Enamel matrix derivative (EMDOGAIN®) in the treatment of intrabony periodontal defects


Abstract. The aim of the present clinical trial was to compare the long-term effect of EMDGAOIN® treatment as an adjunct to modified widman flap (MWF) surgery with the effect of MWF and placebo treatment. The investigation was a placebo-controlled, randomized multicenter trial involving 33 subjects with 34 paired test and control sites. The protocol required 2 interproximal sites, appropriately separated, in the same jaw with probing pocket depths ≥6 mm and an associated intrabony defect with a depth of ≥4 mm and a width of ≥2 mm as measured on a radiograph. Only predominantly 1- and 2-wall defects were included. Clinical attachment gain and radiographic bone gain were used as primary outcome variables. Assessments were made at baseline, 8, 16 and 36 months. Mean values for clinical attachment level gain in test and control sites at 8 months were 2.1 mm and 1.5 mm, respectively; at 16 months, 2.3 mm and 1.7 mm, respectively; and at 36 months 2.2 mm and 1.7 mm, respectively; and the differences were statistically significantly different at each time point (p<0.01). The radiographic bone level continued to increase over the 36 months at the EMDGAOIN®-treated sites, while it remained close to the baseline level at the control sites. The statistically significant (p<0.001) radiographic bone gain at 36 months of 2.6 mm at EMDGAOIN®-treated sites corresponded to 36% gain of initial bone loss or 66% defect fill. The present trial has demonstrated that topical application of EMDGAOIN® onto diseased root surfaces associated with intrabony defects during MWF periodontal surgery will promote an increased gain of radiographic bone and clinical attachment compared to control (placebo application) surgery in the same patient. There was no evidence to indicate any clinical adverse effects from application of EMDGAOIN® conjunction with periodontal surgery.

Key words: enamel proteins; periodontal regeneration; intrabony defects; clinical trial

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organ, have a secretory phase during which enamel related matrix proteins are secreted and temporarily deposited onto the root surface providing an essential surface for the expression of cementum-forming cells. Subsequently, when cementum has been laid down onto the enamel matrix covered dentin surface, the proper attachment apparatus will develop. The discovery of the enamel matrix layer between the peripheral dentin and the developing cementum and its function provided the fundamental concept for enamel matrix derivatice (EMDOGAIN®)-supported tissue engineering in regenerative periodontal therapy (Hannamstarr 1997).

The aim of the present clinical trial was to compare the long-term effect of EMDGAIN® treatment as an adjunct to modified Widman flap (MWF) surgery with the effect of MWF surgery and placebo.

**Material and Methods**

The present clinical trial was designed as a placebo-controlled randomized multicenter trial with a split-mouth design. The inclusion criteria required 2 interproximal sites appropriately separated, i.e., requiring 2 separate surgical procedures, in the same jaw, with probing pocket depths $\geq 6$ mm and an intrabony defect with a depth of $\geq 4$ mm and a width of $\geq 2$ mm as measured on a periapical radiograph after at least 1 month of supervised oral hygiene. 33 patients, having an average age of 48 years (range 33–68 years), met the inclusion criteria of the trial and were recruited. One of these patients contributed two test and two control sites, i.e., 34 surgeries were performed in 34 pairs of sites. Periodontal treatment (including periodontal surgery) could have been performed in the selected sites previously.

Further design criteria included:

- only predominantly 1- or 2-wall defects were included, i.e., strict or predominantly 3-wall defects were not included
- test and control treatments were assigned at random and all measurements were performed by the same, blinded and trained investigators
- treatment of test and control sites were performed during the same surgical session
- the post-surgical care was directed at maintenance of wound stability and infection control

**Clinical assessments**

The surgeon performed all the required measurements during surgery, but he was not assigned to do any other examinations. A trained examiner, who was unaware of the treatment assignments, performed all examinations prior to and following surgery throughout the study at each center.

**Pre-operative screening**

The patient's medical history was recorded and a physical examination performed before the patient was enrolled in the study.

