Case Series

A Prospective, Case-Controlled Study Evaluating the Use of Enamel Matrix Derivative on Human Buccal Recession Defects: A Human Histologic Examination

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Background: Connective tissue grafts (CTGs) and coronally advanced flaps (CAFs) do not regenerate periodontal attachment apparatus when used to treat gingival recessions (GRs). Instead of generating new bone, cementum, and inserting periodontal ligament fibers, CTG+CAF repairs through a long epithelial junction and connective tissue attachment. Enamel matrix derivatives (EMDs) have demonstrated proof-of-principle that periodontal regeneration can be achieved, although data are limited.

Methods: Three patients, each requiring extraction of four premolars before orthodontic treatment, were enrolled in a randomized, open-label study. Two months after induction of Miller Class I and II GR, each patient received EMD+CAF for three teeth and CTG+CAF for one tooth for root coverage. Nine months after root coverage, all four premolars from each of the three patients were surgically extracted en bloc for histologic and microcomputed tomography (micro-CT) analysis, looking for evidence of periodontal regeneration. Standard clinical measurements, radiographs, and intraoral photographs were taken over prescribed time points.

Results: Seven of the nine teeth treated with EMD+CAF demonstrated varying degrees of periodontal regeneration, detailed through histology with new bone, cementum, and inserting fibers. Micro-CT corroborated these findings. None of the three teeth treated with CTG+CAF showed periodontal regeneration. Clinical measurements were comparable for both treatments. One instance of root resorption and ankylosis was noted with EMD+CAF.

Conclusions: EMD+CAF continues to show histologic evidence of periodontal regeneration via human histology, this being the largest study (nine teeth) examining its effect when treating GR. The mechanism of action, ideal patient profile, and criteria leading to predictable regeneration are in need of further exploration. *J Periodontol 2016;87:645-653.*

KEY WORDS

Alveolar bone; biomimetics; dental cementum; gingival recession; histology; periodontal regeneration.

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indicates supplementary video in the online *Journal of Periodontology*.

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Ideal coverage of gingival recession (GR) defects should include: 1) restoration of the protective functional morphology of the mucogingival complex; 2) recreation of the esthetic balance between marginal tissues and the adjacent tooth root and crown; and 3) regeneration of the lost attachment apparatus, including formation of new cementum with inserting connective tissue fibers, and supporting alveolar bone.[1] Researchers and clinicians have diligently pursued these goals.[2,3] Systematic reviews show connective tissue grafting (CTG) under a coronally advanced flap (CAF) to be the "gold standard" for recession coverage.[4,5] However, the clinical outcome is repair through attachment via long junctional epithelium (JE) and connective tissue attachment, whereas true "periodontal regeneration" is new bone and new cementum joined by inserting periodontal fibers at the root surface.[6]

The less-than-ideal CTG+CAF healing and desire for lower-morbidity alternatives has led investigators to explore new approaches to regenerating periodontium, including the use of bioactive modifiers. The rationale is that biologics can provide a localized boost of active stimuli to the patient's own microenvironment and promote regeneration.[7,8] One such biologic modifier is enamel matrix derivative (EMD),[‡] a Food and Drug Administration (FDA)-approved therapy delivered to treated root surfaces to promote healing and regeneration through angiogenesis and osteogenesis. EMD+CAF provides clinical benefits comparable to CTG+CAF in the treatment of GRs[7,8] and is superior to CAF alone in achieving complete root coverage.[4] A meta-analysis found that the highest probability of complete root coverage came from CTG+CAF and EMD+CAF.[9] These clinical results were confirmed at 10 years, with no difference in multiple clinical parameters for both CTG+CAF and EMD+CAF treatments in nine patients.[10]

With the efficacy of EMF+CAF in root coverage well established, the remaining hurdle is histologically demonstrated periodontal regeneration. EMD was shown to enhance the formation of a new connective tissue attachment, cementum, and new alveolar bone in human patients.[11-13] Together, these studies verified the postulate that EMD can regenerate periodontium. In an effort to move past proof-of-concept, presented here is the largest human histologic case study (to the authors' knowledge) examining periodontal regeneration with EMD+CAF versus CTG+CAF in recession defects.

