

A Multicenter Study Evaluating the Sensitization Potential of Enamel Matrix Derivative After Treatment of Two Infrabony Defects

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Background: Several studies reported some success toward regeneration in infrabony defects using enamel matrix derivative (EMD). Clinically and statistically significant improvements in probing depth reduction, clinical attachment levels, and bone fill have been demonstrated. This multi-center study evaluated the potential for sensitization to EMD in a subgroup of periodontal patients treated at least twice with at least 2 months between treatments.

Methods: Three hundred seventy-six (376) patients in 11 university-based postgraduate periodontics programs and five private practices were selected. Surgeries were performed on infrabony defects. Following reflection of mucoperiosteal flaps and debridement of the root surface and defect, root conditioning (either citric acid pH = 1 or 24% EDTA) was performed and the site was irrigated with sterile saline. Enamel matrix derivative was reconstituted and applied to the exposed root surface and the bony defect. Flaps were sutured and pressure applied for 5 minutes. The second test defect was treated in a similar manner at least 8 weeks after the first surgery. The patient was given a diary card where any subjective adverse events (erythema, swelling, itching, headache, root hypersensitivity, or pain) were recorded at weeks 1 and 2 post-surgery. In addition, objective adverse events (gingival inflammation, ulcers, abscess, cratering, and lesions) were recorded by the investigator on an adverse event form.

Results: No clinical adverse reactions to multiple applications of EMD were noted. Of 376 patients, two were referred to a dermatologist for evaluation, but neither had signs indicating any adverse events due to EMD treatment. Instead their reactions were classified as a small local abscess and *tinea cruris*. The single immunoassay performed (on the patient with a small local abscess) did not demonstrate any EMD-reactive antibodies, neither IgE nor IgG. Other subjective/objective reactions that occurred during this study were of the type that are commonly experienced by patients immediately following periodontal surgery, but were not related to EMD. They included headache, swelling, itching, pain, and root hypersensitivity.

Conclusions: This study demonstrated a lack of clinical adverse reactions following two separate applications of EMD. Any subjective/objective adverse reactions experienced by the patient were typical complications following routine periodontal surgery and were not directly related to the use of enamel matrix derivative. *J Periodontol* 2004;75:1001-1008.

KEY WORDS

Enamel matrix derivative/adverse effects; multicenter studies; periodontal diseases/therapy; risk factors; wound healing.

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Enamel matrix derivative (EMD) is the hydrophobic protein assembly of amelogenins extracted from porcine enamel matrix.¹ The potential for some regeneration of lost periodontium with EMD has been demonstrated in several human histological investigations which are considered proof of principal.²⁻⁶ Moreover, results from other clinical studies indicated significant improvements in probing depth reduction, clinical attachment levels, and bone fill in infrabony defects treated with EMD.⁷⁻¹¹ Controlled clinical trials have reported statistically significant improvements in the above clinical parameters when infrabony sites were treated with open flap debridement (OFD) plus EMD compared to those treated with OFD alone.^{7,11}

In addition, several investigations have reported that there were no statistically significant differences in clinical results in infrabony defects treated with EMD or with guided tissue regeneration with either nonabsorbable or absorbable membrane barriers.¹²⁻¹⁶

Enamel matrix derivative has been used in combination with autogenous bone,¹⁷ demineralized freeze-dried bone allograft,¹⁸ anorganic bovine bone,^{19,20} and bioactive glass²¹ in the treatment of infrabony defects. In all previous studies that used EMD alone, in conjunction with bone grafts or bone graft substitutes, there were no reports of any wound healing complications or adverse events. Specifically, the safety of EMD has been demonstrated in two separate studies.^{22,23} One multicenter investigation assessed EMD's sensitization potential in 107 patients following multiple periodontal surgical procedures with EMD. No increase in IgE, IgG, IgM, or IgA reactive to EMD was detected.²² The other study (N = 32) also reported that no adverse events occurred after multiple exposures to EMD in the treatment of infrabony defects.²³ The authors of both studies concluded that EMD is a clinically safe product and that multiple uses do not have a negative impact on periodontal wound healing.²³ The purpose of this present investigation was to assess the potential for sensitization to EMD. A subgroup of periodontal patients was treated in at least two sites with at least 2 months between treatments in 16 different study centers.

MATERIALS AND METHODS

Study Design

Three hundred seventy-six patients (192 females and 184 males; age ranging from 15 to 80; mean 49 years) were included in this study. Patients were recruited from 11 university-based postgraduate periodontic programs and five private practices. The patients agreed to participate in this study and gave their informed written consent on Institutional Review Board approved forms.

Patients selected for the study did not have any medical condition that would contraindicate routine periodontal surgery and all had the ability to maintain good oral hygiene.

This was an open label safety study in patients who received two EMD^{†††} treatments at least 2 months apart. Information regarding the patient's age, gender, ethnicity, smoking habit, health status (especially regarding allergies), and prescription/over-the-counter medication as well as use of antibiotics/antiseptics was recorded on a case report form (CRF).

Blood sampling for immunoassays was performed on patients who reported possible allergic response after treatment. When reactions were noted by the investigator during surgery or at any of the scheduled visits, they were documented on an adverse event form. A diary card was given to each patient to document any subjective adverse events. Whenever possible, the response or reaction was documented by photography, and the patient was referred to a dermatologist for examination.

Patient Demographics

A total of 376 patients were enrolled in the sensitization study (Table 1). Each patient underwent two surgical procedures with EMD, with the exception of one patient who had only one surgery. The mean time span between surgeries was from 2 to 3 months. One patient had only 1 month between surgeries, but was kept in the evaluation based on the "intent to treat."

The health status, allergies, smoking habits, and use of prescription medications were recorded. Eighty-seven (23%) of the patients (47 males and 40 females) were current smokers, with a mean tobacco consumption of 17 cigarettes per day (range 2 to 60).

A vast majority (86%) of the patients stated that they were healthy, although more than 40% used prescription medications or hormone replacement therapy. There was no obvious correlation between the health statement and the type of medication used; i.e., one patient with controlled diabetes, asthma, and high blood pressure was recorded as "healthy," while another patient was recorded as being "unhealthy" with high cholesterol as the only reported problem.

Eighty-eight (21%) of the patients had a known allergic predisposition. Of these, 76% reported an allergy to medications (penicillin, sulfa, aspirin, codeine, etc.); 9% had seasonal hay fever; 9% were sensitive to animals, dust, or unspecific causes giving asthma; 3% were sensitive to soap or detergents; and 3% to foodstuffs or spices.

Prescription Medicines Not Related to Dental Treatment

Of the 192 females, 33 (17%) were taking either estrogen replacement or contraceptive pills. One male reported using sildenafil.^{§§§}

Other prescription medications were classified into eight therapy groups: medication used for 1) hyperten-

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§§§ Viagra, Pfizer, New York, NY.

sion; 2) diabetes; 3) allergy/asthma; 4) arthritis; 5) cardiovascular conditions; 6) thyroid hyper- or hypofunction; 7) epilepsy; and 8) other indications (high cholesterol, glaucoma, colitis, migraine, liver cirrhosis, pain, and gastric reflux).

More than 10% of the patients were treated for hypertension, and several had other medical problems (angina pectoris, asthma, or arthritis) in addition to the hypertension.

Systemic corticosteroids were used by three patients (prednisone, 10 mg) and non-steroidal anti-inflammatory drugs (NSAIDs) were taken by another 12 patients, in addition to being frequently prescribed by the investigators as analgesics after surgery.

Treatment

The first surgery was performed in February 1997, and the last follow-up visit was completed in September 2000.

Surgical Procedures and Use of Antibiotics

The surgical and post-surgical procedures were performed according to the general practice of each center and the needs of each patient; i.e., there were differences between the sizes of the flaps raised; the number of teeth treated; and the use of antiseptics, analgesics, and antibiotics. The mean number of teeth treated was 1.9 (range 1 to 6) in the first surgical session and 2.0 (range 1 to 6) in the second.

A record was made in cases where systemic antibiotics were prescribed in conjunction with surgeries 1 and 2. Use of antibiotics varied between centers. In some centers, antibiotics were prescribed with every surgery in all patients, while in other centers, no antibiotics were used at any time in any patient.

The percentage of patients receiving antibiotics was 56% in the first surgery and 52% in the second. Doxycycline (46%, five centers), tetracycline (25%, one center), amoxicillin (13%, one center), and penicillin V (12%, one center) were used. The prescription varied from a single tablet prior to surgery to daily use during 2 weeks or more post-surgery. At eight centers, no antibiotics were prescribed.

Surgeries were performed on infrabony defects at separate visits at least 8 weeks apart. Following administration of local anesthesia, full mucoperiosteal flaps were reflected and the defect and root surfaces were debrided with hand and ultrasonic instrumentation. Following irrigation, root conditioning was performed. At the discretion of the investigator, either citric acid pH = 1 was applied to the root surface with a cotton pellet for 15 seconds or 24% EDTA was applied with a cotton pellet for 2 minutes. The area was then irrigated with sterile saline for 1 minute. Enamel matrix derivative which had been reconstituted in 1 ml of propylene glycol alginate at least 15 minutes prior to use was then applied to the exposed root surface and bony defect. Flaps were sutured with interrupted sutures and pressure was applied for 5 min-

Table 1.

Patient Demographics*

	Number (%)
Gender	
Male	184 (49%)
Female	192 (51%)
Ethnicity	
Caucasian	236 (63%)
African American	72 (19%)
Hispanic	52 (14%)
Asian	11 (3%)
Other or not defined	5 (1%)
Age	
15-40 years	91 (24%)
41-60 years	222 (59%)
>60 years	63 (17%)

* N patients = 376.

utes. At the discretion of the operator, a periodontal dressing was placed over the surgical sites. The second test defect was treated in a similar manner at least 8 weeks after the first surgery.

Patients returned 7 days post-surgery for suture removal. At 7 and 14 days post-surgery, patients returned the diary card that documented any symptoms and/or reports of any allergic response (erythema, swelling, itching, pain). The treatment areas were examined to assess wound healing. Patients returned post-surgery at days 7, 14, 28, and 42 for professional maintenance of the test area.

Post-surgically, all patients were instructed to rinse twice daily with 0.12% chlorhexidine gluconate and, at the discretion of the investigator, antibiotics were prescribed. At eight treatment centers, no antibiotics were prescribed, and one of four antibiotics (doxycycline, tetracycline, amoxicillin, or penicillin) was prescribed at the other centers.

Assessment

Surveillance during surgery was documented in the case report form (CRF). The patient received a diary card with questions concerning adverse events experienced post-surgery during weeks 1 and 2. This diary was initialed and dated by the patient and returned to the investigator at the 1- and 2-week follow-up. Any adverse events reported or observed were recorded by the investigator on the adverse event form (AEF). At days 7, 14, 28, and 42 post-surgery, the surgical area and surrounding tissues were examined.

Based on the diary and the four post-surgery visits, if any indication of an allergic reaction or an unexpected reaction was noted, the investigator decided whether to exit the patient from the study. Any indication of an allergic or untoward response required that the investigator

immediately contact the sponsor to report referral of a patient to a health care unit. Any visible adverse events, and especially those indicative of an allergic response, were also documented with color photographs.

Blood samples were drawn and serum prepared and frozen according to standard hospital procedures. The frozen serum samples were shipped under dry ice to the sponsor for analysis regarding the presence of IgG or IgE antibodies. The serum, diluted in phosphate buffered saline (PBS) was incubated for 1 hour in plastic tubes^{||||} coated with EMD protein. After a series of rinsing steps, the adsorbed IgG or IgE antibody was detected with ¹²⁵Iodine labeled anti-human IgG or IgE antibody. Quantification was performed by measuring the captured radioactivity by gamma counter.^{###} Donor pool reference samples were analyzed with each run in order to allow comparison of results from different runs. All results were tabulated both as measured count per minute (cpm) and percentage of pool.

Data Management and Statistical Analysis

All Case Report Forms were completed, dated, and signed by the investigator who performed the surgeries. Completed forms were checked independently by the clinical research monitor and retained at the clinics for 2 years from the end of the study.

All patient data were entered using statistical software.^{###} Demographic data and baseline data were summarized. The primary safety assessment was analysis of EMD-reactive IgE in patients with visible adverse reactions indicative of an allergic response. Correlations to patient factors such as age, gender, ethnic group, known allergy, and smoking history were also tested. Furthermore, analyses were performed to investigate correlations to surgery-related factors such as complaints or adverse events after the first treatment with EMD, time between treatments, and use of systemic antibiotics.

RESULTS

Objective Adverse Events

No EMD-reactive antibodies were found in the immunoassays performed. However, two patients reported allergic reactions and underwent dermatological evaluation. One patient had an abscess incised and drained 1 week after the first surgery and "hard nodules" had formed. The dermatologist concluded that it was not a contact dermatitis but rather small local abscesses. A blood sample was taken and serum prepared for immunoassay. No antibodies (IgE or IgG) were found to be contributable to EMD compared to the untreated blood donor pool.

The second patient had red lesions appear on the scrotum. The dermatologist diagnosis was tinea cruris, unrelated to EMD treatment.

Twenty-two (6%) patients had an objective finding noted by the investigator after the first surgery and 24 (6%) after the second surgery. Table 2 summarizes the

Table 2.

Most Frequently Reported Post-Surgery Objective Adverse Events Documented by Investigators (N = 376 patients)

Event	First Surgery	Second Surgery	Total
Local			
Gingival inflammation, redness, soreness, and swelling	10	8	18
Aphtha or palatal ulcer	4	9	13
Herpes-like blister	0	3	3
Abscess at surgical site	1	2	3
Erythema, hematoma	2	1	3
Cratering	0	1	1
Granulation tissue	1	0	1
White spongy material removed	1	0	1
Internal pressure; no thermosensitivity	1	0	1
Extraoral			
Hematoma on cheek	1	0	1
Red lesions on scrotum	1	0	1
Total	22	24	46

most frequently reported post-surgery objective findings (local and extraoral). There were no statistically significant differences between the number of objective reports after first and second surgery, neither in the whole group, nor in the subgroups with allergy medication or within gender.

Table 3 summarizes the most frequently reported objective adverse events by severity and their causal relationship to EMD (possible, unlikely, or not classified) as reported by the investigators. Fifteen cases were classified as possibly related, 20 as unrelated, and two were not classified.

One case of soreness was classified as serious but not related to EMD. Five cases were classified as moderate and possibly related (two cases reported swelling or gingival inflammation; one case of abscess at surgical site; one case of granulation tissue; and one case of internal pressure without thermosensitivity).

Subjective Adverse Experiences

The subjective adverse events were noted by the patients each day in the diary, and discussed with the investigator at the follow-up visits.

Seventy-three percent of the patients reported post-surgical complaints after the first surgery and 65% after

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SPSS Version 9.1 for Windows, SPSS Inc., Chicago, IL.

Table 3.
Most Frequently Reported Objective Adverse Events
by Severity and Casual Relationship to EMD

