

The Feasibility of Demineralized Bone Matrix and Cancellous Bone Chips in Conjunction with an Extracellular Matrix Membrane for Alveolar Ridge Preservation: A Case Series



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An investigation was conducted to test the feasibility of demineralized bone matrix and cancellous bone chips in a reverse-phase medium carrier (DynaBlast) in concert with an extracellular matrix membrane (DynaMatrix) to provide hard and soft tissue regeneration for the purpose of a ridge preservation procedure. Nine patients requiring extraction of 30 maxillary teeth were grafted with DynaBlast and DynaMatrix. Twenty sites attained primary flap closure over the grafted area (primary healing intention group), while 10 sites were assigned randomly to the secondary healing intention group, in which primary flap closure over the membrane was not achieved. Clinical and radiographic evaluations at 6 months revealed comparable bone formation for both groups. Histologic analyses of 21 harvested soft and hard tissue core biopsies revealed absence of the remnant membrane and consistent patterns of new bone formation. The efficacy and safety of DynaBlast and DynaMatrix have been validated clinically and histologically to preserve the dimensions of the alveolar process. (Int J Periodontics Restorative Dent 2011;31:39–47.)

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Resorption of the postextraction alveolar ridge has been reported to result in unfavorable morphogenic and volumetric changes.^{1–11} The predictability of ridge preservation procedures employing bone graft materials in conjunction with barrier membranes to prevent bone resorption is especially important in prominent rooted maxillary anterior teeth.¹⁰

Demineralized freeze-dried bone allograft (DFDBA), also known as demineralized bone matrix (DBM), has been considered previously to simulate the osteogenic effects of autologous bone.¹² The biologic activity of DBM is presumably attributable to proteins and various growth factors present in the extracellular matrix and made available to the host environment by the demineralization process. The efficacy of DFDBA or DBM in ridge preservation procedures has been documented clinically.^{13–20} DBM has been formulated with both synthetic and natural carriers, such as poloxamer 407, collagen, gelatin, glycerol, and sodium hyaluronate, to provide improved handling and delivery properties.



Fig 1 (left) Patient with large caries lesions and failing root canal treatments on the maxillary central and lateral incisors. Implant treatment was requested to replace these unrestorable teeth.

Fig 2 (below) Atraumatic extraction was performed to preserve the thin buccal plates, and the sockets were thoroughly curetted.



An exclusionary barrier membrane promotes selective osteogenic cells to proliferate, since it prevents downgrowth of soft tissue entities. Therefore, the structural integrity of the membrane and its biocompatibility to the underlying graft material are essential for early wound healing. To date, instances of early membrane exposure have demonstrated impaired regenerative outcomes resulting in compromised bone quality and quantity.^{21,22} Soft tissue dehiscences resulting in spontaneous membrane exposures have been reported to range from 28% to 40%.^{23,24} Furthermore, most bioabsorbable barrier membranes are not intended to be left exposed during the healing period.²⁵

Clinical and radiographic success of regenerative biomaterials that can be verified through histologic and histomorphometric evaluations provide a high level of confidence to the clinician. The primary objective of this case series was to demonstrate the feasibility of DBM and cancellous bone chips in a reverse-phase medium carrier (DynaBlast, Keystone Dental), in conjunction with an extracellular matrix membrane obtained from the submucosa of the small intestine of pigs (DynaMatrix, Keystone Dental), to provide hard and soft tissue regeneration for a ridge preservation procedure. The secondary objective was to determine the safety and efficacy of hard tissue regeneration in sites that were augmented with an exposed DynaMatrix membrane compared to sites that received primary flap closure over the DynaMatrix membrane.

Method and materials

Nine patients (four men, five women; age range, 32 to 61 years) requiring localized ridge preservation procedures after extraction of maxillary teeth (n = 30) participated in this outpatient study. All subjects required removal of one or more teeth, and subsequent implant therapy was planned for tooth replacement (Fig 1). Patients were systemically healthy with no surgical contraindications. Oral and written explanations of the study, including the risks, benefits, and alternative therapies, were provided. All patients volunteered for the protocol and signed an informed consent form based on the Helsinki declaration of 1975, as revised in 2000. Preoperative periapical radiographs and computed tomography (CT) scans were taken for all subjects prior to the initiation of the study.



Fig 3a DynaBlast was injected and filled to the level of the adjacent crest.



Fig 3b DynaMatrix was used as a barrier membrane to protect the underlying grafting material.



Fig 4 The membrane was left exposed intentionally at the maxillary left central incisor site (secondary intention healing group), while primary flap closure was obtained at the left lateral incisor (primary healing intention group).

The surgical sites were anesthetized with 2% xylocaine with 1:100,000 epinephrine. An intrasulcular incision was followed by a buccal full-thickness flap. Atraumatic extractions were performed and the postextraction buccal plates were investigated for dehiscence and fenestration (Fig 2). Those with a thin or partially missing labial plate were selected to undergo the proposed ridge preservation procedure.