**Baseline examination**

Pocket depths. The depths of the periodontal pockets were measured with a periodontal probe (PCP-12, HuFriedy, Chicago, IL, USA; probe tip diameter 0.4 mm). Buccal and lingual pocket depth measurements were recorded. Interproximal pocket depth measurements were recorded at the line angles (with the probe kept parallel with the long axis of the tooth), as well as at the deepest point probeable from both buccal and lingual aspects. When probing the deepest point on the approximal surfaces, the probe was placed as close as possible to the contact point. Thus, up to 10 pocket depth measurements around each experimental tooth were recorded. However, at sites where the neighboring tooth (teeth) was missing, an additional pocket depth measurement with the probe tip parallel to the long axis of the tooth was made at a midproximal location, i.e., 5 instead of 4 approximal measurements were performed in such cases. This was duly noted in the patient record forms.

The number of surfaces measured for each experimental tooth site was determined by the location and morphology of the osseous defect. All pocket depth measurements were adjusted to the nearest mm.

**Clinical attachment levels.** Probing clinical attachment was measured with a periodontal probe at the same time as probing pocket depths were recorded and at the same locations around each experimental tooth. The measurements were made from well-defined landmarks (e.g., the cemento-enamel junction, a groove in the tooth surface or the margin of a crown or other restoration) on the tooth surfaces at the experimental sites or from a customized stent, which covered the experimental tooth and one tooth on either side.

**Bleeding**

Bleeding or absence of bleeding was recorded while measuring probing pocket depths and clinical attachment levels at all examination intervals.

**Plaque scores**

Tooth sites adjacent to the gingival margin exhibiting soft deposits, which could easily be removed with the side of the probe were scored as having plaque. The presence of plaque was documented by a local plaque score, based upon the surfaces involving the experimental sites and expressed as a percentage of the total number of surfaces under evaluation.

**Radiographic examination and measurements**

Commercially available film holder devices designed to hold a vertically oriented X-ray film, were used to obtain standardized radiographs of all tooth regions included in the study. Each film holder device was adapted to the occlusal surfaces in an experimental tooth region.

Radiographs were digitized using an image processor (Argus 10, Hamamatsu, Hamamatsu City, Japan) and linear measurements of root length and bone height were made. The anatomic reference points used were native bone level, coronal margin of the intrabony defect, apical bone level (bottom of defect) and apex of tooth. Native bone level was defined as a point located 1 mm apical to the cemento-enamel junction (Lindhe & Karring 1989). Radiographic bone level was measured from apex to bottom of defect and initial bone loss was measured from native bone level to bottom of defect. Readings of all
radiographs were performed by a separate, blinded examiner and in a randomized fashion.

Treatment

Study devices EM DOGAIN® from BIORA AB, Malmö, Sweden (Enamel Matrix Derivative 30 mg, to be reconstituted with 1.0 ml of propylene glycol alginate solution, PGA) was used as test device. The vehicle, PGA, was used as placebo control. EM DOGAIN® is a resorbable, implantable material. It consists of hydrophobic enamel matrix proteins extracted from developing embryonal enamel of porcine origin. It is supplied in sterile, lyophilized form. The vehicle supplied is a sterile aqueous solution of propylene glycol alginate, with a suitable viscosity to facilitate application of EM DOGAIN® onto root surfaces exposed during periodontal surgery.

Randomization

At the time of periodontal surgery, and only after the first surgical site was fully prepared, the envelope containing the randomization code was opened to expose the treatment assignments. The sites were distinguished by their tooth number (18 through 48) and the randomization code specified the treatment assignment for the site with the lowest as well as highest tooth number. The randomization process targeted one of the sites for test treatment and the other site for control treatment. Patient numbers were assigned in chronological order as patients were enrolled in the trial.

Regenerative periodontal surgery

The areas selected for surgery were anesthetized (Xylocain® adrenalin, Asta A B) and disinfected. Following buccal and lingual intracrevicular incisions with a vertical releasing incision(s) extending out into the alveolar mucosa on the buccal aspect of the surgical areas, full-thickness (muco-periosteal) buccal and lingual access flaps were raised. Granulation tissue and pocket epithelium were removed from the inner surface of the flaps by curettage. Granulation tissue adherent to the alveolar bone of the associated intrabony defects was removed to provide full access and visibility to the root surfaces. Any visible, remaining subgingival plaque and calculus were removed by gentle scaling. Root planing was kept to a minimum. The surgical areas were rinsed with sterile saline and the exposed root surfaces were “etched” (not more than 15 seconds) with 37%ortho-phosphoric acid (DeTrey, Dentsply) to remove the “smear layer” (Polson & Proye 1982, Polson et al. 1984, Blomlof & Lindskog 1995, Blomlof et al. 1996), thereby allowing the enamel matrix proteins to precipitate onto a root surface devoid of organic debris (Gestrelius et al. 1997). Following acid application root surfaces were thoroughly rinsed with saline. In test sites, EM DOGAIN® was immediately applied to the exposed root surface, starting at the most apical bone level and covering the entire root surface. In control sites the vehicle, PGA, was used for placebo application. The flaps were replaced and sutured appropriately with Goretex™ sutures. Both test and control sites were treated during the same surgical session and all surgical procedures, within each center, were performed by one and the same surgeon.

Other therapy

The patients received systemic antibiotherapy for a period of 3 weeks postsurgery. The regimen consisted of oral administration of doxycycline (Vibramycin, Pfizer), 200 mg the first day and then 100 mg daily. In addition, the patients were advised to rinse with 0.2% chlorhexidine solution (Hibitane® Dental, ICI) for weeks 4-6 postsurgery. Mechanical tooth cleaning was not allowed in the surgical areas for the first 6 weeks. Patients were also instructed to avoid chewing in the surgical areas during the same postsurgical period.

Examination during surgery, intrabony defects

Number of tooth surfaces involved and number of osseous walls

The number of tooth surfaces involved at the apical portion of a defect was recorded. The defects were visually recorded according to dominant form as to 1- or 2-wall intrabony defect.

Description and categorization of intrabony defects

Intrabony defects usually have varying numbers of osseous walls depending on their topography in the vertical direction (for example, 1-wall or combined 1- and 2-wall defects often have an apical 3-wall component). Therefore, the osseous defect depth was recorded after debridement, so that the surgical site could be properly described and categorized. Measurements were made using the same periodontal probe and reference points as used for preoperative measurements.

Re-examination

The sites selected for surgery were re-examined at 8, 16 and 36 months post-treatment. At the follow-up examinations all parameters studied at baseline were recorded. All re-examination measurements were made by the same blinded investigator, who made the initial measurements.

Adverse events (AEs)

Adverse event definition

In this context, an adverse event (AE) was any unfavorable unintended event (signs, symptoms, etc.) temporally associated with the administration of the test device whether or not considered device related.

Procedures

AEs were recorded at all follow-up examinations. The documentation included all adverse events/discomforts reported by the patients. Distinctions were made between postoperative effects/reactions routinely encountered after periodontal surgery and those not normally expected. The methods used for collecting data regarding AEs were subjective symptoms, e.g., open questioning, and objective findings, e.g., clinical evaluation done by the examiner. Intensity and possible causal relationship of an adverse event to the test device were recorded as were actions taken and interventional outcomes.

Efforts to control investigational procedures

The study centers were visited periodically during the data collection period. The monitors checked/determined that the clinical facilities remained acceptable, that the investigating team was adhering to the protocol, that the results of the study were recorded accurately in the case report forms and collected case record forms. The investigator and other relevant staff were available at these visits. Editing of the forms at the clinical site with the investigator occurred before data was entered.
Data management and statistics

The data entry system used was SPSS data entry II. The data management and statistical analysis were performed using the SAS 6.04 system and SPSS/PC+ version 4.01. The results of the assessments were evaluated at baseline, 8, 16 and 36 months postoperatively. All outcome variables were expressed as differences from baseline and all statistical analyses utilized change from baseline.

A 'Per Protocol' approach to the analysis of the data was used. Thirty-three patients from three centers were included (one female patient had 2 test and 2 control sites) and none of these patients terminated the study before 8 months. At 16 months 30 patients having 31 paired sites were reexamined and at 36 months 26 patients having 27 paired sites were reexamined.

Demographic data and baseline data for all patients were summarized. The comparisons of the effectiveness of the two treatment procedures were evaluated by analyzing reduction of periodontal pocket depths, gain of clinical attachment level and gain of radiographic bone. The primary efficacy variables were radiographic bone gain and gain of clinical attachment.

The statistical computations were based upon site values, implying that an average was used for sites in which several measurements were made. Thus, averages of facial and lingual measurement were used in all cases where the average baseline pocket depth was ≥6 mm. In cases where the pocket depth was ≥6 mm only at the facial or lingual side, the appropriate measurement (facial or lingual) was used in the calculations instead of the average.

Differences between the treatments were assessed using the Student t-test (2-sided, split-mouth design). P-values less than 0.05 were considered statistically significant.

Sample size determination

The primary objective was to establish the existence of an advantage for the test device EM DOGAIN® over the placebo control, PGA. The sample size was calculated to detect a 1 mm difference (assuming 1 mm SD) in clinical attachment gain and radiographic bone gain between EM DOGAIN® treatment and control treatment with a power (1-β) of at least 90%. This approach of confidence intervals was according to the method described by Pocock (1983).

Ethical requirements

The study was approved by the Medical Ethics Committee of the Karolinska Institute, Stockholm, Sweden before initiation of the study and was performed in accordance with the principles stated in the Declaration of Helsinki. All patients were given written information about the study design and their right to withdraw from the study at any time, and information stating that a decision to withdraw would in no way affect their right for future treatment. The patients’ written informed consent was obtained before they were included in the study.

The patients were informed that the data from this study would be processed in a strictly confidential manner and that information from this study must be available on request for an inspection by reviewing representatives from authorized governmental agencies.

Results

Demographics

Patients

33 patients (7 men and 26 women), all of which had 2 available experimental sites, were included in the study. The mean age was 48 years (range 33 to 68 years). One of the patients had 4 experimental sites available. Thus, in all 34 test/control pairs of data were included in the analysis. A t each of the three participating centers, 10–13 patients were included.

All patients included in the trial had been subjected to systematic periodontal treatment including repeated mechanical debridement and, in some patients, therapy supplemented with antimicrobial as well as surgical treatment of the experimental sites. These therapeutic regimens had been provided over long periods of time, which was often several years.

30 patients having 31 sites completed the 16-month evaluation. 2 patients had both test and control teeth extracted before the 16-month evaluation as part of treatment with complete dental implant prostheses. These 2 patients were characterized as having very severe adult periodontitis. A 3rd patient had suffered an accident and was not available during the examination period. 26 patients (7 men and 19 women) having 27 sites were re-examined at 36 months.

Other diseases (medication)

Most of the patients (31 out of 33) were considered healthy, even though eleven (11) were prescribed one or several of the following medications: analgesics, betablockers, antibiotics, insulin, Levaxin R and anti-inflammatory drugs.

Smoking habits

At baseline, 16 (48%) of the patients were recorded as current smokers.

These patients had been smoking for 10 to 40 years. 13 patients were former smokers.

Table 1. Defect characteristics as measured during surgery

<table>
<thead>
<tr>
<th>Intrabony component</th>
<th>Test sites</th>
<th>Control sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-wall (mm±SD)</td>
<td>2.0 (1.4) 43%</td>
<td>1.4 (1.2) 26%</td>
</tr>
<tr>
<td>2-wall (mm±SD)</td>
<td>1.4 (1.4) 29%</td>
<td>2.5 (1.7) 48%</td>
</tr>
<tr>
<td>3-wall (mm±SD)</td>
<td>1.4 (1.0) 29%</td>
<td>1.2 (0.8) 26%</td>
</tr>
<tr>
<td>total defect depth (mm±SD)</td>
<td>4.8 (1.5) 100%</td>
<td>5.0 (1.4) 100%</td>
</tr>
</tbody>
</table>

Table 2. Postsurgical evaluation of randomization on type of defect

<table>
<thead>
<tr>
<th>Treatment of sites</th>
<th>Predominant type of intrabony defect no of sites</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-wall</td>
<td>2-wall</td>
</tr>
<tr>
<td>EM D</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>23</td>
</tr>
</tbody>
</table>

(p<0.06)

A trend towards an uneven distribution of defects was found for control sites using the binomial test.
Table 3. Mean values for pocket depth, clinical attachment level and radiographic bone level

<table>
<thead>
<tr>
<th>Variable/examination</th>
<th>Test sites</th>
<th>Control sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>8 months</td>
</tr>
<tr>
<td></td>
<td>(n=34)</td>
<td>(n=34)</td>
</tr>
<tr>
<td>pocket depth (mm)</td>
<td>7.8 (1.1)</td>
<td>4.4 (1.0)</td>
</tr>
<tr>
<td>pocket reduction (mm)</td>
<td>-</td>
<td>3.3** (1.3)</td>
</tr>
<tr>
<td>attachment level (mm)</td>
<td>9.4 (1.5)</td>
<td>7.1 (1.0)</td>
</tr>
<tr>
<td>attachment gain (mm)</td>
<td>-</td>
<td>2.1** (1.5)</td>
</tr>
<tr>
<td>initial bone loss (mm)</td>
<td>7.1 (2.2)</td>
<td>0.9*** (0.6)</td>
</tr>
<tr>
<td>radiographic bone gain (mm)</td>
<td></td>
<td>0.9*** (0.6)</td>
</tr>
<tr>
<td>% of initial bone loss</td>
<td>13%</td>
<td>31%</td>
</tr>
</tbody>
</table>

(*)/**/*** Denotes statistical difference between test and control sites with p<0.08, p<0.05, p<0.01 and p<0.001

Data on intrabony defects
In the test group, most of the patients had 1 tooth surface involved in the osseous defect. The circumference of the defects ranged between 90 and 250°. In the control group, most of the patients had 1 tooth surface involved in the osseous defect. The circumference of these defects ranged between 60 and 270°.

The predominant type of defect at all surgical sites in all subjects was a 1- or 2-walled defect as required by the inclusion criteria. The characterization performed during surgery is given in Table 1. Both test and control sites had a mean intrabony component close to 5 mm, with about 1/4 being a 3-wall component at the apical part of the defect. The residual 3/4 of the defect was mainly 1-wall (43%) in the test sites but 2-wall (48%) in the control sites.

When the distribution of defects was analyzed, it could be demonstrated that in the EMDOGAIN®-treated sites 17 were predominantly 1-wall defects and 17 predominantly 2-wall defects. In the control sites, however, there were twice as many 2-wall defects as 1-wall defects included (23 compared to 11; Table 2). 7 patients had test/control pairs with predominantly 1-wall defects and 14 had test/control pairs with predominantly 2-wall defects. The remaining pairs had differing types of defects at the test and control site. There were 15 pairs of defects in the maxilla and 19 pairs of defects in the mandible.

Effectiveness
Post-trial power calculation
The post-trial calculated power and the 95% confidence intervals for the difference between EMDOGAIN® and control at 8 months were calculated to be 0.94 for the 34 paired probing measurements (0.26–0.98 mm) and 0.99 for the 29 paired radiographic measurements (0.71–1.27 mm).

Baseline measurements
The mean pocket depth at both test and control sites was 7.8 mm at the baseline examination. The mean attachment level was 9.4 mm for the test sites and 9.3 mm for the control sites. The mean radiographic bone level was 7.5 mm (as measured from the apex of the tooth) and the initial bone loss was 7.1 mm (as measured from the native bone level) for the test sites and 8.4 mm and 6.5 mm for the control sites, respectively.

There were no statistically significant differences between the EMDOGAIN®-treated sites and the control sites with respect to mean baseline measurements of probing pocket depths, attachment levels or radiographic bone levels (Table 3).

Re-examinations at 8, 16 and 36 months pocket reduction. Regarding pocket depth reduction (Table 3) there was a statistically significant difference between test and control sites within patients at 8 months postsurgery (mean values 3.3 mm in test versus 2.6 mm in control; p<0.01) for all 34 pairs of sites. 16 months postsurgery, mean pocket depth reduction was still significantly different between test and control (3.3 versus 2.6 mm; p<0.01). The mean pocket depth reduction at 36 months...
As shown in Table 3, the mean radiographic bone level at 8 months showed a statistically significant increase ($p < 0.01$) for the EMDOGAIN®-treated defects by 13% of initial bone loss or 0.9 mm, while the control defects showed an average loss of 2% or 0.1 mm. At 16 months, mean radiographic bone gain in EMDOGAIN®-treated defects was 31% of initial bone loss or 2.2 mm, while the control defects showed a mean radiographic bone loss of 4% or 0.2 mm, which constituted a statistically significant difference ($p < 0.001$).

