Histomorphometric and 3-D Cone-Beam Computed 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.

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ABSTRACT:

**Purpose:** The purpose of this study was to evaluate the results of socket preservation after extraction using human particulate mineralized cancellous bone (MCAB) and Type I porcine collagen membranes (PCM) as a guided bone regeneration barrier.

**Materials and Methods:**
Thirteen patients were selected that had a diagnosis of one or more unsalvageable teeth with a treatment plan to replace them with implant-supported single crown restorations. Extractions were done atraumatically by sectioning teeth for removal to avoid damage to the socket walls, and immediately placing mineralized cancellous allograft bone (MCAB) graft to fill the sockets. The socket opening was covered with porcine collagen membranes (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 computed tomographic scanning (CBCT).

**Results:** The formation of new bone in the treated sites averaged 19.4% with a range of 17% to 43% in bone biopsies trephined from the center of the grafted socket sites. The mean density measured with cone-beam computed tomography scans in Hounsfield Units (HU) was 537 HU with a range of 420 to 822.

**Conclusions:** The results indicated that in socket graft preservation using particulate MCAB and PCM, soft tissue ingrowth was blocked by the porcine collagen barrier. The resulting new bone regeneration averaged 19% at 16 weeks. Socket wall defects did not appear to influence the histological results, but the number of sites was too small to determine their significance.

**Key Words:** allograft bone, extraction site, porcine collagen, guided bone regeneration
The goal of socket preservation is to prevent the inevitable bone remodeling and resorption that takes place after extractions. Studies have documented that 12 months after extraction the width of the alveolar ridge decreased by 50%, 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 three-dimensional alveolar bone volume allows for ideal implant positioning that is required for esthetic and functional restorations.

Autogenous bone is the first choice for augmentation due to the inherent osteogenic, osteoconductive and osteoinductive properties. Limited quantities 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 non-membrane socket healing in a split-mouth study. Non-membrane extraction sites lost 4.6 mm in width, and 1.5 mm of vertical dimension, while sites treated with a resorbable membrane alone showed 1.32 mm loss in width and 0.38 mm loss in vertical dimension.

Significant differences were seen in a following study when using socket grafting was combined with covering the graft with a resorbable collagen membrane. Non-grafted 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 data base searches demonstrated that non-treated extraction sites compared to sites augmented and covered with a barrier membrane showed less vital bone and more resorption vertically and horizontally. Treated sites required less augmentation, and larger diameter implants could be placed.

A recent review reported that there are 144 bone substitutes identified, of which 93 were allografts, 30 were alloplasts and 21 were xenografts. Vital, vascular bone provides the initial mechanical support for implants and is required for sustained long term osseointegration, so human allograft bone is desirable as it generates a higher per cent of vital vascular bone earlier in socket grafts than alloplasts and xenografts.

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 to 32.5% in sites without PDGF after 4 months.

A recently published case series reported that rhBMP-2 on a collagen sponge and placed over socket sites produced 46.8% vital bone at 4 months with no filler material of any type placed into the sockets.

The purpose of this consecutive case series was to obtain densitometric, histologic and histomorphometric results from 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, autoimmune disorders, taking bisphosphonate medications for osteoporosis, having congenital or metabolic bone disorders, uncontrolled diabetics, smokers and pregnant females. One male patient and seven female patients participated in this study. Surgeries were carried out with monitored intravenous sedation using an automatic pulse oximeter displaying heart rate, electrocardiogram, oxygen saturation and blood pressure. Sedation was initiated with intravenous injection of medications that were titrated to induce and maintain the desired level of conscious sedation. Two percent (2%) lidocaine with 1:100,000 epinephrine (Novocol, Septodont Inc., Ontario, Canada) was used for block and local anesthesia.

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 Incorporated, Columbus, OH) fissure burs, periotomes and elevators. Sockets were debrided of epithelial remnants (Figure 2) and grafted to fill the sockets with particulate MCAB, particle size 1000 to 2000 microns (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 cover completely bone wall defects (Figure 4). The membranes were hydrated with sterile saline for one minute, according to the manufacturer’s recommendations. Flaps were then released with full thickness dissection and tissue spreading using curved scissors and periosteal release. Wherever possible, flaps were completely closed passively over each site with continuous mattress PTFE 4-0 (Figure 5) (Cytoplast™, Osteogenics, Lubbock, TX). Augmentin (Glaxo Smith Kline, UK) antibiotic and hydroxycodeine with ibuprofen for analgesia were prescribed for 5 days for all subjects post-surgically. 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 three-dimensional cone-beam computed tomographic scan (Prexion, San Mateo, CA) (Figure 7) were taken prior to implant placement surgery for surgical guidance, implant size selection, evaluation of bone width, height and measurement of the bone density in Hounsfield units. After a soft tissue punch access was completed (Figure 8), a 10 mm length trephine with a 2.0 mm internal diameter (Salvin Dental Specialties, Charlotte, NC) was used to harvest bone cores for histomorphometric analysis as the first step in the implant osteotomy (Figure 9). The osteotomy was then completed with 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 AL) was stable upon seating to a maximum torque of 50 newton-centimeters (Figure 10). Uncovering was done after 4 months with a soft tissue punch and healing abutments were placed (Figure 11).