Thirty extraction sockets were divided randomly into two treatment groups. All sites received grafting with DynaBlast and DynaMatrix. DynaBlast was injected and filled to the level of the adjacent crest with minimal compression to allow for passive fill, and a barrier membrane (DynaMatrix) was trimmed and contoured to fit over the grafted site (Figs 3a and 3b). The buccal flap was mobilized to allow for tension-

free primary flap closure over the membrane for the primary healing intention group (group A, 20 extraction sockets) (Fig 4). The secondary healing intention group (group B) consisted of 10 grafted extraction sockets, and no attempts were made to advance the flap to cover the entire membrane. The size of the exposed areas varied from approximately 2×5 mm to 5×7 mm. Simple interrupted 3-0 silk sutures (Ethicon) were used to position the flap over the augmented area and followed with verbal and written postoperative instructions. Appropriate antibiotics (500 mg amoxicillin, 3 times daily for 5 days or 300 mg clindamycin, 3 times daily for 5 days), analgesics (800 mg ibuprofen, 3 times daily for 5 days), and mouth wash (0.12% chlorhexidine digluconate, 2 times daily for 2 weeks) were prescribed. Sutures were removed

7 to 10 days postsurgery, and patients were seen at regular intervals during the 6 months of healing.

A postoperative CT scan was obtained 6 months after the ridge preservation procedure prior to implant surgery. Soft and hard tissue biopsies of the grafted area were obtained using a 3-mm trephine bur (Biomet 3i) along the long axis of the treated site prior to implant placement ($n = 21$; PrimaConnex Straight Implants, Keystone Dental). Twenty-one cores were obtained: 13 from group A and 8 cores from group B. They were placed immediately in formalin and shipped to a histologist for detailed histologic and histomorphometric evaluations.

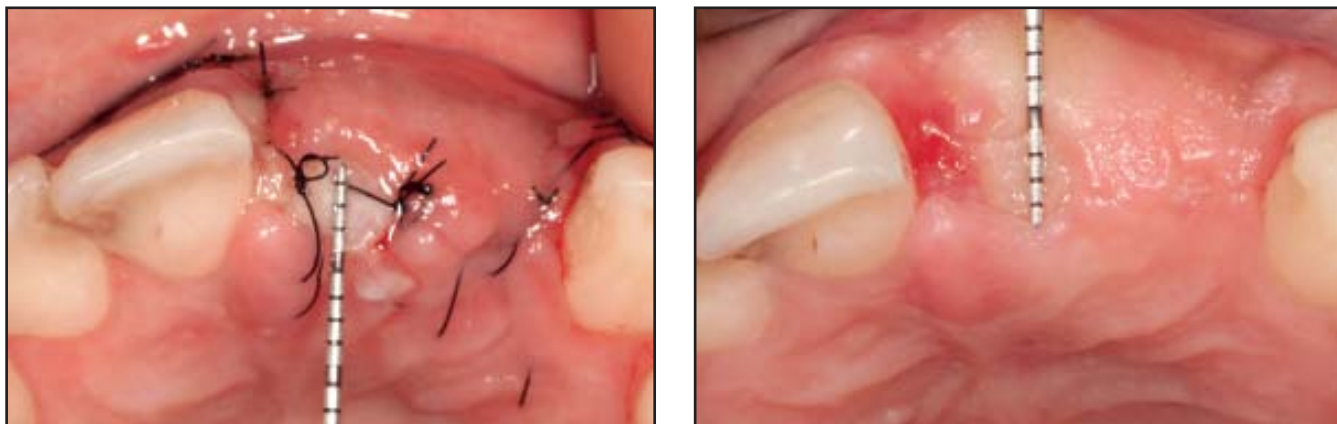


Fig 5 Early wound healing observed throughout the healing period at (left) day 7 and (right) day 18 without any obvious signs of complication.

Light microscopy

Histologic procession was carried out on 21 biopsies. The cores were embedded following complete dehydration in ascending grades of ethanol in a light-curing one-component 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 cut by using a diamond blade saw and 50 to 100 g of pressure. A 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 toluidine blue/pyronin G staining without deplastication.

Results

Clinical and radiographic evaluations

A total of nine patients requiring extraction of 30 maxillary teeth underwent simultaneous ridge preservation procedures with DynaBlast and DynaMatrix. All subjects completed the study, and no adverse events or complications were reported. Throughout the course of the healing phase, both primary (group A) and secondary healing intention sites (group B) underwent a similar clinical and radiographic healing. Group B demonstrated early epithelization over the exposed membrane at 2 weeks (Fig 5). The healed tissue over the exposed membrane sites



Fig 6 Six-month postsurgical CT scans revealed preserved ridge dimensions of the sockets, as well as radiographic evidence of bone formation and maturation.



