenous injection of medications that were titrated to oxygen saturation, and blood pressure. Sedation was initiated with intravenous injection of medications that were titrated to oxygen saturation, and blood pressure. Sedation was initiated with intravenous injection of medications that were titrated to oxygen saturation, and blood pressure. Sedation was initiated with intravenous injection of medications that were titrated to oxygen saturation, and blood pressure. Sedation was initiated with intravenous injection of medications that were titrated to oxygen saturation, and blood pressure.

After a soft tissue punch access was completed (Figure 8), a 10-mm-long trephine with a 2.0-mm internal diameter (Salvin Dental Specialties) was used to harvest bone cores for histomorphometric analysis as the first step in the implant osteotomy (Figure 9). The osteotomy was then completed using a flapless technique, increasing in size up to the final drill corresponding to the diameter of the implant chosen for the site. Each implant (Internal RBT Laser-Lok, BioHorizons, Birmingham, Ala) was stable upon seating to a maximum torque of 55 Ncm or less (Figure 10). Uncovering was performed after 4 months with a soft tissue punch, and healing abutments were placed (Figure 11).

SURGICAL METHOD
Atraumatic extraction of unsalvageable teeth was performed by elevating a full-thickness flap and sectioning horizontally to remove the clinical crown (Figure 1). The roots were then separated with Piezoelectric inserts (Piezosurgery Inc, Columbus, Ohio), fissure burs, periotomes, and elevators. Sockets were debrided of epithelial remnants (Figure 2) and filled with human particulate mineralized cancellous allograft bone (MCAB), particle size 1000 to 2000 μm (OraGRAFT, LifeNet Health, Virginia Beach, Va). Sterile normal saline was used to wet the particulate bone graft. The mixture of bone and liquid was then placed with light compression to completely fill the extraction site (Figure 3).

Porcine collagen membranes (PCM) (Renovix, Salvin Dental Specialties, Charlotte, NC) were cut to the appropriate shape to cover each socket site, extending 5 mm past the socket rim when used to cover intact sockets, and extended as necessary to completely cover bone wall defects (Figure 4). The membranes were hydrated with sterile saline for 1 minute, according to the manufacturer’s recommendations. Flaps were then released with full-thickness dissection, and tissue spreading was performed using curved scissors and periosteal release. Wherever possible, flaps were completely closed passively over each site with continuous mattress polytetrafluoroethylene 4-0 sutures (Figure 5) (Cytoplast, Osteogenics, Lubbock, Tex). Augmentin (Glaxo Smith Kline, Brentford, UK) antibiotic and hydrocodone with ibuprofen for analgesia were prescribed postsurgically for 5 days for all subjects. Each socket site was allowed to heal for 16 weeks before reentry. At the time of reentry for implant placement, a digital periapical radiograph (Figure 6) and a 3-dimensional cone-beam computerized tomographic scan (Prexion, San Mateo, Calif) (Figure 7) were taken before implant placement surgery. The scans were converted to DICOM (Digital Imaging and Communications in Medicine) 3 format and were used for surgical guidance. Implant dimensions were chosen after measuring the width and height of each site. Proprietary software was used to position an implant outline completely within the surrounding bone in each region of interest. The density in Hounsfield units (HUs) was then read using Prexion’s proprietary software. The area of the graft selected for density measurement was within the schematic implant outline chosen, using the coronal slice view.

Histologic Preparation Description
Bone specimens contained within the trephines were placed immediately into 10% buffered formalin, then dehydrated in an ascending series of alcohol rinses and embedded in methyl methacrylate resin. They were then thick sectioned longitudinally (coronal to apical) to collect 3 slides per specimen and ground and polished to approximately 35 μm thick using Donath’s method. 17,18

All ground sectioned slides were stained with Stevenel blue and Van Gieson picrofuchsin for light microscopy and histomorphometry analysis at ×40 magnification. One slide
from each thin-sectioned specimen was processed with hematoxylin and eosin, Goldner Trichrome, and Von Kossa/ MacNeal Tetrachrome staining. The histomorphometric analysis distinguished vital from nonvital bone by the presence of cells in the lacunae and red staining of vital bone (Figure 12).

RESULTS

This private practice–based case series included 14 patients, 12 women and 2 men. Twelve extraction sites were molar teeth and 2 were premolar teeth. None of the membranes was exfoliated prematurely, and none developed infection, including the maxillary sites where flap closure was not complete. Most of the unsalvageable teeth were endodontically treated and became unsalvageable due to root fractures. All augmented sites were reentered for bone trephine biopsy as the first step in implant placement after a minimum of 16 weeks following grafting. Because the reentry was flapless, the trephined specimen could have included septal bone in the molar sites. The trephined bone cores were composed of a combination of vital bone, nonvital residual graft material, connective tissue, and fibrous tissue. The histomorphometric data showed a mean value of 11.2% new bone with a range of 1.8% to 43%. Mean was 543 HU (range = 473–822 HU). Average seating torque was 46.8 Ncm (range = 40–55 Ncm). An Excel spreadsheet (Microsoft, Redmond, Wash) was used to calculate all values (Table).