MATERIALS AND METHODS

Study Design

The primary objective of this prospective, open-label, randomized case series is to evaluate periodontal regeneration in Miller Class I and II surgically created recession defects[14] after treatment with EMD+CAF or control CTG+CAF. This is assessed in teeth extracted at 9 months post–root coverage surgery, through histologic and microcomputed tomography (micro-CT) analysis. Secondary outcomes include standard clinical measurements assessed at 7 days; 4 weeks; and 3, 6, and 9 months post-treatment. Patients continued to be monitored for 4 weeks after the 9-month (tooth extraction and grafting) time point for evaluation of adverse events.

Recruitment was performed from April 11, 2011 to May 7, 2012 by contacting referring local practitioners. Patients were compensated through a one-time payment to their orthodontist, to be used toward orthodontic therapy. All patients signed written consent and Health Insurance Portability and Accountability Act notification forms. The study protocol was approved by an institutional review board (Western Institutional Review Board, Puyallup, Washington) and was conducted according to the applicable code of federal regulations and good clinical practice.

Study Execution

Patients scheduled to undergo orthodontic treatment with planned extraction of a minimum of two premolars were considered at screening. Three female patients (aged 24, 29, and 57 years; two African American, one of Asian ethnicity; no history of tobacco use) were enrolled after they gave written consent and met all of the inclusion and exclusion criteria (see supplementary Table 1 in online *Journal of Periodontology*). All three patients required extraction of four premolars. Full medical and dental history and concomitant medications of the patients were recorded. Pregnancy testing and supportive periodontal therapy were administered as necessary.

Patients underwent a surgical procedure to induce GR on all premolars, shown in Figure 1. Following administration of local anesthetic, each recession was created by removing all but 2 to 3 mm of keratinized tissue. A full-thickness flap was elevated by connecting an intrasulcular incision to vertical releasing incisions, which were mesial and distal to the study teeth. The coronal portion of the exposed cortical plate was removed through ostectomy such that the new osseous crest lay 2 to 3 mm apical to the new gingival margin (GM). The flap, sutured with 5-0 chromic gut, was apically repositioned such that the recession defect was ≥3 mm. Patients were assessed 6 to 8 days following surgery.

GR defects healed for 2 months, after which patients were randomized to receive EMD+CAF or CTG+CAF. All sites were treated in the same visit according to a randomization plan; three teeth received EMD+CAF, and one received CTG+CAF. To serve as baseline demarcations for future measurements, notches were made in the root surface at the free GM and alveolar

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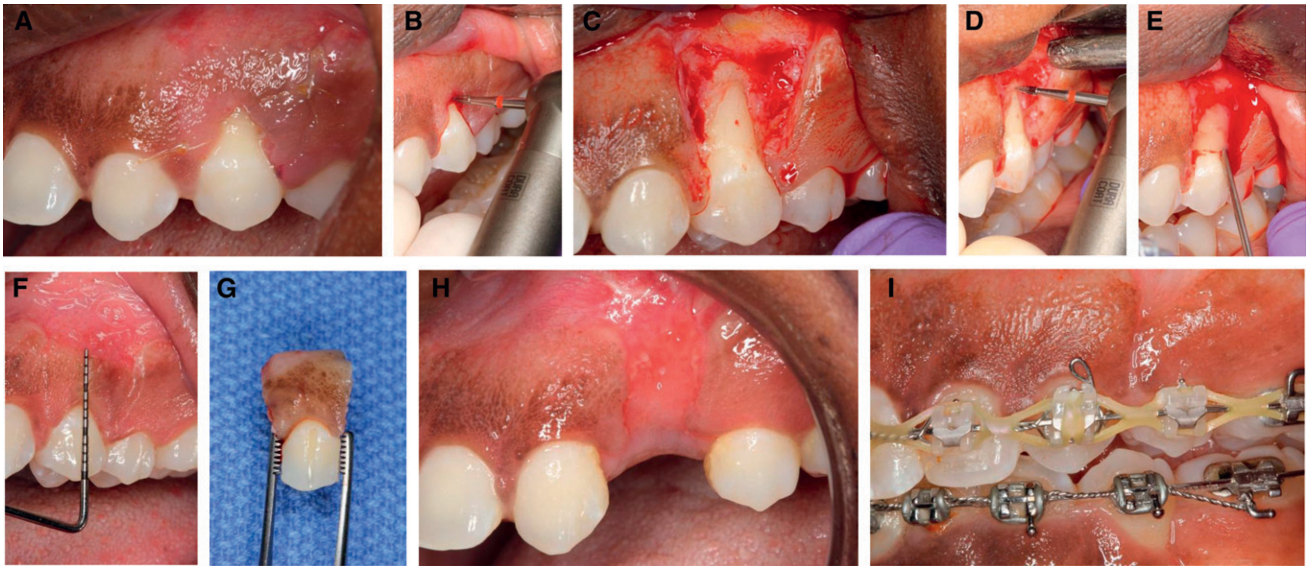