Event	Severity									Total		
	Mild			Moderate			Severe					
	P	U	NC	P	U	NC	P	U	NC	P	U	NC
Gingival inflammation, redness, soreness, and swelling	8	7	0	2	0	0	0	1	0	10	8	0
Aphtha or palatal ulcer	2	2	0	0	0	0	0	0	0	2	2	0
Herpes-like blister	0	2	0	1	0	0	0	0	0	1	2	0
Abscess at surgical site	0	2	0	0	0	1	0	0	0	0	2	1
Erythema, hematoma	0	3	0	0	0	0	0	0	0	0	3	0
Cratering	0	1	0	0	0	0	0	0	0	0	1	0
Granulation tissue	0	0	0	1	0	0	0	0	0	1	0	0
White spongy material removed	0	0	1	0	0	0	0	0	0	0	0	1
Internal pressure; no thermosensitivity	0	0	0	1	0	0	0	0	0	1	0	0
Hematoma on cheek	0	1	0	0	0	0	0	0	0	0	1	0
Red lesions on scrotum	0	1	0	0	0	0	0	0	0	0	1	0
Total	10	19	1	5	0	1	0	1	0	15	20	2

P = possibly; U = unlikely; NC = not classified.

Table 4.
Most Frequently Reported Post-Surgery
Subjective Adverse Events Documented
in Patients' Diaries

Event	First Surgery		Second Surgery	
	N	(%)	N	(%)
Headache	64	(17)	52	(14)
Root hypersensitivity (to hot, cold, etc.)	75	(20)	73	(19)
Swelling	95	(25)	85	(23)
Tooth pain	30	(8)	27	(7)
Itching	8	(2)	5	(1)
Other (bleeding, tooth mobility, throbbing/tenderness)	6	(1)	4	(1)
Total	278	(73)	246	(65)

the second. This decrease in subjective complaints from first to second surgeries is statistically significant for the whole group, as well as for females only.

Table 4 summarizes the most frequently reported post-surgery subjective adverse events and Table 5 summarizes the most frequently reported post-surgery subjective adverse events by severity and casual relationship to EMD.

Transient swelling was the most frequently reported adverse event, followed by root hypersensitivity and headache. Root hypersensitivity (e.g., to hot or cold food) and headache were noted with the highest intensity (Table 6) and root hypersensitivity was the adverse event with the longest duration (Table 7). The frequency of high intensity and long duration decreased slightly from the first to second surgeries. However, this was not statistically significant.

DISCUSSION

This study as well as other investigations^{7,22,23} found no clinical adverse reactions to multiple applications of EMD. In addition, Zetterstrom et al.²² found no increase in EMD-reactive IgG, IgE, IgA, or IgM prior to and 4 weeks after multiple exposure to EMD.

Out of 376 patients in the current study, two were referred to a dermatologist for evaluation, but neither had signs indicating any adverse events due to EMD treatment. Instead their reactions were classified as a small local abscess and tinea cruris, respectively. The single immunoassay performed (on the patient with a small local abscess) did not demonstrate any EMD-reactive antibodies, neither IgE (which had been expected for a sensitization reaction) nor IgG. All other post-surgery findings are common after periodontal surgery and no therapy was necessary other than prescribing analgesics.

In general, the patients were taking a wide variety of prescription medications not related to dental treatment. In addition to the large number of diabetics, there were patients with arthritis being treated with NSAIDs, as well as high doses of corticosteroids, and one patient was treated with an anticoagulant. These patients had no post-surgery problems.

Symptoms experienced by patients following periodontal surgery can vary significantly.²⁴ Common post-

surgery subjective symptoms mentioned by patients included pain, headache, swelling, tenderness, itching, and root hypersensitivity. A distinction needs to be made between normal post-surgical healing responses experienced by the patient and adverse events related to a material. An allergic episode is a type of adverse reaction. Signs and symptoms of an allergic reaction are different from the normal healing response following periodontal surgery. Tooth pain may occur due to trauma induced by surgery. Minor swelling of soft tissues is a normal response to surgical manipulation. Severe swelling beyond the third or fourth post-surgical day may be indicative of an infection. However, soft tissue swelling is not usually a sign of an allergic reaction.

Root hypersensitivity is commonly experienced by patients after periodontal surgery and headaches are

usually stress induced. Itching may be caused by the sutures and not necessarily reflect an allergic reaction.