Fig 7 Restored dentition at 1 year.

was pink in color, without evidence of tissue infection or inflammation at 4 weeks. None of the exposed membranes showed signs of infection nor had to be removed prematurely.

The CT scan evaluation at 6 months after ridge preservation demonstrated various degrees of radiographic bone maturation (Fig 6). Some grafted areas were indistinguishable from the neighboring native bone, while others revealed dispersed areas of radiolucency, indicating immature bone formation.

The soft tissue that formed over the exposed membrane area appeared to be firm in texture at the 6-month soft and hard tissue biopsies. The regenerated bone also appeared to be dense and firm and offered significant resistance to the

trepine bur. A full-thickness flap was elevated to clinically reenter the grafted site after the biopsy. Clinical reentry revealed that the dimensions of the ridge appeared to be maintained in both groups. Internal socket bone fill was evident for both groups, and most augmented sites were indistinguishable from the original neighboring bony tissue. The buccolingual dimensions of the augmented alveolar ridge allowed placement of dental implants that were at least 4 mm in width (implant diameters ranged from 4 to 5 mm; lengths ranged from 10 to 13 mm). Implants were placed successfully in all sites, irrespective of the treatment. All implants were placed uneventfully and have been restored (Fig 7). Overall, clinical and radiographic examinations revealed

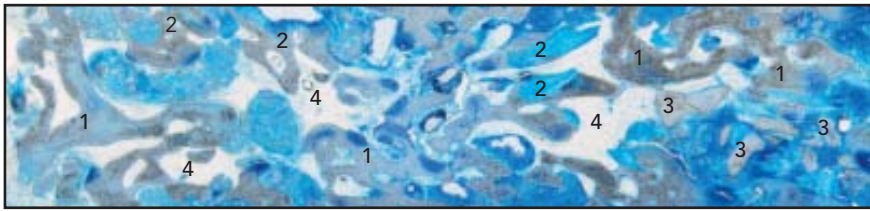


Fig 8a Ground section of a specimen at 6 months (secondary healing intention group) showing (1) newly formed bone, (2) DBM particles undergoing mineralization, (3) cancellous bone chips, and (4) marrow space.

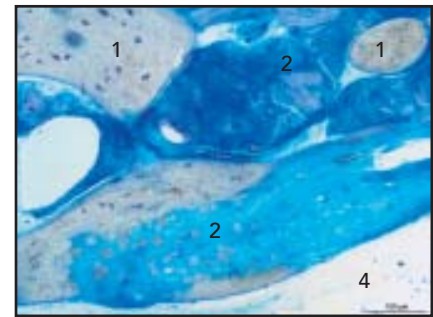


Fig 8b (right) Higher magnification view of Fig 7a revealed (1) subsequent new bone formation and (2) an acellular remineralization of DBM. 4 = marrow space.



Fig 9 (top) Light microscopic overview and (bottom) backscatter scanning electron microscopy overview showing cores obtained from another secondary healing intention group. Note the presence of soft connective tissue (CT) covering the mineralized augmented area composed of (1) newly formed bone, (2) DBM particles undergoing mineralization, (3) cancellous bone chips, and (4) marrow chambers. Also note the perceived bone density improvement proceeding from the coronal to apical direction.



that this combination of biomaterials appeared to prevent buccal plate resorption, even in sockets with obvious dehiscences and fenestrations.

Light microscopy

Histologic analysis was implemented to determine the presence of the membrane, signs of inflammation or adverse reaction of the surrounding tissues, new bone formation, and remodeling of the grafted area under the membrane (Figs 8 to 10). No obvious signs of local inflammation or other adverse effects on the

surrounding tissues, or membrane remnants, were observed (Fig 10b). The connective tissue was devoid of inflammatory cell infiltrates but was characterized by the presence of a dense network of collagen fibers. There was perceived bone density improvement proceeding from the coronal to apical direction. Evidence of more mature bone formation was found in the primary healing intention group (Figs 10a, 10c, and 10d).

Three distinctive patterns of mineralization processes that were reported previously by Groeneveld et al with DFDBA in sinus augmentation²⁶ and by Kim et al with DynaBlast

also in sinus augmentation²⁷ were observed in the current study. The first mineralization pattern revealed osteoconduction on the surface of cancellous bone chips and DBM and bridging between the two biomaterials. A second mineralization pattern observed demonstrated remineralization of the DBM particle starting in the particle center, whereby the mineralization front was following the lamellar organization of the DBM particle. A third mineralization pattern demonstrated the remineralization process starting at the periphery of DBM particles and advancing toward the inner area.



Fig 10a Ground section of a specimen at 6 months (primary healing intention group) demonstrating a robust regeneration of newly formed bone and minimal amounts of remaining graft particles (DBM). E = keratinized epithelium; CT = connective tissue; B = bone.

