Equine-Derived Bone Mineral Matrix for Maxillary Sinus Floor Augmentation: A Clinical, Radiographic, Histologic, and Histomorphometric Case Series

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The objective of this proof-of-principle multicenter case series was to examine the bone regenerative potential of a newly introduced equine-derived bone mineral matrix (Equimat\textsuperscript{2}) to provide human sinus augmentation for the purpose of implant placement in the posterior maxilla. There were 10 patients requiring 12 maxillary sinus augmentations enrolled in this study. Histologic results at 6 months demonstrated abundant amounts of vital new bone in intimate contact with residual graft particles. Active bridging between residual graft particles with newly regenerated bone was routinely observed in intact core specimens. A mean value of 23.4% vital bone formation was observed at 6 months. This compared favorably with previous results using xenografts to produce bone in the maxillary sinus for the purpose of dental implant placement. Both the qualitative and quantitative results of this case series suggest comparable bone regenerative results at 6 months to bovine-derived xenografts.


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Sinus augmentation surgery for treating the atrophied posterior maxilla prior to implant placement is now considered the standard of care in surgical practice. Critical to successful outcomes is the regeneration of well-vascularized, healthy bone. Variables influencing regenerative outcomes in maxillary sinus augmentation surgery include the duration between subantral grafting and implant placement,\textsuperscript{1–11} the type of graft material used,\textsuperscript{1–10} the presence or absence of occlusive membranes over the lateral window osteotomy site,\textsuperscript{12–16} and whether resorbable or nonresorbable membranes are placed over the lateral osteotomy.\textsuperscript{12}

Although originally designed with autogenous bone as the graft source, bone graft substitutes, including allografts, xenografts, and alloplasts, have largely replaced autogenous grafts as effective alternatives in subantral grafting.\textsuperscript{1–11,17–20} In particular, bovine-derived xenograft bone mineral has been extensively used, either alone or as a composite graft with autogenous bone or other bone graft substitutes, in sinus augmentation procedures.\textsuperscript{1–3,6,7,9,12,15,16}
Multiple systematic reviews appear to verify excellent implant survival following sinus augmentation with 100% bovine-derived xenografts. In addition to implant survival, multiple case series studies have examined the quality and quantity of bone regeneration at numerous time points in xenograft-grafted sinuses. Reported values of bone regeneration vary from 13% at 3 to 4 months to 70% at 1 year or longer. Due to relatively low rates of resorption, the percent of residual xenograft particles generally remains high, a finding that may explain reduced graft slumping in bovine xenograft augmented sinuses.

A number of recently published studies have examined the safety and efficacy of equine-derived bone graft substitutes in treating significant periodontal defects, in postextraction ridge preservation procedures, and in augmenting the atrophied alveolar ridge. One such equine-derived bone graft substitute, Equimatrix (Equine Bone Mineral or EBM, Osteohealth), appears similar in structure and composition to other xenografts. EBM is a sterile, natural, nonantigenic, porous bone mineral matrix produced by removal of all organic compounds (proteins) from equine bone and is physically and chemically comparable to the mineralized matrix of human bone. The mineral matrix of EBM has a macro- and microporous structure similar to human bone, with a trabecular architecture that appears to favor the osteoconductive formation and in-growth of new bone.

The purpose of this proof-of-principle study was to examine histologically and histomorphometrically the bone regenerative potential of EBM in human sinus augmentation procedures for the treatment of significant posterior maxillary ridge atrophy.

Method and materials

Ten healthy patients (5 women and 5 men), ages ranging from 20 to 65 years (mean age, 55.4 years), were recruited from six different centers for this prospective case series study. Informed consent was reviewed with each patient at a separate consultation appointment, and each patient signed a consent form based on the Helsinki Declaration of 1975, as revised in 2000. Patients with 5 mm or less of posterior maxillary sub sinus alveolar bone height who requested implant-supported restorations were included in this study. Acute or chronic sinus disease, untreated periodontal disease, and acute or chronic systemic disease excluded patients from participating in this study.

At baseline, a comprehensive oral examination, full-mouth periapical and panoramic radiographs, clinical photographs, and maxillary computed tomography (CT) scans were performed (Fig 1). Under local anesthesia, following elevation of a full-thickness mucoperiosteal flap, a traditional maxillary lateral wall osteotomy approach to the sinus was accomplished. Piezosurgical instrumentation was used to create the lateral window osteotomy and to assist in elevation of the sinus membrane. Approximately 2 g of large particle EBM, saturated with sterile saline, were incrementally placed in each subantral space. A resorbable collagen membrane was then placed over the lateral window osteotomy site, and the mucoperiosteal flap was primarily closed with multiple expanded polytetrafluoroethylene sutures (CV-5, Gore-Tex, WL Gore & Associates). Patients rinsed with a 0.12% chlorhexidine solution and refrained from brushing or flossing the surgical sites until sutures were removed.

Patients were seen for postoperative follow-up at 1, 2, 4, 8, and 12 weeks and every 4 weeks thereafter until core biopsy specimens were obtained at 6 months following sinus grafting. No serious adverse events occurred during the course of the study. Core biopsy specimens 2 mm in diameter were obtained at implant insertion from the augmented alveolar ridge and were preserved and prepared for histologic evaluation. One to four implants were placed without incident in each posterior maxillary augmented site.

