Case Series

A Retrospective Case Series Comparing the Use of Demineralized Freeze-Dried Bone Allograft and Freeze-Dried Bone Allograft Combined With Enamel Matrix Derivative for the Treatment of Advanced Osseous Lesions

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Background: *Combined regenerative approaches have been used for treating advanced osseous lesions around teeth. The aim of combining treatments is to enhance both clinical predictability and regenerative outcome compared to a monotherapeutic approach. This case series from a private practice reports on the clinical efficacy of an enamel matrix derivative (EMD) combined with either demineralized freeze-dried bone allograft (DFDBA) or freeze-dried bone allograft (FDBA) in the treatment of advanced infrabony lesions. The advanced lesions were veneered by a rapidly formed absorbable polymer barrier of poly(DL-lactide) to enhance graft containment.*

Methods: *A total of 22 consecutive patients, each contributing one infrabony lesion, are reported. After patients completed presurgical preparation, the infrabony lesions were surgically treated with a combined approach that included root surface treatment with citric acid. The two groups differed in their composite graft; one received DFDBA-EMD (n = 10) and the other received FDBA-EMD (n = 12). Patients followed a stringent postoperative protocol and were evaluated 6 months postsurgery. Clinical outcomes were assessed by changes in clinical attachment level (CAL) and probing depth (PD) from pretreatment. Surgical re-entry of several sites was possible in each group.*

Results: *CAL at pretreatment measured 9.2 ± 1.3 mm and 9.1 ± 1.9 mm for DFDBA-EMD and FDBA-EMD groups, respectively, with corresponding PD of 8.4 ± 1.6 mm and 8.9 ± 2.0 mm for each group. At 6 months post-treatment, CALs were reduced to 4.7 ± 1.3 mm and 3.8 ± 1.0 mm for DFDBA-EMD and FDBA-EMD groups, respectively; with corresponding PD decreased to 3.0 ± 0.8 mm and 3.2 ± 1.0 mm. Relative improvements in CAL for the DFDBA-EMD and DFDBA-EMD groups were 49.1% ± 11.0% and 57.3% ± 9.4%, respectively (P <0.07).*

Conclusions: *This case series demonstrates the clinical benefits of using a combined therapeutic approach in which a biologic mediator (EMD) was combined with either DFDBA or FDBA. In this limited case series, a trend was observed towards greater improvement in clinical attachment level gain in advanced infrabony defects when EMD was combined with FDBA as compared to DFDBA. Larger prospective controlled clinical trials are needed to determine if differences exist in the relative efficacy of DFDBA versus FDBA in combination with EMD.*
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KEY WORDS

Bone, demineralized; bone, freeze-dried; periodontal regeneration; guided tissue regeneration; enamel matrix derivative; grafts, bone; polylactic acid/therapeutic use.

Regeneration of the periodontium to its pre-disease state is the optimal therapeutic goal for clinicians. Treatment modalities that have demonstrated this capability in humans include autogenous bone graft,[1-3] demineralized freeze-dried bone allograft (DFDBA),[4,5] guided tissue regeneration using expanded polytetrafluoroethylene (ePTFE),[6,7] citric acid,[8] osteogenin combined with DFDBA,[9] enamel matrix derivative (EMD) on a conditioned root surface,[10,11] among others.

Combined regenerative approaches have been used where a bone replacement graft of DFDBA has been covered by either a non-absorbable[12,13] or bioabsorbable[14] barrier in an effort to achieve clinical results that are both superior and more predictable than either the barrier[12,13,15] or the bone replacement graft[16,17] alone. By "layering" techniques, there is added assurance that wound stability,[18] space maintenance,[19,20] epithelial exclusion,[21,22] and graft containment[23] may be achieved more predictably—all of these factors having a positive association with an enhanced regenerative outcome.

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Recent efforts have focused on the potential for biologic mediators to enhance regenerative outcomes while reducing the technique sensitive nature of regenerative therapy. A material comprised of enamel matrix (amelogenin) proteins derived from the enamel organ of developing porcine teeth (EMD)‡ is available commercially. In two separate studies, both Mellonig[10] and Yukna and Mellonig[11] demonstrated proof-of-principle that EMD applied to a conditioned root surface is capable of regenerating new bone, cementum, and a functional periodontal ligament on a diseased root surface. Case series reports have demonstrated clinical efficacy of this technique.[24,25] Comparison studies have demonstrated EMD to be superior to flap debridement[26-28] and comparable to other periodontal regenerative modalities,[28-30] as measured by probing depth reductions, clinical attachment level gains, and, in some studies, reentry procedures. A possible limitation to the regenerative capability of this material, however, may be its inability to maintain space in larger areas due to flap collapse into the defect. Lekovic et al.[31] have recently reported improvements in clinical outcomes by combining EMD with a graft material, overcoming the problem of space maintenance when using EMD alone.

Studies[4,5] have shown that DFDBA is capable of predictable regenerative outcomes in humans. Bowers et al.[9] demonstrated that combining DFDBA with osteogenin (bone morphogenic protein-3) enhanced the formation of new attachment apparatus beyond the use of DFDBA alone. While DFDBA may be a regenerative material because of its inductive nature, one potential drawback to its clinical efficacy may relate to the limited ability of this particular allograft to maintain space in non-contained lesions (i.e., less than 3 walls). Results of a clinical trial have suggested that freeze-dried bone allograft (FDBA) may be more suitable for maintaining space than DFDBA, as it is better contained.[32]

This clinical case series reports on combination therapy in which EMD was used with a bone allograft (DFDBA or FDBA) in the treatment of advanced infrabony lesions in consecutively treated patients. Additionally, treatment included citric acid root conditioning and the in situ placement of an absorbable polymer barrier of poly(DL-lactide)§ to enhance graft containment.

CASE SERIES DESCRIPTION AND RESULTS

This case series reports on the clinical outcome of 22 consecutively treated patients (8 female and 14 male; average age, 53.1 years) who were referred to a private practice limited to periodontics. Each patient contributed one infrabony defect for evaluation at 6 months postsurgery (Table 1). The majority of sites represented either 1-, 2- or combined 1-2-wall defects (Table 1). Infrabony defects that contained a 3-wall component measuring more than 2 mm were not included in the analysis. Selective reentry was performed for several sites to evaluate the results and permit supplemental treatment (Figs. 1A through F and 2A through D). The infrabony lesions were treated by either DFDBA/EMD or FDBA/EMD in combination with a bioabsorbable polymer barrier.

All patients were in good systemic health and underwent initial therapy in either the office of their general dentist or a periodontist. Plaque control orientation was performed in a periodontal practice until an excellent level was achieved, with deposits being either absent or minimal. Occlusal therapy included selective odontoplasty and/or splinting of the teeth to reduce excessive mobility or fremitus patterns. The periodontal examination included assessments of probing depth (PD), clinical attachment level (CAL), and mobility. The PD measurement represented the greatest distance from the gingival margin to the base of the pocket, whereas CAL measured the corresponding distance from the cemento-enamel junction, crown, or restoration margin to the base of the pocket.

Patients rinsed immediately prior to the surgery with a 0.12% chlorhexidine mouthwash. All measurements were performed by the surgical operator (PSR) using a calibrated 15 mm probe and rounded to the nearest millimeter. A sulcular incision with full thickness flap was employed. The defects were thoroughly debrided and the roots scaled and planed with ultrasonic and hand instruments. Infrabony defects were categorized by their morphology.[33,34] Prominent osseous ledges or exostoses in the surgical area were removed/reduced through osteoplasty to aid in primary closure. Rotary high-speed instrumentation with flame-shaped finishing burs‖ was used for additional root debridement. A saturated solution of citric acid (pH 1) was vigorously applied by rubbing the roots with moistened cotton pellets for approximately 1 minute for further root preparation. The surgical site was then thoroughly irrigated with sterile water prior to the topical applica-

‡ Emdogain, Biora, Chicago, IL.
§ Atrisorb, Collagenex, Newtown, PA.
‖ Brasseler USA, Savannah, GA.

tion of EMD for approximately 30 seconds. The EMD was mixed at the beginning of the surgery according to the manufacturer's instructions. Defects were treated with a composite graft of DFDBA or FDBA mixed with enough EMD to wet the graft material. These composite grafts were placed with light incremental pressure to fill the osseous lesions. A polymer barrier was applied directly over the graft by expressing it from its syringe through a 20-gauge, 1″-long, blunt needle. After placement, a high-speed handpiece or ultrasonic instrument was used to deliver an atomized spray of sterile water (20 to 30 seconds) to form the barrier. Formation was clinically determined by opacification of the material. Any portion of the composite graft not covered with the initial application received additional polymer to fill any voids or gaps. The flaps were positioned over the defects to obtain primary closure. Suturing was performed with interrupted monofilament #4-0 suture.¶ Additional EMD was flowed over the area and allowed to remain, undisturbed, for approximately 1 minute prior to the placement of a periodontal dressing. This dressing was changed approximately 7 to 10 days postsurgery. The patient was prescribed amoxicillin, 2 gm, at the time of the surgery, then 500 mg, 3 times daily, for 10 days. This was followed by doxycycline, 100 mg, 2 tablets the first day, and 100 mg per day for an additional 9 days. For patients allergic to amoxicillin, doxycycline was administered for a total of 14 days. The patients were seen every 7 to 10 days for postoperative treatment during the first month. Sutures were removed at either the second or third postoperative visit. Patients were then seen every other week for the second month and every month for up to 6 months. Postoperative treatment included plaque removal, selective stain polishing, and oral hygiene reinforcement. Patients used 0.12% chlorhexidine as a postoperative oral rinse for the first 30 days. After 30 days, patients rinsed with an essential oil mouthrinse twice daily. Patients were instructed to neither brush nor

Table 1.

Summary of Patient Data

Patient	Age (years)	Age (male/female)	Tooth (Surface)	Pretreatment CAL	Pretreatment PD	Graft	Lesion (walls)	6 Months CAL	6 Months PD
1	51	F	26(D)*	9.0	7.0	D‡	2	5.0	2.0
2	52	M	18(D)	10.0	10.0	D	MOAT	4.0	4.0
3	72	M	8(D)	9.0	7.0	D	1-2	5.0	2.0
4	69	F	6(D)	9.0	9.0	D	1	4.0	3.0
5	76	F	11(M)†	12.0	12.0	D	1-2-3	6.0	4.0
6	56	F	13(D)	10.0	7.0	D	1-2-3	7.0	4.0
7	40	F	31(M)	8.0	7.0	D	2	3.0	2.0
8	42	M	31(D)	8.0	8.0	D	MOAT	3.0	3.0
9	56	M	8(M)	8.0	8.0	D	1-3	5.0	3.0
10	52	M	15(D)	9.0	9.0	D	2	5.0	3.0
11	36	M	8(M)	8.0	8.0	F	1-2	3.0	2.0
12	46	F	19(D)	11.0	11.0	F§	1-2	5.0	5.0
13	43	F	30(D)	7.0	7.0	F	2	3.0	3.0
14	52	M	30(M)	11.0	11.0	F	1-2	4.0	3.0
15	46	M	31(D)	12.0	12.0	F	MOAT	3.0	3.0
16	48	F	3(D)	7.0	7.0	F	2	3.0	3.0
17	54	M	18(D)	11.0	11.0	F	MOAT	5.0	5.0
18	54	M	19(D)	9.0	9.0	F	1-2	4.0	3.0
19	58	M	9(D)	9.0	7.0	F	1-2	6.0	2.0
20	57	M	14(D)	7.0	7.0	F	1-2	3.0	2.0
21	53	M	30(M)	10.0	10.0	F	1	4.0	4.0
22	56	M	3(M)	7.0	7.0	F	1-2	3.0	3.0

Measurements are in millimeters
* Distal.
† Mesial.
‡ DFDBA.
§ FDBA.