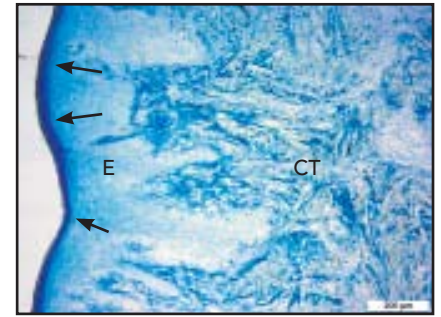
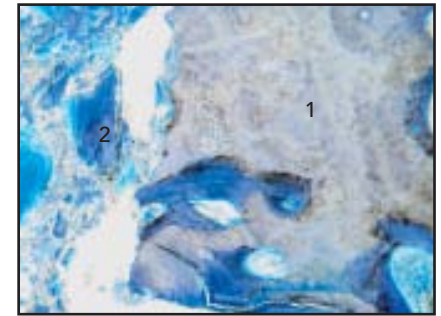
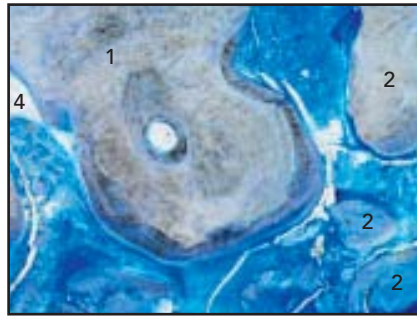


Fig 10b (right) High-magnification view of the soft tissue covering the grafted area in the primary healing intention group (see Fig 10a). Note the presence of a keratinized epithelium (E) with a well-developed keratinized layer (arrows) and the presence of a dense network of collagen fibers in the connective tissue (CT). Also note that the connective tissue is devoid of inflammatory cell infiltrates. Membrane remnants were not found.

Figs 10c and 10d Higher magnification view of the primary healing group specimen showing (1) newly formed bone along with (2) the presence of DBM undergoing the remineralization process. 4 = marrow space.



Discussion

Preservation or reconstruction of the extraction socket according to the principles of guided bone regeneration allows sufficient alveolar bone volume and favorable ridge architecture for implant placement. Even though favorable results have been reported with a wide array of biomaterials currently being used in ridge preservation procedures, it is essential to test new biomaterials in a clinical setting to rule out slow graft resorption and remodeling processes that can be detrimental in bone regeneration. A well-contained four-

wall extraction socket defect with a good vascular supply is an ideal site for osteoconductive bone graft material. Areas with buccal plate dehiscence or fenestration may require both osteoconductive and osteoinductive materials to achieve acceptable results for implant placement.

DynaBlast has been found previously to be a safe and efficacious augmentation material for the sinus augmentation procedure by the means of histologic and histomorphometric analyses.²⁷ There is a clinical and radiographic confirmation of new bone formation via three distinctive mineralization patterns. The

ultimate bone substitute should conduct or induce new bone formation and eventually show time-dependent resorption and replacement by the new bone.

All barrier membranes used in ridge preservation procedures should provide barrier biocompatibility, tissue integration, cell occlusion, space-making ability, shape adaptability, and simple application.²⁸ The DynaMatrix extracellular matrix membrane is obtained from the submucosa of the small intestine of pigs using a process that retains the natural composition of matrix molecules, such as collagens (types I, III, IV, and VI), glycosaminoglycans, glycoproteins, proteoglycans, and growth factors.^{29,30} A study that tested the feasibility of DynaMatrix compared to an autogenous gingival graft in increasing the width of attached keratinized tissue revealed that both procedures were effective and predictable.³¹ A unique finding from the current study was that some ridge preservation sockets did not receive primary coverage over the membrane but still obtained similar clinical and radiographic findings when compared to the primary flap closure group. Spontaneous exposure of the membranes in both primary and secondary healing intention groups, which has been commonly reported for cross-linked and non-cross-linked collagen materials,^{23,24} was not observed. It appears that DynaMatrix (exposed or not) can serve as a barrier to sufficiently support hard tissue regeneration.

The clinical and histologic findings observed in this study seem to reveal that commercially available

DynaBlast has the potential to demonstrate both osteoconductive and osteoinductive potentials for appositional new bone growth, as well as promoting migration of osteoprogenitor cells to the DBM.

Conclusion

Clinical and histologic evaluations of the DynaBlast bone grafting material and DynaMatrix membrane used in this investigation promoted the preservation of the postextraction ridges after a 6-month healing period to receive dental implants. Grafted sites that healed by secondary healing intention revealed similar clinical and radiographic findings as sites healed by primary healing intention. Exposed areas of the barrier membrane appeared to be clinically re-epithelialized 2 to 4 weeks after membrane exposure.

Acknowledgments

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