Figure 1.
A) *Test GR defect 8 weeks after creation of GR defect.* ***B)*** *Histologic marker being placed into root surface at the position of free GM after root planing and application of EDTA.* ***C)*** *Full-thickness flap creating recipient bed showing notch at GM and relationship to the alveolar bone crest (ABC).* ***D)*** *Insertion of histologic notch into root surface at ABC.* ***E)*** *Application of EMD over root surface.* ***F)*** *Healing at 9 months with 100% root coverage for EMD+CAF graft.* ***G)*** *Test tooth removed en bloc.* ***H)*** *One-month healing after bloc resection and ridge augmentation.* ***I)*** *Healing and orthodontic closure of extraction site.*

bone crest (ABC). Root coverage was performed using a standard technique for the both groups.[15] Patients were given standard postoperative instructions and assessed per supplementary Table 2 and Video 1 in online *Journal of Periodontology*.

Clinical measurements included: 1) plaque index;[16] 2) inflammation; 3) bleeding on probing;[17] 4) GR depth; 5) GR width; 6) keratinized tissue height; 7) probing depth (PD); 8) probing attachment level; 9) proximal PD; 10) proximal attachment loss (AL); 11) root dentin hypersensitivity; and 12) percent root coverage. Examination of defects at surgery included alveolar bone level, width of bone defect, and surgically positioned GM. All measurements were performed by the same calibrated examiner (Rebecca Showalter, RDH, Perio-Health Professionals), using a calibrated probe.[§]

The examiner made assessments for wound healing,[18] color, and texture match. Intraoral photographs and radiographs were taken. Patient-reported outcomes were noted at each visit.

Study Endpoints

Treated teeth were removed en bloc at 9 months. Following local anesthetic, a full-thickness incision was made outlining the collar of tissue to be removed. A full-thickness flap was reflected apically, mesially, and distally. A piezosurgical incision was used to extend the incision through the bone and into the root. The tooth with the block section ("block") was elevated and removed.

Sites were grafted with mineralized freeze-dried bone allograft,[‖] EMD, and a collagen membrane.[¶] Patients were monitored for uneventful healing at 7 days and 4 weeks post-extraction.

Histotechnical and Micro-CT Processing

Extracted blocks were processed using standard histology techniques. Evaluation was performed by a masked examiner (PS). For micro-CT, blocks were scanned using a high-resolution micro-CT system[#] in multislice mode at a resolution of 16 μm.

RESULTS

Primary Outcome

The primary outcome of this study was achieved, as seven of the nine sections for the EMD+CAF group showed histologic evidence of periodontal regeneration, corroborated by micro-CT analysis. Regeneration was noted in all three teeth from patients 1 and 2, and in one tooth from patient 3. No regeneration was visible in any CTG+CAF control teeth. Figure 2 provides a summary of images, and full quantitative results are shown in Table 1.

EMD+CAF group. Seven of nine EMD+CAF test teeth showed regeneration, but to varying degrees. Patient 1 possessed excellent regeneration: 4.2 mm

§ PCP 12 Hu-Friedy Probe, Chicago, IL.
‖ Allograft GC, Straumann, Andover, MA.
¶ Bio-Gide, Geistlich, Wolhusen, Switzerland.
\# μCT 40, Scanco, Brüttisellen, Switzerland.

