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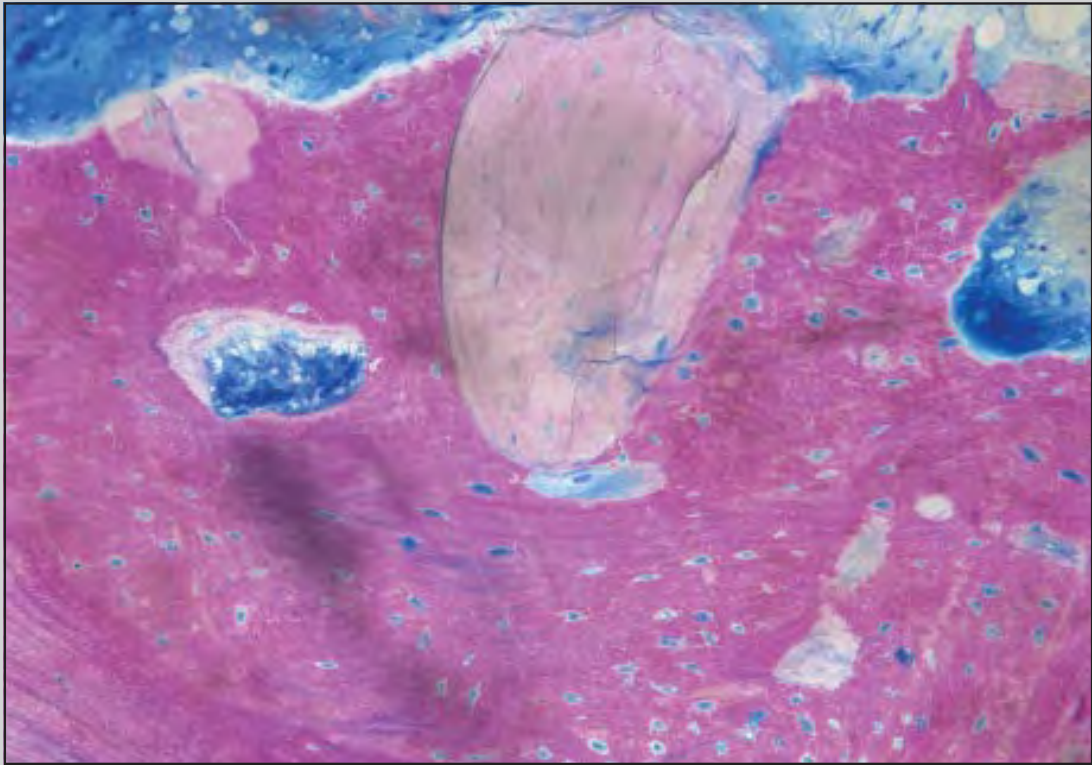
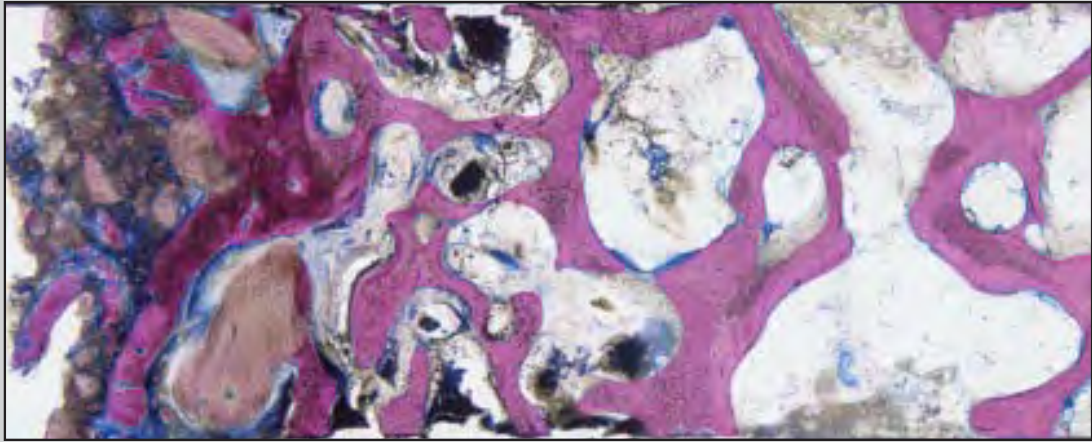
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Equine-Derived Bone Mineral Matrix for Maxillary Sinus Floor Augmentation: A Clinical, Radiographic, Histologic, and Histomorphometric Case Series



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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 (Equimatrix) 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. (Int J Periodontics Restorative Dent 2013;33:483–489. doi: 10.11607/prd.1728.)