HISTOLOGIC PREPARATION DESCRIPTION:

Bone specimens contained within the trephines were processed and embedded into methyl methacrylate (MMA) resin, thick sectioned longitudinally to collect 3 slides per specimen, and ground and polished to approximately 35 microns thick. All ground sectioned slides will be stained with Sanderson’s Rapid
Bone Stain and VanGieson picrofuchsin for light microscopy and histomorphometry analysis. One slide from each thin-sectioned specimen will be subjected to H and E, Goldner’s Trichrome and VonKossa/MacNeal’s Tetrachrome staining. The histomorphometric analysis 19% new bone, with vital bone distinguished from non-vital bone by the presence of cells in the lacunae (Figure 12).

RESULTS:

This private practice-based case series included 8 patients, 7 female, 1 male. 5 extraction sites were molar teeth, 3 extraction sites were premolar teeth. A majority 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. The trephined bone cores were composed of a combination of vital bone, non-vital residual graft material, connective tissue and fibrous tissue. The histomorphometric data showed a mean value of 19% new bone with a range of 19% to 43%. The density in Hounsfield units had a mean value of 537 HU. None of the PC membranes were exfoliated prematurely, and none developed infection, including the maxillary sites where flap closure was not complete.

DISCUSSION:

The xenograft membrane and allograft particulate bone used for socket preservation in this case series are treated to remove cellular components in order to avoid rejection or infection:

The mineralized cancellous bone allograft (MCAB) is prepared by a solvent cell extraction, ultrasonicification and a centrifugation process with hypotonic reagents and antimicrobial solutions.

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

Bone regeneration in extraction sockets proceeds with angiogenesis and ingrowth of osteogenic cells from peripheral bony walls. This vascularized tissue serves then as scaffolding for the development of woven bone maturing to lamellar bone throughout the space. There are multiple positive clinical benefits from utilization of barrier membranes over grafted extraction sockets. They prevent soft tissue ingrowth that would disrupt the ingress and maturation of osteogenic and endothelial cells into socket spaces.¹⁷

Loss of bone volume is 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.¹⁸

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 implants¹⁹.

Primary closure was achieved whenever possible, since this is important in preventing infection complications which can interfere with the maturation of woven bone, and result in decreased bone fill.²⁰

Materials used as barriers over grafted extraction sites can be resorbable or non-resorbable products. Resorbable membranes include xenografts from bovine and porcine sources, polylactide, pericardium and allograft acellular dermis matrix. Non-resorbable types include polytetrafluoroethylene and titanium mesh. These non-resorbable barrier membranes can lead to a high percent of complications due to
infection and dehiscence. A second surgery can be necessary for removal of the membrane, and this risks loss of bone fill gained and healing complications.\(^2^1\)

Porcine collagen used as a socket graft barrier can generate increased height of keratinized gingival tissue.\(^2^2\) This thicker keratinized tissue is desirable around implants, as reports show that a lack of keratinized gingiva is associated with significantly more gingival inflammation, more plaque accumulation, adverse esthetic appearance and more gingival recession.\(^2^3\)

There were fenestration or dehiscence defects in 4 of the treated sites. The influence of wall defects compared to intact bony walls on socket graft regeneration is not possible to determine within the limits of this study, but it is recommended to investigate this with a larger sample study with comparable bony wall defects in molar extraction sites. None of the membranes were exfoliated, and all sites healed well without discernible complications. The particulate allograft was contained well without loss of particles of bone during the healing phase.

The new bone gain of 19% compares favorably to results from a comparable study by Barone et al. Two types of bovine xenografts were compared. Vital bone recorded for the test group was 28.5% ± 20% and for the control group, 31.4% ± 18%. However these results were obtained after 6 months for the socket grafts.\(^2^4\)

A longer time period between socket grafting and implant placement would most 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.\(^2^5\)

In this case series, cone-beam c.t. scans were taken for all sites just prior to implant placement surgery. The c.t. scans were very valuable as they provided identification of anatomic landmarks, including sinus and inferior alveolar nerve locations, and allowed 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 that graft material was well integrated. With these parameters identified, 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.\(^2^6\)

This limited case series was designed to gather densitometric, histologic, histomorphometric and computed tomographic data from trephined bone specimens harvested from healed molar 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 can produce significant new bone regeneration after 16 weeks in molar extraction sites.

**DISCLOSURE:** Materials and financial support for this case series were provided by LifeNet Health, Salvin Dental Specialties and BioHorizons Inc.
Figure 1. Sectioned tooth prior to extraction

Figure 2. Defect after atraumatic extraction #30

Figure 3. Socket grafted with mineralized cancellous allograft

Figure 4. Renovix® fully covering grafted socket

Figure 5. Flap closed with PTFE 4-0 suture

Figure 6. Radiograph of grafted site at 16 weeks
Figure 7. 3-dimensional cone beam
C.T. scan

Figure 8. Tissue punch used to access
grafted site

Figure 9. Trephine with bone biopsy

Figure 10. Restored implant at #30 site

Figure 11. Healing abutment placed

Figure 12. Histological analysis shows
new vital bone

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REFERENCES:


