A Histologic and Clinical Evaluation of Ridge Preservation Following Grafting with Demineralized Bone Matrix, Cancellous Bone Chips, and Resorbable Extracellular Matrix Membrane

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In an attempt to reduce postextraction alveolar bone resorption, ridge preservation grafting procedures with or without resorbable membranes have become standard-of-care treatments following tooth removal. This prospective case series examined histologic and clinical outcomes following socket grafting with a syringeable paste allograft and a resorbable extracellular matrix membrane at three different time periods following postextraction grafting: 6, 12, and 24 weeks. At each time period, bone core specimens were retrieved for microscopic examination, and implants were placed. Following prosthetic restoration, implants were monitored under long-term occlusal function. At all three time periods, histologic results revealed active bone regeneration. At 6 weeks, localized areas of woven bone were evident, although nonmineralized osteoid was the dominant feature. At 12 and 24 weeks, regenerated woven bone dominated the histologic landscape, with increasing amounts evident in the latter specimens. Regardless of when implants were placed following grafting, implant survival under function occurred. (Int J Periodontics Restorative Dent 2012;32:543-552.)

Successful implant placement requires adequate alveolar ridge volume. Effective management of postextraction sockets and any associated defects is necessary to provide sufficient bone for implant placement. Multiple causes of postextraction bone resorption are known, including bundle bone resorption, thin and often dehisced labial or buccal bone, preexisting periodontal disease, traumatic extractions, and soft tissue flap reflection during tooth removal.1-10

Crestal bone resorption is an unavoidable consequence of tooth loss, with much of the remodeling occurring within the first 3 months following tooth removal.1,2 In the maxillary esthetic zone, volumetric bone loss is particularly severe during the first 6 months following tooth extraction and continues over time, with as much as an additional 11% of volumetric loss during the following 5 years.11,12 After 1 year without treatment, up to 50% loss of alveolar crestal bone width will likely occur, especially in the esthetically critical anterior maxilla.5,13-15 In a recent systematic
review, alveolar bony dimensional changes following tooth removal were examined. Across multiple studies, reductions in alveolar width and height consistently occurred, with loss of width being more severe than loss of height.3

Postextraction bone resorption can significantly compromise implant placement, implant-related esthetics, and long-term implant survival under function. Although immediate placement of implants following tooth removal is an accepted protocol when warranted, various ridge preservation protocols are often indicated prior to implant insertion. Multiple case reports and case series have examined the effectiveness of these protocols in diminishing postextraction bone resorption and preserving the hard tissue morphology critical for effective implant placement.1,5,8,13,16-22 Common to these protocols is the use of osteoconductive and, at times, osteoinductive matrices, including autogenous bone, mineralized and demineralized freeze-dried or irradiated allograft, bovine bone mineral, alloplasts, and others.16,20,23-34 Along with varying graft matrices, time from graft placement to implant insertion varies widely and is generally reported to be from 2 to 12 months.26,35 Although robust bone regeneration is a shared goal of all postextraction grafting protocols, sparse information is available describing histologic differences at various time intervals following ridge preservation grafting. In addition, little is known regarding implant survival related to different implant insertion times following ridge preservation procedures.

The primary purpose of this case series was to examine the bone regenerative outcomes at three different time intervals following ridge preservation grafting with DynaBlast (Keystone Dental), an FDA-cleared demineralized allograft bone matrix, and DynaMatrix extracellular matrix resorbable membrane (Keystone Dental). DynaBlast (DBM) is a composite graft material of de-mineralized bone and mineralized cancellous bone chips derived from the same donor and configured as either a putty or an injectable paste in a reverse phase resorbable medium. The 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 types I, III, IV, and VI collagens, glycoproteins, proteoglycans, glycosaminoglycans, and growth factors.36,37 The secondary purpose of this study was to examine the survival of implants placed at each of the different time intervals of abutment connection and at later time points under occlusal function.

Method and materials

Seventeen patients (5 men, 12 women; age range, 32 to 69 years) who met the inclusion criteria were included in this study. All patients either had no significant systemic disease or were medically well controlled and taking no steroids, bisphosphonates, or chemotherapeutic drugs and had stopped cigarette, cigar, or pipe smoking at least 3 weeks prior to study initiation. Each patient required a single tooth extraction followed by socket grafting for ridge preservation. Third molar and mandibular incisor teeth were excluded, as were acutely infected teeth. Patients were assigned randomly to one of three treatment groups depending on the time interval between socket grafting and implant placement and core retrieval: group A (6 weeks), group B (12 weeks), or group C (24 weeks). Implant survival was examined at abutment connection and under function at various long-term time intervals.

Following thorough discussion of all surgical procedures as well as potential risks, each patient agreed to proceed, and an informed consent form was signed based on the Helsinki Declaration of 1975, as revised in 2000.

Defect characterization and initial examination

Treated sites were limited to single maxillary or mandibular extraction sockets. The majority of sites had intact buccal walls with adjacent teeth that had little to no interden tal bone loss. Two sites exhibited severe buccal cortical bone loss and two had the coronal third missing. Tooth extractions in all cases were considered minimally traumatic, with mucoperiosteal flap reflection allowed as needed to
position the membrane 2 to 3 mm over the bone margin and under the soft tissue margin. Those sites with more extensive bone loss required greater mucoperiosteal flap reflection to facilitate proper membrane placement over the graft and bone margins. No attempt was made to obtain primary soft tissue closure.

For all patients, a standard head, neck, and oral clinical examination was performed. Periapical radiographs, computed tomography scans, and preoperative photographs of the proposed extraction site were obtained at the screening visit and at later time points during the study.

**Tooth extraction, ridge preservation, and core retrieval procedures**

Following administration of local anesthesia, teeth were removed atraumatically, followed by thorough socket debridement. Using a no. 1/4 round bur, internal cortical socket walls were perforated to induce moderate venous bleeding. DBM paste was then syringed into the socket to the alveolar crest (Fig 1). An extracellular membrane was then placed to cover the grafted sites and host bone margin, and the flaps were repositioned, but not closed primarily, via multiple 4.0 resorbable sutures (Fig 2).
Depending on the randomly selected group, bone core removal, including the overlying epithelium, and implant placement were completed at 6, 12, or 24 weeks ± 7 days following socket grafting (Fig 3). Retrieved bone cores remained in the trephines and were placed immediately into formaldehyde and sent for microscopic examination. Bone and tissue level implant insertion (Keystone Dental) was facilitated by the EasyGuide (Keystone Dental) computer-generated implant placement system, which is capable of generating a rigid surgical implant osteotomy guide. A primary implant stability of at least 35 Ncm was achieved for all 17 implants.

Abutment connection and implant restoration time intervals varied from 2.5 to 6 months following implant placement. Figure 4 shows the 6-month radiograph of a group B subject in whom abutment connection and implant restoration occurred 3 months following implant placement. In this example, the total time for tooth extraction, socket grafting, and definitive implant-supported restoration delivery was 6 months.

**Light microscopy**

All cores from groups A, B, and C were fixed in formaldehyde, dehydrated in an increasing series of alcohol rinses, infiltrated with Tecnovit 7200 + BPO resin (Heraeus Kulzer), and embedded. Undecalcified ground sections (one for each trephine) were made with a thickness of approximately 50 μm and stained with Sanderson's Rapid Bone Stain (Dorn & Hart Microedge). Slides were digitally photographed using a Leica DM 6000B light microscope.

**Backscatter scanning electron microscopy**

The remaining resin block containing the longitudinally cut trephine was polished and sputter-coated with a 6-nm carbon layer using a Bal-Tec SCD 500 sputter device. The specimens were evaluated with the backscatter detector using a Zeiss 40BP scanning electron microscope. The resulting images were compiled to create an overview image.

**Results**

**Clinical findings**

Immediate postgrafting healing for all patients was uneventful, with minimal swelling and inflammation and no signs of postoperative infection. No adverse events occurred during the interval between ridge preservation grafting and implant insertion.

At implant placement, re-epithelialization at the alveolar crest was evident in all patients. Soft tissues remained healthy, with minimal to no inflammation and without signs of infection. Following conservative mucoperiosteal flap reflection, clinical evidence of bone regeneration was readily evident in groups B and C. At both 12 and 24 weeks, implant insertion proceeded uneventfully. Group A patients at 6 weeks exhibited less volume of regenerated coronal bone, although implant placement with primary apical fixation in native bone proceeded without complication in each patient in group A.
Six weeks following tooth removal and grafting, evidence of early woven bone formation coronal to dense native bone was seen. NAB = native bone.

At 6 weeks, osteoid began to bridge gaps between early forming bony trabeculae. NB = new bone.

At 6 weeks, one core biopsy specimen evidenced significant vital bone formation in the apical and middle thirds. DB = demineralized bone.

At abutment connection, regardless of the time of implant insertion, 16 of 17 implants successfully osseointegrated, each tolerating a 35-Ncm torque test. One implant in group B required removal secondary to a significant periapical infection of endodontic origin that developed in an adjacent tooth.

Of the 16 patients with successfully integrated implants, 15 were available for postloading examination. Follow-up times extended from 6 to 17 months following prosthetic restoration. Both clinical and radiographic examination revealed continued bone maturation under functional load without signs or symptoms of inflammation, infection, or loss of osseointegration.

Histologic findings

Group A
At 6 weeks following guided bone regeneration (GBR), evidence of early woven bone associated with significant osteoblastic activity was noted. A representative core biopsy specimen from an intact socket revealed early regeneration of woven bone coronal to dense native apical bone. Few DBM particles appeared in the core specimen, and little evidence of more coronal bone regeneration was evident (Fig 5a). Higher magnification revealed osteoid forming adjacent to the woven bone trabeculae and bridging the gaps between trabeculae (Fig 5b). One group A specimen with a 3 × 5-mm initial buccal plate dehiscence evidenced unusually dense and abundant bone formation at 6 weeks in the middle and apical thirds of the core biopsy specimen. Trabecular bone bridging was noted throughout the specimen, and vital osteocytes were readily apparent within the regenerated bone. In addition, partial remineralization of demineralized allograft particles was readily seen at higher magnification (Figs 5c and 5d).
Group B
At 12 weeks following GBR, significant vital bone formation had occurred. An intact core from a mandibular second molar site with an initial 3-mm buccal furcation bone loss revealed actively regenerating woven bone in both the middle and apical thirds of the retrieved core (Fig 6a). Higher magnification demonstrated newly formed woven bone surrounding and replacing particles of demineralized allograft (Fig 6b). At higher power, regenerated bone was seen forming against surfaces of pores found within DBM particles (Fig 6c).

In a mandibular first molar site with an initial Class III furcation defect, intense osteogenesis was readily apparent at 12 weeks. Advancing fronts of osteoblasts actively secreted significant amounts of osteoid that bridged the gaps between grafted DBM particles, suggesting ongoing regeneration of vital bone. As in the 6-week specimens, partial remineralization of demineralized allograft particles was noted at higher power (Fig 7).
Fig 8a  Robust bone regeneration with interconnecting trabeculae was seen throughout this 24-week core specimen. E = epithelium; CT = connective tissue; CBC = cancellous bone chips; NB = new bone; DB = demineralized bone; M = marrow.

Fig 8b  At higher power at 24 weeks, regenerated bone could be seen forming coalescing trabeculae, often surrounding residual graft particles. CBC = cancellous bone chips; NB = new bone; DB = demineralized bone.

Fig 8c  Backscatter electron microscopy demonstrates large amounts of newly formed bone. Arrows point to foci of mineralization adjacent to the periphery of intact demineralized allograft undergoing active remineralization. NB = new bone; CBC = cancellous bone chips.

Fig 8d  Increased magnification of backscatter electron microscopy suggests significant amounts of de novo mineralization along the borders of residual remineralizing allograft (arrows).