Light microscopy and histomorphometric analysis

The bone cores were embedded following complete dehydration in ascending grades of ethanol (60%, 80%, 96%, and absolute ethanol)
in a light-curing one-component composite resin (Technovit 7200 VLC, Heraeus Kulzer). Polymerized blocks were initially ground to bring the tissue components closer to the cutting surface. A 100-μm-thick section attached to the second slide was sawed with a diamond blade. The final thickness of 40 μm was achieved by grinding and final polishing with 1,200-, 2,400-, and 4,000-grit sandpaper. Sections from each block were used for Sanderson’s Rapid Bone Stain and acid fuchsin counterstain. Light microscopic overview images of the cores were taken digitally with a Leica M16 stereomicroscope (Leica Microsystems). Histomorphometric measurements were performed by using software (ImageAccess, Imagic) to calculate the percentages of mineralized bone, soft tissue components (connective tissue and/or bone marrow), and residual graft particles.

Results

In this proof-of-principle study, 12 maxillary subantral augmentation surgeries were performed. Healing was uneventful, with minimal soft tissue inflammation and no signs of infection. At 6 months, sufficient regenerated bone was present at each site for successful implant placement (Figs 2 and 3). Figures 4 and 5 show representative histologies of core biopsy specimens that
demonstrate the range of bone regenerative results seen at 6 months in this case series study.

Figure 4a represents an intact core obtained at the time of implant placement 6 months following subantral grafting. Large areas of newly regenerated bone surround and interconnect with intact EBM particles. Active bridging of newly formed bone is seen throughout the apical portion of the core specimen. Occlusally, native sub sinus alveolar bone is surrounded by broad areas of healthy marrow. No evidence of an inflammatory infiltrate is present in this core specimen. At higher magnification, well-formed vital bone is seen bridging intact EBM particles (Fig 4b). Vital osteocytes are seen throughout the newly regenerated bone. Intense osteogenesis is evidenced by areas of recently secreted osteoid originating from advancing fronts of adjacent osteoblasts. Healthy marrow is again noted throughout the specimen. At still higher magnification, osteocytes, indicative of healthy, vital bone, are readily apparent throughout the newly regenerated bony area. Osteoid is again noted along the regenerated bone margins, indicative of ongoing osteogenesis. Intact graft particles are
seen in intimate contact with newly regenerated bone. As in lower magnified views, inflammatory cells are notably absent (Fig 4c).

A second representative intact core demonstrates significant quantities of dense, mostly lamellar, newly regenerated bone in the apical portion of the specimen (Fig 5a). As in the first core, active bridging of newly regenerated bone is readily apparent. At higher magnification, newly formed bone is seen in intimate contact with residual EBM particles. Lacunae with vital osteocytes are seen throughout areas of regenerated bone, verifying the vitality of this newly formed bone (Fig 5b). Another higher magnified view emphasizes the intimate contact between EBM particles and recently regenerated bone. Of particular note are the abundant numbers of osteocytes present in this specimen, again emphasizing the health and vitality of the regenerated bone (Fig 5c).

**Histomorphometric results**

At 6 months following subantral grafting, histomorphometric quantitative results support the qualitative histologic findings. The mean histometric results of analyzed
cores are as follows: mean percent bone was 23.35%, mean percent residual graft particles was 15.68%, and mean percent marrow/connec-
tive tissue was 60.97%.

Discussion

Long-term clinical success of max-
illary subantral augmentation pro-
cedures is in large part dependent
upon the regeneration of vital,
well-vascularized bone.1–5,7–9,16,20,33
Bovine-derived bone mineral xe-
nografts have consistently dem-
onstrated successful long-term
implant survival when used alone
or in combination with other ma-
trices in sinus augmentation pro-
cedures.13,21–24 Evidence further
documents a range of values for ef-
effective percent new vital bone for-
mation at various time points when
bovine xenografts are used in sinus
augmentation procedures.1–3,6–8,10

The earliest documented time
point following subantral grafting
is generally 6 months, with mean
regenerated bone values rang-
ing from approximately 12.5% to
24%.1,2,12,16,34,35

In this proof-of-principle case
series, a newly introduced equine-
derived bone mineral matrix, with
physical and chemical characteris-
tics similar to other xenografts, was
used in multiple sinus augmenta-
tion procedures to increase poste-
rior maxillary alveolar ridge height
prior to implant placement. Study
outcomes included histomorpho-
metric and histologic findings at
6 months following grafting. At 6
months, newly regenerated bone
was surrounded by and in intimate
contact with residual EBM parti-
cles. Active bridging between EBM
particles with newly formed bone
was routinely observed in intact
core biopsy specimens. No histo-
logic evidence of an inflammatory
cellular infiltrate was evident in any
of the biopsy sites.

Histomorphometric values of
percent vital bone proved com-
parable to reported mean values
of bovine-derived bone mineral
xenografts. Ranging from 16.3% to
33.6%, with a mean value of
23.4% vital bone formation, EBM
in this initial case series appears
comparable to other bovine bone
mineral xenografts in terms of its
osteoconductive ability to support
new bone formation at 6 months in
sinus augmentation procedures.

Although the results of this
study are promising, longer-term
studies are needed to deter-
mine bone regenerative trends at
later time points following sinus
augmentation grafting. In addi-
tion, clinical studies examining
long-term implant survival under
function are needed to gain a com-
prehensive understanding of the
role EBM may play in correcting
maxillary posterior ridge atrophy.

Conclusion

Clinical and histologic evidence
supported the suitability of EBM
for maxillary sinus augmentations
that allowed subsequent dental
implant placement after a 6-month
healing period.

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