With regard to reactions reported after administration of EMD in other investigations, Zetterstrom²² et al. reported that 7% of their patients treated with EMD experienced root sensitivity and 70% reported severe swelling. Heijl et al.⁷ found no incidence of severe symptoms in patients treated with EMD. Heard et al.²³ noted 1.3% of patients had severe root hypersensitivity and 9.5% had severe swelling. In contrast, the present study found the occurrence of severe swelling (with a duration >5 days) to be 4% and 5% (first and second surgery, respectively) and tooth pain to be 2% and 2% (first and second surgery, respectively). The occurrence of severe headache was 4% and 3% (first and second surgery, respectively). The occurrence of severe

root hypersensitivity was 7% and 6% (first and second surgery, respectively). This is slightly lower than the range of root sensitivity (8% to 35%) documented by others following periodontal surgery.²⁵ Differences in reports of root sensitivity or pain between centers may be due to a variety of factors. For example, the amount of root planing or the management of soft tissue during the surgical procedures may have influenced the symptoms reported. In addition, it has been shown that psychological preparation for repeated

Table 5.

Most Frequently Reported Post-Surgery Subjective Adverse Events by Severity and Casual Relationship to EMD

Surgery	Severity									Total		
	Mild			Moderate			Severe					
	P	U	NC	P	U	NC	P	U	NC	P	U	NC
First	59	99	2	32	54	1	13	17	1	104	170	4
Second	45	89	2	37	47	1	6	19	0	88	155	3
Total	104	188	4	69	101	2	19	36	1	192	325	7

P = possibly; U = unlikely; NC = not classified.

Table 6.

Adverse Events Reported as Severe as Documented in Patients' Diaries (N = 376 patients)

Event	First Surgery	Second Surgery
Headache	8	9
Root hypersensitivity (to hot, cold, etc.)	13	8
Swelling	6	7
Tooth pain	3	1
Itching	0	1
Other (bleeding, tooth mobility, throbbing/tenderness)	1	0
Total	31 (8%)	26 (7%)

Table 7.

Most Frequently Reported Adverse Events with a Duration >5 Days as Documented in Patients' Diaries (N = 376 patients)

Event	First Surgery	Second Surgery
Headache	15	10
Root hypersensitivity (to hot, cold etc.)	26	21
Swelling	15	17
Tooth pain	7	6
Itching	1	1
Other (bleeding, tooth mobility, throbbing/tenderness)	4	1
Total	68 (18%)	56 (15%)

periodontal surgery was associated with a reduction of pain.²⁶ However, in a clinical study such as the present one, there is a tendency to stress the study "risks" in order to downplay expectation and satisfy the required IRB consent process. This might be a hidden source of stress for the patient with an increase in subjective symptoms reporting following surgery.

In conclusion, the present safety study did not show any signs of sensitization to EMD as a result of repeated use, even in the subgroup predisposed to allergies. In addition, patients with many different types of medications, including high dose steroid therapy and anticoagulant therapy, did not react negatively to the treatment.

The present study found that occurrences of signs and symptoms, complications, and pain after surgical treatment with EMD were similar to typical complications following other forms of periodontal surgery.²⁴ The present study also had a lower occurrence of post-surgical pain than previously reported following periodontal surgery.²⁴ This may have been due to the surgical methods utilized in the present study, since there was no removal of osseous tissue and no mucogingival surgery employed, both of which have been shown to increase the incidence of post-surgical complications such as pain and swelling.²⁴

SUMMARY AND CONCLUSIONS

In this multicenter study of 376 patients who received multiple surgical treatments with EMD, the following conclusions can be drawn.

1. The use of EMD in conjunction with periodontal surgical procedures produced no greater adverse reactions than those associated with routine periodontal surgery.

2. None of the variety of non-dentally related medications taken by the patients in this study appeared to have any effect on the post-surgical healing responses of EMD treated sites.

3. Two of the 376 treated patients were referred to dermatologists for evaluation. Neither had any adverse reactions related to EMD treatment.

4. EMD may be considered a safe material when used for single or multiple treatments of periodontal osseous defects.

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