Group C
At 24 weeks after GBR, robust bone regeneration was seen. A representative core specimen from an intact maxillary second premolar extraction site revealed active bone formation, including in the coronal third (Fig 8a). Newly formed bone actively bridges gaps between residual mineralized and demineralized allograft particles. At increased magnification, regenerated woven bone was seen coalescing into contiguous masses of vital tissue, often surrounding particles of grafted de-mineralized bone (Fig 8b).

In addition to robust bone regeneration evidenced under light microscopy, backscatter electron micrographic study of this group C specimen suggested advancing fronts of mineralization occurring de novo along the borders of residual demineralized allograft particles that were themselves almost completely remineralized (Figs 8c and 8d).
Discussion

Following tooth removal, crestal bone resorption begins almost immediately and is the inevitable consequence of alveolar bone's dependence on teeth to maintain anatomically driven volumetric stability.\(^1\) In an attempt to minimize postextraction bone resorption and maintain essential crestal bone morphology prior to implant placement, ridge preservation procedures have become the standard of care following tooth removal. However, few studies have examined human histologic healing of grafted extraction sockets at very early time points, i.e., 6 weeks postgrafting, followed by long-term clinical follow-up of implant survival.\(^2\)\(^,\)\(^3\)\(^,\)\(^8\)\(^,\)\(^38\) This case series was one such attempt at examining these parameters.

In this study, new bone formation was examined histologically at three different time points after tooth extraction and socket grafting with a composite demineralized and mineralized allograft matrix used in conjunction with an overlying resorbable collagen membrane. Significant differences in bone regeneration were seen at each of the three time points. Core specimens at 6 weeks (group A) revealed significant osteoblastic activity leading to nonmineralized osteoid with areas of early woven bone formation. One early responder in group A exhibited a more advanced regenerative response compared to other group A subjects at 6 weeks. At both 12 and 24 weeks, osteoid production was also accompanied by significant woven bone formation. At 12 weeks postgrafting (group B), new bone regeneration, while present elsewhere, was rarely noted at the coronal third in the core biopsy specimens. In contrast, by 24 weeks (group C), evidence of new vital bone formation was apparent throughout the core specimen.

Of particular interest was the finding of dot-like foci of mineralization noted via backscatter electron microscopy in one group C specimen occurring along the borders of remineralizing demineralized allograft particles. First described by Groenveld et al.,\(^39\) acellular remineralization within demineralized allograft particles in areas remote from vital bone appears as a necessary prerequisite for subsequent remodeling and replacement of allograft with newly regenerated vital bone. The dot-like foci of mineralization occurring along the borders of the allograft particles appear spatially related to varying fronts of active mineralization (ie, osteoid to mineralized bone, predentin to dentin). Active mineralization of demineralized allograft particles was also noted in the 12-week histologic sections of group B and in the regenerative advanced 6-week group A specimen.

Regardless of the duration between socket grafting and implant placement, 16 of 17 implants survived at abutment connection as well as under full occlusal function, with the one unsuccessful implant resulting from acute periapical infection of an adjacent tooth. Osseointegration occurred independent of when grafting occurred and showed no evidence of compromise throughout the study. The presence of intense osteoblastic activity, especially at earlier time points, suggests that active ongoing bone regeneration may have contributed to implant survival. Results of this study suggest that staged protocols of tooth extraction, GBR with effective graft matrices and barrier membranes, and delayed implant placement may allow for earlier implant insertion times without compromising long-term implant survival. Other variables such as adequate primary stability, the type of implant surface at the bone-to-implant interface, and vectors of occlusal force are equally important determinants for long-term implant survival, especially in the accelerated implant placement and loading protocols highlighted in this study.

The bone matrix examined in this case series along with the resorbable extracellular matrix membrane appeared effective for GBR ridge preservation procedures at all three time periods, followed by stable long-term implant survival. This case series study, while promising, was insufficiently powered to allow for quantitative histomorphometric analysis. Further prospective randomized trials with both test and control groups and sufficient power to allow for both qualitative and quantitative examination are needed to fully evaluate the potential of both the graft matrix and membrane used in this study.
References