¶ W.L. Gore and Associates, Inc., Flagstaff, AZ.

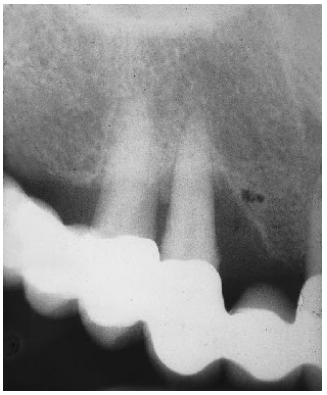

Figure 1A.
Pretreatment radiograph shows advanced osseous lesion at the mesial of the maxillary right canine.

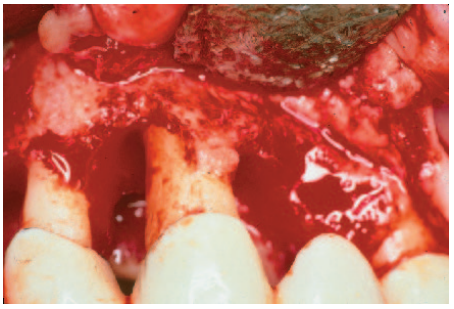

Figure 1B.
Surgical exposure of the site reveals a combined 1-2 wall lesion that wraps around to the distal of the canine in this 69-year old female.

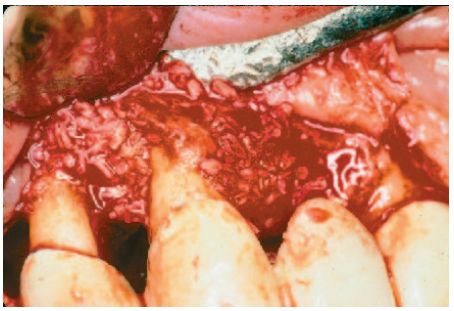

Figure 1C.
Following root preparation with scaling, planing, citric acid detoxification, and enamel matrix derivative application, composite DFDBA-EMD graft is placed.

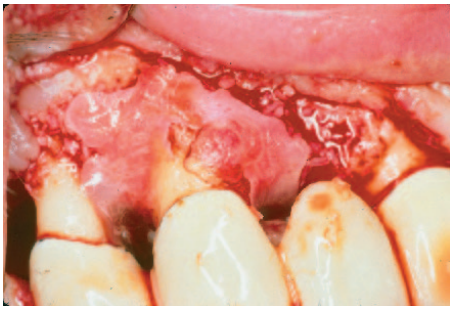

Figure 1D.
Poly(DL-lactide) barrier is flowed over the graft and formed using an atomized spray of sterile water.

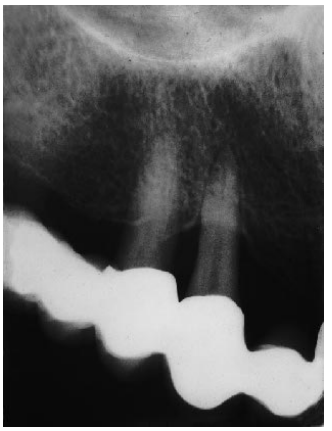

Figure 1E.
Radiograph taken 6 months postsurgery suggests favorable improvement of the area. There has been a gain in CAL of 5 mm.

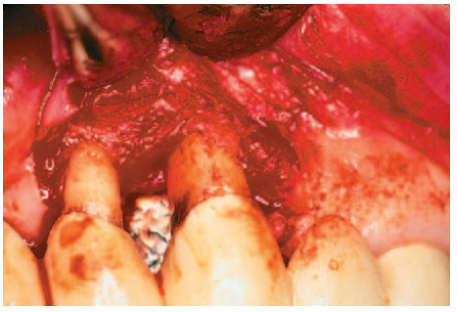

Figure 1F.
Reentry procedure performed at 8 months confirms defect fill with hard tissue.

floss the surgical area for the first 4 to 5 weeks and continued to topically apply the chlorhexidine to the site. Reexamination of CAL and PD was performed at 6 months postsurgery.

Statistical comparisons of mean CAL and PD measurements between sites (FDBA/EMD versus DFDBA/EMD) and within sites (pretreatment versus post-treatment) were made using Student and paired Student *t* test, respectively, with an alpha level of $P \leq 0.05$.

Individual case data are summarized in Table 1. No significant differences in clinical measures were found at baseline between treatment sites. The average CAL at pretreatment was 9.2 ± 1.3 mm (7 to 12 mm) for DFDBA-EMD sites and 9.1 ± 1.9 mm (7 to 12 mm) for FDBA-EMD sites. PD at pretreatment was 8.4 ± 1.6 mm (7 to 12 mm) for the DFDBA-EMD sites and 8.9 ± 2.0 mm (7 to 12 mm) for the FDBA-EMD treated sites. At the 6-month evaluation, sites treated with DFDBA-EMD exhibited a residual CAL of 4.7 ± 1.3 mm (3 to 7 mm) and PD of 3.0 ± 0.8 mm (range 2 to 4 mm). These changes represent a mean gain of 4.5 ± 1.1 mm for CAL and a mean reduction of 5.4 ± 1.3 mm for PD (Table 2). Similarly, sites treated with FDBA/EMD demonstrated a residual CAL of 3.8 ± 1.0 mm (3 to 6 mm) and PD of 3.2 ± 1.0 mm (2 to 5 mm). These changes represent a mean gain of 5.3 ± 1.7 mm for CAL and a mean reduction of 5.8 ± 1.6 mm for PD (Table 2). These clinical improvements represent relative gains in CAL of 49.1% ± 11.0% and 57.3% ± 9.4% for sites treated with DFDBA-EMD and FDBA-EMD,

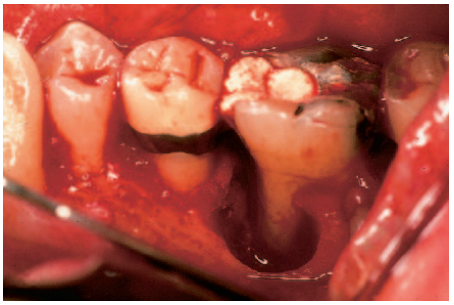

Figure 2A.
Lesion is exposed at the mesiolingual aspect of mandibular right first molar in this 52-year-old male. There is no furcation involvement.

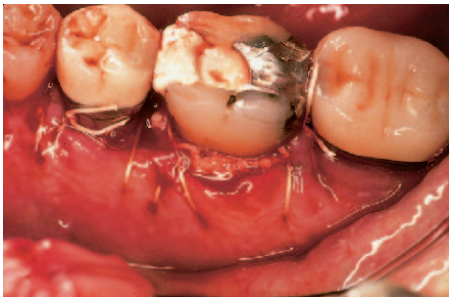

Figure 2C.
Graft is veneered by poly(DL-lactide) barrier that is formed in situ and the flaps are sutured with a monofilament suture of ePTFE.

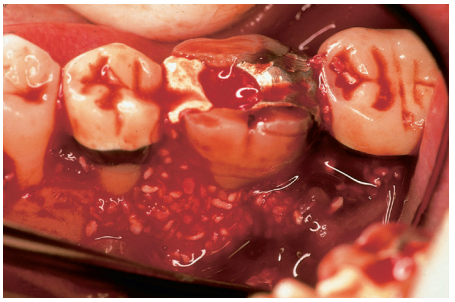

Figure 2B.
Root is treated with citric acid and EMD following scaling and planing and a composite graft of freeze-dried bone allograft mixed with EMD is placed.

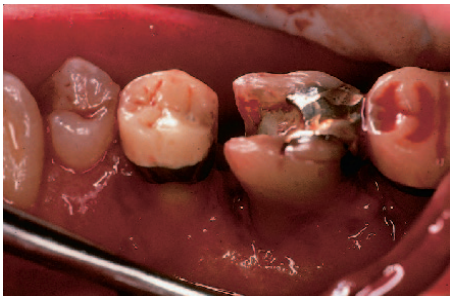

Figure 2D.
Reentry procedure at 7 months postsurgery suggests favorable fill with hard tissue. There was a gain in CAL of 7 mm.

respectively ($P \leq 0.07$; Table 2). Relative probing depth reduction, calculated as a percentage of the initial depth of the lesion, were similar for both groups, with reductions of 64.2% ± 8.5% and 64.4% ± 8.4% for the DFDBA/EMD and FDBA/EMD sites, respectively.

DISCUSSION

The 6-month results of this case series demonstrate favorable clinical outcomes for both DFDBA-EMD and FDBA-EMD, with relative improvements in CAL of 49% and 57%, respectively. Given the advanced and challenging nature of the initial osseous lesions, these improvements represent clinically significant outcomes.

The average CAL gain in these consecutively treated cases compares favorably with results of studies employing combined regenerative approaches[12,14] and appears more favorable than EMD[26-31] or osseous graft alone[16] and EMD complexed with bovine porous bone mineral.[31] However, direct comparisons between studies are difficult due to differences in multiple factors, including patient- and defect-related characteristics, treatment protocols, and outcome criteria. The comparative results of this retrospective case series analysis must be interpreted carefully, given the potential for bias due to lack of randomization and masking of the examiner.

Probing depth reductions mirrored the clinical attachment gains. Eighteen of the 22 sites treated had 6-month reductions of 5 mm or greater. The abil-

Table 2.

Clinical Measures at Pretreatment and 6 Months Post-Treatment for DFDBA (N = 10) and FDBA (N = 12) Sites

	CAL				PD			
Treatment	Pretreatment (mm)	6 Months (mm)	Mean Change	% Change	Pretreatment (mm)	6 Months (mm)	Mean Change	% Change
DFDBA	9.2 ± 1.3	4.7 ± 1.3	4.5 ± 1.1	49.1 ± 11.0	8.4 ± 1.6	3.0 ± 0.8	5.4 ± 1.3	64.2 ± 8.5
FDBA	9.1 ± 1.9	3.8 ± 1.0	5.3 ± 1.7	57.3 ± 9.4*	8.9 ± 2.0	3.2 ± 1.0	5.8 ± 1.6	64.4 ± 8.4

Values represent mean ± standard deviation.
* $P \leq 0.07$.

ity to predictably achieve shallow probing depths is clinically important because deeper sites are more likely to demonstrate ongoing loss of clinical attachment and increases in probing depth.[35]

The use of citric acid was chosen for root surface modification versus EDTA, as recommended by the manufacturer, for several reasons. Human histologic evidence has demonstrated regeneration of new bone, cementum, and periodontal ligament on previously diseased root surfaces with citric acid use, making it a regenerative technique onto itself.[8] The low pH of citric acid is bactericidal[36] and may kill residual bacteria in the dentinal tubules,[37] a possible reservoir for reinfection. Like EDTA, citric acid will remove the smear layer[38] which may aid in regeneration. The clinician must be cautious when using citric acid, since denaturing of the collagen fibers is possible with prolonged use.[39]

DFDBA was combined with a biologic mediator based upon the work of Bowers et al.,[9] who showed that osteogenin (bone morphogenic protein-3), combined with DFDBA enhanced the regenerative capabilities of DFDBA in humans. Whether this same principle would apply to EMD, a different biologic mediator, when combined with DFDBA remains to be demonstrated by histological evaluation. The recent work of Boyan et al.[40] suggests that there is a synergistic interaction for osteoinduction between active DFDBA and EMD.