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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,^{1–11} the type of graft material used,^{1–10} the presence or absence of occlusive membranes over the lateral window osteotomy site,^{12–16} and whether resorbable or nonresorbable membranes are placed over the lateral osteotomy.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.^{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.^{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.^{13,21-24}

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.^{1-3,6-8,10} 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.²⁵⁻³² 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 subsinus 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 sub-antral 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)



Fig 1 CT scan reveals an enlarged maxillary sinus with an alveolar crestal height of 2 to 3 mm.



Fig 2 Six-month CT scan reveals significant increase in bone height with no evidence of slumping.



Fig 3a Six months following sinus augmentation, two Biomet 3i Prevail implants, 5/4 × 11.5 mm, are placed into the grafted posterior maxilla.

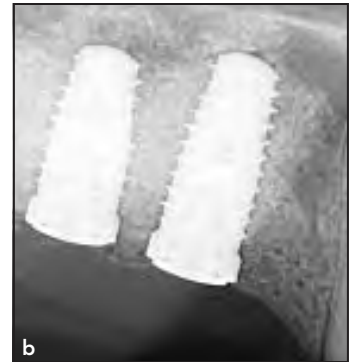


Fig 3b Periapical radiograph at 6 months confirms excellent implant position.

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- μ m-thick section attached to the second slide was sawed with a diamond blade. The final thickness of 40 μ m 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

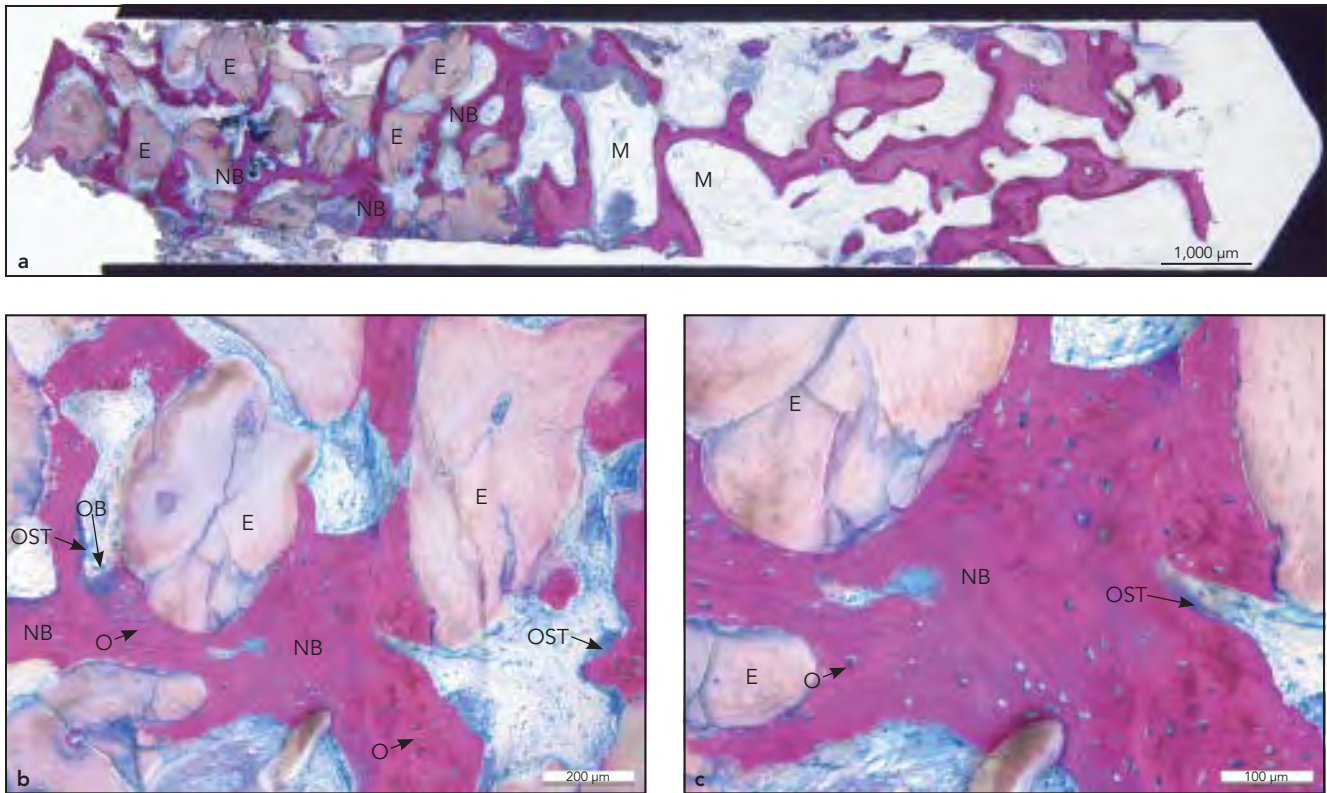


Fig 4a Implant biopsy core at 6 months reveals large areas of newly formed lamellar bone surrounding and interconnecting with intact EBM particles. E = EBM particle; M = marrow; NB = new bone.