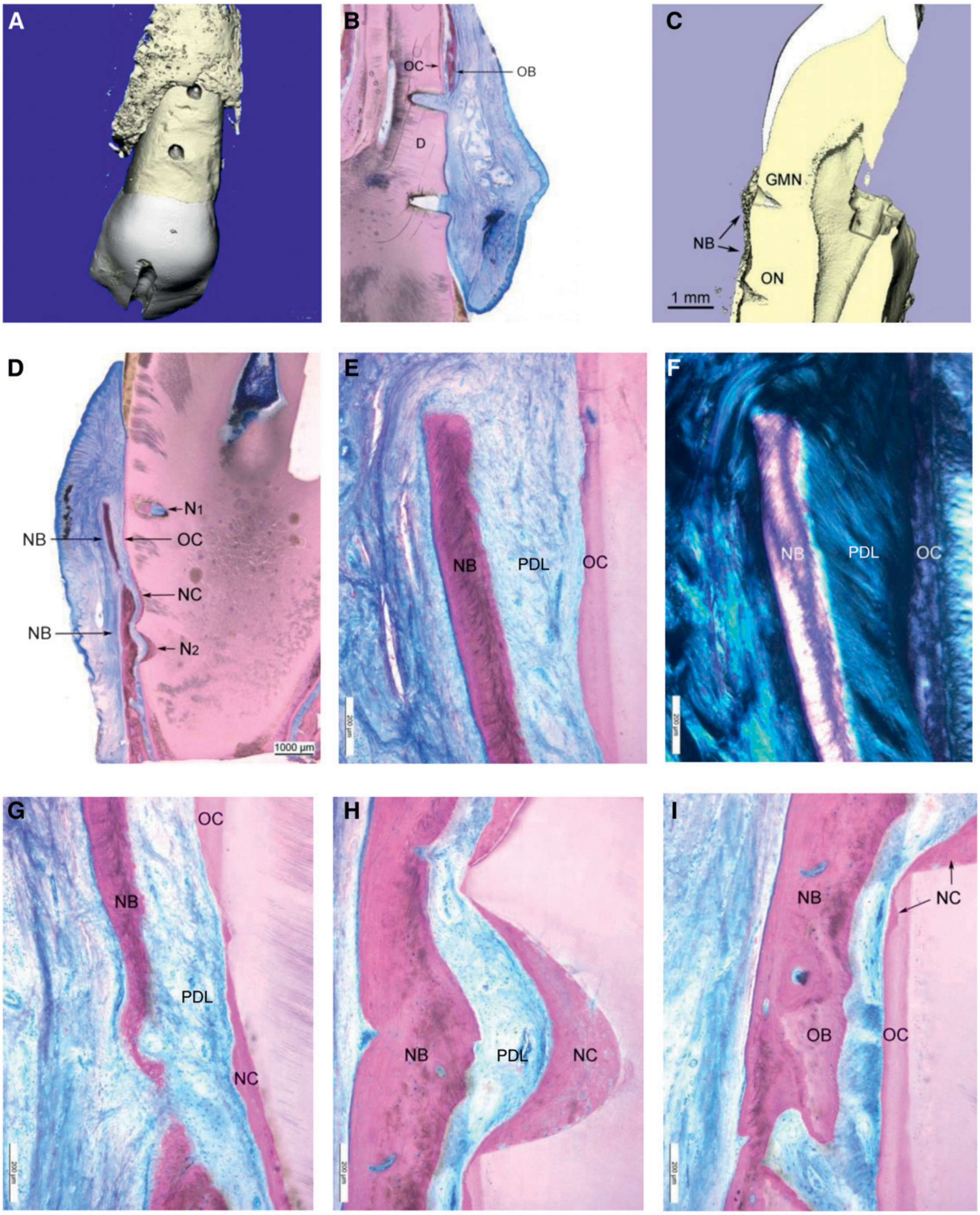

Figure 2.
Representative specimens. ***A)*** Micro-CT of CTG control tooth showing notch at ABC and notch at original free GM. ***B)*** Ground section of CTG control tooth showing 100% root coverage over notch at original osseous crest and notch at original GM mediated by long JE and connective tissue adaptation along with old bone, old cementum, and dentin. ***C)*** Micro-CT showing longitudinal cross-section through EMD+CAF test tooth and both notches (ON= osseous notch; GMN= gingival margin notch). ***D)*** Low-power ground section demonstrating both notches, newly formed bone, and old and new cementum. The remaining images represent higher power images of D, moving from the newly created osseous crest (new bone) down the root surface to the junction with the original osseous crest (old bone) N1 = GMN; N2 = ON. ***E)*** Ground section showing new bone separated from old cementum by newly-formed periodontal ligament. ***F)*** Identical section to E under polarized light demonstrating newly formed periodontal ligament fibers between newly formed bone and old cementum. ***G)*** New bone covering previously exposed root surface. Root surface is covered by both old and new cementum. ***H)*** Continuing down the root surface, new bone, periodontal ligament, and cementum can be seen covering the notch at the original GM. ***I)*** Continuing apically, new bone, new cementum, and periodontal ligament can be seen covering the notch at the original ABC. New bone can be seen extending from the original osseous crest. Old and new cementum are seen. OB = old bone; OC = old cementum; D = dentin; NB = newly formed bone; NC = new cementum; PDL = periodontal ligament.

Table 1.
Quantitative Results

EMD+CAF								
Patient	Tooth	T	JEP	CT	NC	NB	Regen	Resorb
#1	#12	6.6	2.4	2.6	1.6	1.7	Yes	No
	#20	7.8	1.9	3.0	2.7	4.2	Yes	No
	#29	5.5	1.4	0.9	1.8	4.5	Yes	Yes*
#2	#5	5.2	2.0	2.6	0.6	1.4	Yes	No
	#12	6.2	2.5	0.5	1.7	1.4	Yes	No
	#28	4.9	2.5	1.8	0.6	0.4	Yes	No
#3	#12	5.9	2.0	3.9	0.0	–0.5	No	No
	#21	9.6	2.2	6.8	0.6	2.3	Yes	Yes
	#28	10.8	2.2	N/A	N/A	N/A	No	N/A
Mean		6.94	2.12	2.76	1.20	1.93		
SD		1.94	0.33	1.84	0.83	1.61		
CTG+CAF								
Patient	Tooth	T	JEP	CT	NC	NB	Regen	Resorb
#1	#5	5.6	2.3	2.7	0.6	0.5	No	No
#2	#21	6.8	2.3	5.3	0.0	–2.3	No	No
#3	#5	6.8	3.7	3.1	0.0	0.0	No	No
Mean		6.40	2.77	3.70	0.20	–0.60		
SD		0.57	0.66	1.14	0.28	1.22		

SD = standard deviation; T = total height of gingiva from osseous crest to GM; JEP = length of JE; CT = length of connective tissue attachment; NC = length of new cementum; NB = new bone formation; regen = presence of periodontal regeneration; resorb = presence of some type of root resorption; N/A = not available.
All measurements are given in mm.
* Ankylosis noted.

of new bone and 2.7 mm of new cementum over old cementum. Patient 2 showed minimal regeneration: 0.4 mm of new bone, 0.6 mm of new cementum. For the EMD+CAF group, bone gain was seen in seven of eight samples (one could not be assessed due to error in methodology) with a mean of 1.93 ± 1.61 mm of new bone, ranging from 0.5-mm loss to 4.5-mm gain. The average new cementum gain for this group was 1.20 ± 0.83 mm, ranging from 0.5 to 6.8 mm. This group quantitatively showed many elements of periodontal regeneration, but with variability.

Qualitative assessment of the seven test cases showed varied hallmarks of periodontal regeneration (Fig. 3). New bone was laid over old bone, separated from the cementum and dentin, positioned apically from the osseous notch. In many cases, new cementum was stratified over old cementum. New cementum was observed directly on the root, including in the notches. Connective tissue spanned the interstitial space and can be observed inserting into adjacent tissues. Across all cases, although there was new cementum, the amount was limited and variable. In most cases, new cementum was found filling the apical notch, but there was only weak fiber connection between new bone and new cementum. Micro-CT analysis corroborated new bone growth and anatomically correct spatial organization. During defect creation of consistently thin buccal bone with intermittent fenestration in each of the patients, both histology and micro-CT supported the findings of the investigators.

Two cases with apparent resorption were noted. Both cases are illustrated in Figure 4. In one case, clear root resorption was evident, as marked by open lacunae and osteocytes (patient 3; no periodontal regeneration). Patient 1 showed apparent signs of ankylosis (and some form of resorption, potentially related to the ankylosis). New bone was seen alongside the root surface and within the osseous groove, separated by a thin, vacant crevice. In examining the full view of the tooth section, the tooth appeared fractured (likely during extraction or processing), and the fracture line propagated up the root surface and separated from what was once new bone ankylosed into the root and groove.

CTG+CAF group. No evidence of periodontal regeneration was seen in any of the three patients (one tooth each; three total). Sites were primarily characterized by a long JE and connective tissue attachment, 2.77 ± 0.66 mm and 3.70 ± 1.14 mm, respectively. Minimal new bone or cementum formation was observed; only one case contained new bone and cementum. Islands of tissue were not connected by inserting fibers and did not exhibit periodontal regeneration. The amount of bone gain/loss included a 2.3-mm loss, no change, and a 0.5-mm gain. There were no cases of root resorption or ankylosis. Areas of root surface appeared pitted, originally flagged as potential root resorption. After careful analysis and discussion, it was decided that these areas did not show any pathologic signs of resorption (i.e., true lacuni or osteocytes). The uneven appearance of the tooth root might have been caused by histologic processing or surgical intervention.

Secondary Outcomes

Although the study was not powered to show statistical differences between groups, no significant clinical differences were seen (Table 2). Both groups performed comparably in terms of achieving complete root coverage and associated clinical measurements, with a trend toward more keratinized tissue with CTG+CAF than with EMD+CAF.

Safety Outcomes

Patients reported no serious adverse events or adverse events related to the EMD treatment. Four events were deemed related or possibly related to surgery, all of which resolved with minimal intervention. One

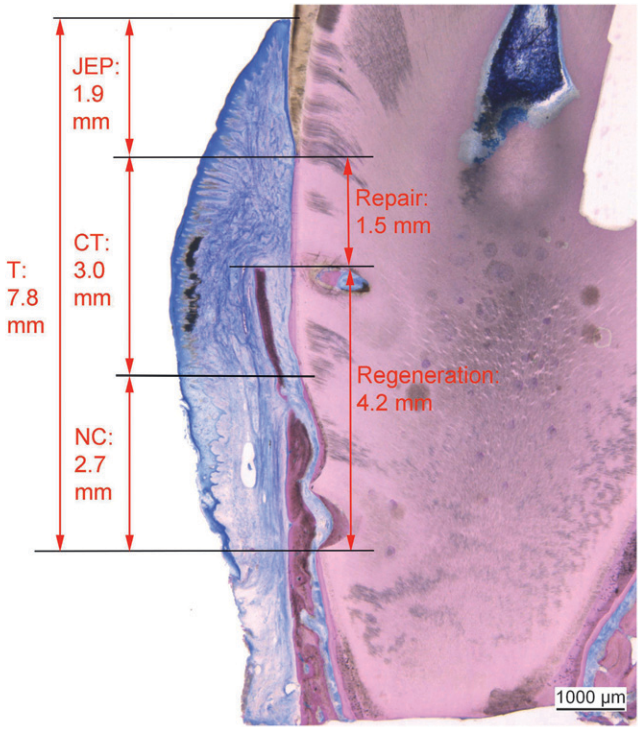

Figure 3.
Quantitative results are listed in Table 1 for each subject, treatment, and tooth. Results are calculated based on histologic measurements according to Figure 2. Mean and standard deviation are calculated for all points in the data set, except those not available. T = total height of gingiva from osseous crest to GM; JEP = length of JE; CT = length of connective tissue attachment; NC = length of new cementum.

exception was a patient who experienced intermittent joint pain due to temporomandibular joint syndrome. During surgery, investigators noted that all patients had generally thin facial bone. Protocol deviations were minor and typically concerned patients seen for follow-up marginally outside of the defined window. No patients withdrew or needed to be replaced; the three enrolled patients completed the study.

DISCUSSION

To the authors' knowledge, this study is the largest body of evidence showing periodontal regeneration in humans with a bioactive modifier. The histologic results advance the premise that EMD+CAF promotes periodontal regeneration beyond the proof-of-concept previously demonstrated in animals[19] and humans.[11-13] This work also reinforces the finding that CTG+CAF results in repair rather than regeneration.

When treating GR, where root coverage is desired, the chosen therapy should restore the function and esthetics. The challenge is to concretely demonstrate true periodontal regeneration: 1) new bone, 2) cementum, and 3) inserting fibers. Connective tissue, despite its predictability in effectively covering roots in an esthetic manner, has not proven to regenerate the periodontal apparatus. Instead, the procedure promotes functional repair of the site through a long JE and connective tissue attachment. Although this repair has become acceptable because of its long-term maintenance of function,[10] adjunctive therapies are used to promote regeneration. In three patients, neither connective tissue nor acellular dermal matrix showed evidence of periodontal regeneration.[20] As opposed to the passive mechanism of action of acellular dermal matrix, bioactive therapies are thought to actively promote regenerative healing. Growth factors such as 1) recombinant human platelet-derived growth factor (rhPDGF); 2) fibroblast growth factor-2 (FGF2); 3) recombinant human growth/differentiation factor-5 (rhGDF-5); and 4) EMD have all demonstrated periodontal regeneration. rhGDF-5, a member of the bone morphogenetic protein (BMP) family, demonstrated periodontal regeneration in animal models when used in conjunction with an absorbable collagen sponge[21] and a β-tricalcium phosphate (β-TCP) carrier.[22] In 10 human patients, regeneration was histologically apparent in a randomized controlled trial using rhGDF-5+βTCP.[23] FGF2 has progressed to Phase II human clinical trials in 253 patients, where significantly more bone fill was shown compared with vehicle alone (cellulose gel).[24] Evidence of periodontal regeneration has been histologically verified for FGF2, but in animals only: Class II furcation defects in beagles[25] and non-human primates.[26] An FDA-approved therapy, rhPDGF+βTCP, was shown to regenerate periodontium through human histology in a surgically induced GR model[27] and for human intraosseous periodontal defects.[28] rhPDGF's first demonstration of histologically confirmed periodontal regeneration was recorded when it was used in conjunction with allogeneic bone in human interproximal intrabony and molar Class II furcation defects.[29,30] Taken together, the peer-reviewed literature supports the proposition that biologically active modifiers advance periodontal regeneration above that of connective tissue alone.

This study was a relatively large, controlled case series designed to examine the potential for periodontal regeneration with EMD. Considering 10-year results, EMD+CAF appears to perform as well as the "gold standard" CTG+CAF in terms of restoration of function and esthetics and avoiding a palatal donor site.[10] Like the other bioactive modifiers, EMD has indeed been shown to regenerate periodontium in different human models: intrabony defects[12,31] and GR.[13] The results reported here demonstrate that although both the control and test treatments effectively achieved root coverage and improved associated clinical measurements, CTG provided a repair outcome, and EMD tended to provide a regenerative

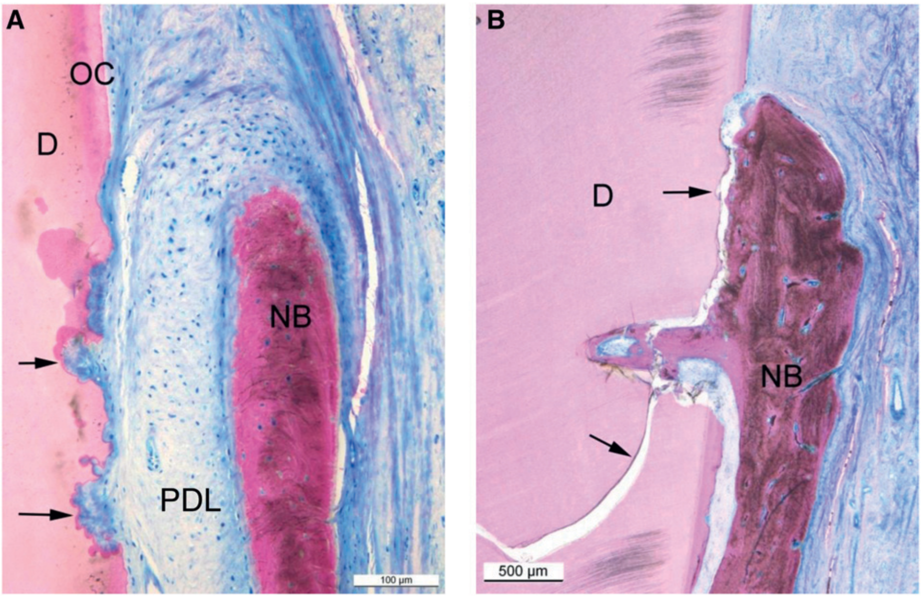

Figure 4.
A) *Representative image of root resorption and regeneration (patient 3, tooth #21). Old cementum, dentin, and resorption lacunae (arrows) on dentin are present on the root surface.* ***B)*** *Representative image of ankylosis and regeneration. Although a gap (superior arrow) is shown between new bone and dentin, it was likely caused during tooth extraction. A fracture line can be seen propagating through the tooth (inferior arrow), new bone (superior arrow), and ankylosis area within the osseous notch. (patient 1, tooth #29). OC = old cementum; D = dentin; NB = new bone; PDL = periodontal ligament.*

outcome. However, as mentioned previously, this study was not powered for statistical differences. Furthermore, surgical creation of GR, where a gingivectomy is used, may not be an appropriate model for making conclusions regarding final keratinized tissue height.