The added benefits of space maintenance and clot stabilization provided by grafts such as DFDBA or FDBA may prove beneficial when used in combination with EMD, since collapse of the flap may diminish the space for achieving regeneration. EMD may provide the graft with predictable biologic activity since previous studies have shown that there can be variability to the osteoinductivity of DFDBA related to donor age, residual calcium content, particle size, and mode of processing.[41,42] Shigeyama et al.[43] demonstrated that the processing of DFDBA diminishes the graft's residual inductivity. Since FDBA is strictly an osteoconductive material, EMD may impart the biologic activity needed for regeneration. Some of the biologic effects attributed to EMD include the ability to enhance the expression of collagen Type I, interleukin-6 and prostaglandin G/H synthase 2,[44] cell attachment, spreading and proliferation of cultured periodontal ligament cells,[45,46] and production of transforming growth factor β_1 by periodontal ligament cells.[46,47] In this case series, FDBA was utilized as a carrier-scaffold for EMD, since a prior study suggests that FDBA is superior to DFDBA in its containment within periodontal defects.[32] The clinical question remains, however, whether the addition of EMD to a human bone allograft imparts any additional benefits beyond those of a graft alone. Similarly, it is unclear to what extent the scaffolding effects provided by a bone allograft enhances the potential of EMD as a biologic mediator.

The use of antibiotics to facilitate postsurgical plaque control in regenerative therapy remains controversial. Studies are inconclusive, with reports describing the benefits[48,49] or lack[50] thereof to antimicrobial therapy. While a recent study has concluded that the postsurgical use of antibiotics may be unnecessary in a protocol utilizing EMD as a monotherapy,[50] caution must be given to extrapolating these results to treatment that involves an implantable device or graft. Postsurgical exposure of the barrier at interproximal sites is a common complication[51] that presumably leads to contamination of the barrier and/or graft. In our patients, mechanical postsurgical plaque debridement was provided at the recall visits for the first 30 days on a stringent 7- to 10-day interval to minimize site contamination while maintaining wound quiescence. Although our basis for using antibiotics during the first 14 to 20 days in these patients was empiric, we consider the potential for minimizing the risk of postoperative infection of the surgical site as important. A controlled clinical trial will be necessary to determine the efficacy of such postoperative care in combination therapy.

The use of a combined approach, graft plus barrier membrane, has demonstrated clinically significant benefits in the treatment of furcations compared with either a barrier[12,13,15] or a graft alone.[52,53] This advantage, however, has not been demonstrated for infrabony lesions. It has been our experience that graft containment of DFDBA at interproximal sites can be difficult due to papillary necrosis, leading to diminished regenerative outcomes, and, in larger, more critical size lesions, space maintenance may not be optimal with graft alone. Containment of the graft has been previously shown to be critical for achieving a favorable regenerative outcome.[23] The use of a barrier is one possible way to gain this advantage. An earlier clinical case series[14] has demonstrated that the combination of DFDBA with an in situ formed polymer barrier lead to favorable clinical attachment level gains and probing depth reductions.

Smoking has been shown to be deleterious to regenerative therapy outcomes.[16,17] This retrospective case series does not analyze smoking as a covariate, since none of the patients were smokers. That

patients in this case series were non-smokers may partially explain the overall favorable clinical outcomes.

This case series demonstrates the clinical benefits of a regenerative approach employing a biologic mediator combined with bone allografts on root surfaces treated with citric acid in combination with an absorbable barrier of poly(DL-lactide). Clinical benefits were measured by gain in CAL and reduction in PD. In these limited number of advanced infrabony lesions, a trend was noted towards greater improvement in CAL at sites where EMD was combined with FDBA versus DFDBA. While the results of this case series are favorable, a number of unresolved questions remain. Would the lesions have equally benefited by other regenerative therapies? Will the gains in CAL and reductions in PD be maintained over time or improve? If so, are these gains more easily maintained than those of traditional regenerative therapies? Are these combined approaches cost effective for the patient? Larger prospective controlled clinical trials are needed to help answer some of these questions and establish the efficacy of this layered regenerative approach.

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