DISCUSSION

The xenograft membrane and allograft particulate bone used for socket preservation in this case series were treated to remove cellular components in order to avoid rejection or infection. The mineralized cancellous bone allograft was prepared by a solvent cell extraction, ultrasonicification, and a centrifugation process with hypotonic reagents and antimicrobial solutions.

The PCM is type I collagen with cross-linking from certified pigs. It is prepared using standardized, controlled manufacturing processes. Sterilization is achieved after double packaging with gamma radiation. The material handles well, can be sutured, and is easily adapted to cover extraction-site defects.

Use of barrier membranes has multiple positive clinical benefits over grafted extraction sockets. They prevent soft tissue ingrowth that disrupts the ingress and maturation of osteogenic and endothelial cells into socket spaces. Loss of bone volume is also prevented, thus allowing for optimal
positioning and placement of larger-diameter implants without encroaching on the 1.8–2.0 mm width of bone that is important to maintain adjacent to implants. A significant clinical benefit from augmenting maxillary molar socket sites is the reduction in the need for sinus grafting in order to be able to place the desired length and width implants.

Primary closure was achieved whenever possible, as this is important in preventing infection complications that can interfere with the maturation of woven bone and result in decreased bone fill.

Resorbable or nonresorbable materials can be used as barriers over grafted extraction sites. Resorbable membranes include xenografts from bovine and porcine sources, polylactide, pericardium, and allograft acellular dermis matrix. Nonresorbable types include polytetrafluoroethylene and titanium mesh. These nonresorbable barrier membranes can lead to a high percent of complications due to infection and dehiscence. A second surgery could be necessary to remove the membrane, which risks loss of bone fill gained and healing complications.

Porcine collagen used as a socket graft barrier can generate increased height of keratinized gingival tissue. This thicker keratinized tissue is desirable around implants, as studies show that a lack of keratinized gingiva is associated with significantly more gingival inflammation, more plaque accumulation, adverse esthetic appearance, and more gingival recession.

Fenestration or dehiscence defects were found in 3 of the treated sites. The influence of wall defects compared with intact bony walls on socket graft regeneration is not possible to

| FIGURES 7–12. FIGURE 7. Three-dimensional cone beam computerized tomographic scan. FIGURE 8. Tissue punch starting osteotomy. FIGURE 9. Trephine with bone biopsy. FIGURE 10. Implant seated. FIGURE 11. Uncovering step at 4 months with healing abutment. FIGURE 12. Microphotograph of typical specimen from #30 site showing osteocytes present in lacunae. The specimen was taken longitudinally (apical to coronal), and stained with Stevenel blue and Van Gieson picrofuchsin. Vital bone stains red, nonvital bone and osteoid stain bright green. |

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<th>Mean values (ranges) for histomorphometric results, bone density in Hounsfield units (HUs), and seating torque for 14 subjects (42 specimens)</th>
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determine within the limits of this study, but it is recommended that this be investigated using a larger sample size with comparable bony wall defects in molar extraction sites. None of the membranes was exfoliated, and all sites healed well without discernible complications. The particulate allograft was contained well, and there was no loss of bone particles during the healing phase.

The new bone gain in the sites with the most bone regeneration compares favorably with results from a similar study by Barone et al in which 2 types of bovine xenografts were compared. Vital bone recorded was 28.5% ± 20% for the test group and 31.4% ± 18% for the control group. However these results were obtained after 6 months of socket graft healing: a longer period between socket grafting and implant placement would likely increase the percent of new bone present in grafted sockets.

Using cross-sectional imaging with all implant cases is identified as the standard of care in a position statement published by the American Academy of Oral and Maxillofacial Radiology. In this case series, cone-beam computed tomographic scans were taken for all sites just before implant placement surgery. The scans were very valuable as they allowed for the identification of anatomic landmarks, including sinus and inferior alveolar nerve locations, and allowed for accurate selection of the exact width and length implant that was optimal for each site. In addition, the density of each site was measured and recorded in Hounsfield units, helping to determine whether the graft material was well integrated. With these parameters identified, a flapless placement technique was used for each site. This has been shown to result in greater width of keratinized tissue and less horizontal bone loss than conventional flap access procedures. However, it must be noted that molar sites could have had sepal bone present in the trephined biopsy specimen because of flapless osteotomy.

This limited case series was designed to gather densitometric, histologic, histomorphometric, and computerized tomographic data from trephined bone specimens harvested from healed extraction sites that had been grafted with mineralized cancellous allograft bone and covered with a xenograft porcine barrier membrane.

**Conclusions**

Within the limits of this case series, porcine collagen membrane used as a barrier over extraction sites grafted with freeze-dried mineralized cancellous particulate allograft bone showed a wide range of new bone regeneration after 16 weeks. The results suggest the need to follow up with a larger sample size to obtain statistical relevance.

**Abbreviations**

HU: Hounsfield units  
MCAB: mineralized cancellous allograft bone  
PCM: porcine collagen membrane  
PDGF: platelet-derived growth factor

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**References**