The present study supports EMD-mediated regeneration of periodontium, but also presents some confounding results: 1) variability of regeneration; 2) possible root resorption; and 3) ankylosis. Variability is not surprising, given the thin bone of all three patients. Most pointedly though, predictable results cannot be the aim of a three-patient study. Showing a degree of regeneration in all three patients (compared with the lack of regeneration in the CTG group) is certainly clinically significant, though larger studies might better define the true amount of variability. The authors speculate that the community will likely never see a large enough study to unequivocally draw conclusions given the need for human histology.

The present study underscores the lack of clarity about which patient types might best respond to therapy. A more thorough understanding of the mechanism of action of the therapy might contribute to the predictability of its use. However, the mechanism of action is multimodal and initiates a complex cascade of biologically mediated events, such that dissecting it completely is nearly impossible. Nonetheless, EMD has been suggested to promote the formation of cementum (through amelogenins)[15] as well as enhancing periodontal ligament cell migration.[32] Recent in vitro research suggests that portions of EMD may act through the BMP pathway via osterix and vascular endothelial growth factor-A,[33] potentially supporting the increased osteogenic response seen here.

What caused the apparent root resorption and ankylosis cases is unknown and could have been related to patient, tooth or treatment, or surgical intervention factors. Although no ankylosis was found in the control group, EMD cannot necessarily be linked to these outcomes: the study was insufficiently powered, and an ankylosed or resorptive outcome is not outside the realm of possibility with surgery. Root resorption has been documented with CTG+CAF along with various root surface biomodifiers.[13,34-36] The current rates of ankylosis and root resorption in root coverage procedures with CTG+CAF with root surface biomodifiers is unknown. The outcomes in this study may have been exacerbated by the recession defects in question being surgically created (iatrogenic). The removal of cementum might have provoked ankylosis. The results where areas of root were devoid of old cementum, yet overlaid with new cementum, support this notion. The unexpected outcomes reported in the present study have not been seen before in similar surgically induced models of recession treated with EMD. These studies were limited in size and might, therefore, have missed the outcomes seen in this larger study. Of course, if one were to attribute the overall surgical intervention as having contributed to these unexpected results, one might also question the regenerative result seen after such interventions. Taken together, it is not clear whether these events were attributable to the materials, surgery, or both.

CONCLUSIONS

This study supports EMD promotion of periodontal regeneration. Histologic analysis showed greater periodontal regenerative results by new connective tissue, cementum, and bone with EMD+CAF. Despite this encouraging result, this study was not without shortcomings, such as the limited number of patients and confounding results (i.e., possible root resorption and ankylosis). The variability of periodontal regeneration

Table 2.

Clinical Measurements (in millimeters)

Group/Time point	GR Depth	GR Width	PD Mesial	PD Buccal	PD Distal	Keratinized Tissue Height	AL Mesial	AL Buccal	AL Distal
EMD: Mean (SD)									
Baseline	2.77 (0.36)	3.44 (0.53)	2.67 (0.5)	1.28 (0.44)	2.39 (0.49)	3.56 (1.76)	5.44 (0.85)	4.06 (0.68)	5.17 (0.5)
9 Months	0 (0)	0 (0)	2.72 (0.57)	1.67 (0.5)	2.61 (0.60)	3.78 (1.18)	0 (0)	0 (0)	0 (0)
CTG: Mean (SD)									
Baseline	2.5 (0.5)	2.83 (1.04)	2.33 (0.58)	1.5 (0.5)	2.17 (0.29)	3.5 (3.04)	5 (0.5)	4.17 (0.29)	4 (1.32)
9 Months	0 (0)	0 (0)	2.33 (1.15)	2.17 (0.5)	2.67 (0.58)	4.67 (2.08)	0 (0)	0 (0)	0 (0)

SD = standard deviation.

might indicate patient selection that could respond differently to therapy. It should be remembered that this was a non-statistically powered study with surgically created GR defects. As such, the conclusions of periodontal regeneration as well as the unexpected outcomes seen should be taken in context. It is typical to conclude such a study by suggesting the need for yet larger clinical trials. However, when the required endpoint is human histology, it may not be feasible to do so. Until less invasive methods for assessing periodontal regeneration are devised, the community will be left to draw its own conclusions from both prior histologic studies and this clinical and histologic controlled case series.

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