Fig 4b Higher magnification demonstrates well-formed vital bone bridging intact EBM particles. E = EBM particle; NB = new bone; O = osteocyte; OB = osteoblast; OST = osteoid.

Fig 4c Osteocytes, indicative of healthy, vital bone, are readily seen in this high power view at 6 months following EBM grafting. E = EBM particle; NB = new bone; O = osteocyte; OB = osteoblast; OST = osteoid.

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 subsinus 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

Fig 5a Six-month core biopsy specimen demonstrates healthy regenerated lamellar bone bridging gaps between EBM particles. E = EBM particle; NB = new bone; O = osteocyte; M = marrow; NAB = native bone.

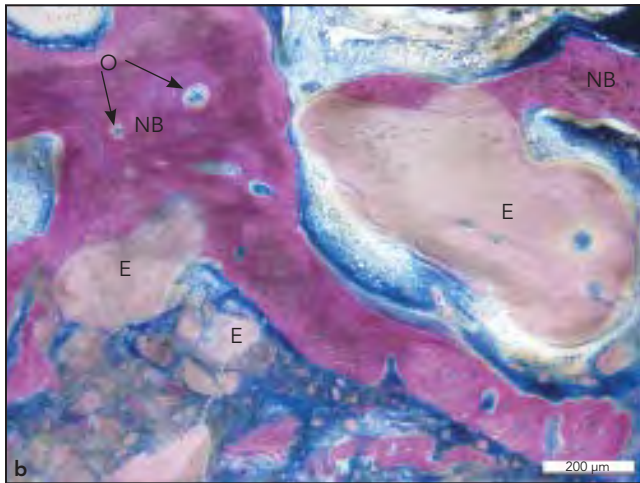
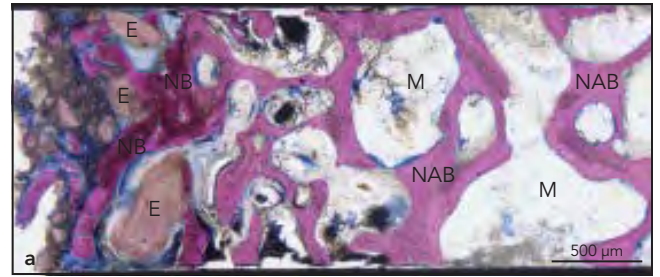


Fig 5b Magnified view reveals healthy osteocytes in all areas of the regenerated, vital bone. E = EBM particle; NB = new bone; O = osteocyte; M = marrow.

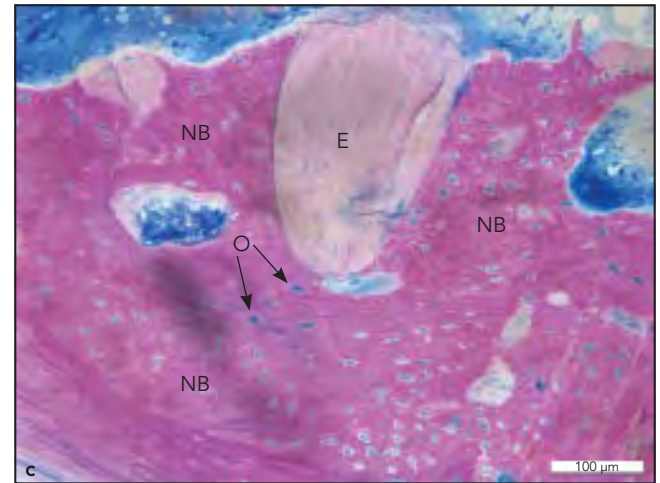


Fig 5c Healthy newly formed bone with many viable osteocytes forms intimate contact with an EBM particle. E = EBM particle; NB = new bone; O = osteocyte.

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. 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/connective tissue was 60.97%.

Discussion

Long-term clinical success of maxillary subantral augmentation procedures is in large part dependent upon the regeneration of vital, well-vascularized bone.^{1-5,7-9,16,20,33} Bovine-derived bone mineral xenografts have consistently demonstrated successful long-term implant survival when used alone or in combination with other matrices in sinus augmentation procedures.^{13,21-24} Evidence further documents a range of values for effective percent new vital bone formation 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 ranging 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 characteristics similar to other xenografts, was used in multiple sinus augmentation procedures to increase posterior maxillary alveolar ridge height prior to implant placement. Study outcomes included histomorphometric and histologic findings at 6 months following grafting. At 6 months, newly regenerated bone

was surrounded by and in intimate contact with residual EBM particles. Active bridging between EBM particles with newly formed bone was routinely observed in intact core biopsy specimens. No histologic evidence of an inflammatory cellular infiltrate was evident in any of the biopsy sites.

Histomorphometric values of percent vital bone proved comparable 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 determine bone regenerative trends at later time points following sinus augmentation grafting. In addition, clinical studies examining long-term implant survival under function are needed to gain a comprehensive 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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