Individual values for radiographic bone gain (RBG) or bone loss at 16 months post-surgery for EMDOGAIN®-treated and control sites ($n = 31$). The frequency of no gain or loss was much higher in the control sites than in the test sites. The mean gain of clinical attachment at 36 months postsurgery, the mean gain at the EMDOGAIN®-treated sites was 2.7 mm or 36% of initial bone loss compared to an unchanged bone level at the control sites. The value of radiographic bone gain at 36 months in the test sites was equal to a mean 66% radiographic bone fill of the original intrabony defects treated.

Fig. 3. Individual data for radiographic bone gain/loss (mm) 16 months post-surgery for EMDOGAIN®-treated and control sites ($n = 31$).

Fig. 4. (a) Radiographs at baseline (BL) and at 16 months post-treatment (16 mo) of a control site at the mandibular right cuspid demonstrating more or less unchanged bone levels at the 16 month follow-up (patient no. 11, female, 68 years, non-smoker). (b) Radiographs at baseline (BL) and at 16 months post-treatment (16 mo) of an EMDOGAIN®-treated site at the mesial surface of the mandibular left first bicuspid. Note regain of radiographic bone at the 16 month examination time point (patient no. 11, female, 68 years, non-smoker).
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favorable in lower jaws, 2-wall defects and non-smokers. However, the results of EMDOGAIN®-treatment, calculated as a difference between test and control sites within the same patient, was not dependent upon jaw, type of defect or smoking habits.

Safety (AEs)

4 subjective adverse events were reported in 4 patients. One of the patients reported “giddiness” on some occasions, another patient reported “stomach disturbances”, a 3rd patient reported “shooting pain” of short duration from one of the surgical sites (test) and a 4th patient had a gingival irritation around one of the surgical sites (control). 2 of these were general, and could not be attributed to test or control sites, one appeared in an EM-DOGAIN® treated site and another in a control site. 2 of the adverse events occurred immediately after surgery, while the other began two to 6 weeks after surgery. There were no other AEs or serious adverse events recorded during the course of the trial.

Discussion

The present clinical trial demonstrated that in patients having good oral hygiene, periodontal flap surgery alone at sites with predominantly 1- and 2-wall ed intrabony defects resulted in clinically beneficial effects similar to those

change at 4 sites and a net loss of bone at 10 sites. Thus, the predictability for radiographic bone gain was much higher in test sites (93%) than in control sites (48%). None of the test sites lost radiographic bone during the 36 month trial, while 37% of the control sites lost radiographic bone during the same period.

Fig. 7 illustrates radiographic bone gain or loss as a function of time. Each column represents mean values in test and control sites at each of the examination intervals, i.e., 8, 16 and 36 months. As can be seen, the control sites show a mean loss of radiographic bone at all time points. The test sites, however, show a continuous gain of bone over time.

Bleeding on probing. Local bleeding scores were maintained at or below 10% throughout the study period. There were no differences between the test and control sites at any of the examination intervals.

Plaque. Local plaque scores were maintained at or below 10% throughout the study period. There were no differences between the test and control sites at any of the examination intervals.

Influence of jaw, type of defect and smoking habits

The radiographic bone gain after 16 months in test as well as control sites varied between upper and lower jaw, between 1-wall and 2-wall defects and between smokers and non-smokers. Both test and control results were more

Fig. 6. (a) Radiographs at baseline (BL) and at 36 months post-treatment (36 mo) of a control site at the distal surface of the maxillary left first bicuspid, showing a change in radiographic bone level between the two examination time points (patient no. 7, female, 42 years, non-smoker). (b) Radiographs at baseline (BL) and 36 months post-treatment (36 mo) of an EMDOGAIN®-treated site at the distal surface of the maxillary right first bicuspid showing regain of bone at the 36 month examination time point. Note the well defined marginal bone compared to the control defect in Figure 6A (patient no. 7, female, 42 years, non-smoker).
reported previously (Lindhe et al. 1982, 1984, Pihlstrom et al. 1983, 1984, Lindhe & Nyman 1985, Westfelt et al. 1985). However, with the adjunctive use of EMDOGAIN®, effectiveness was significantly better. In regard to radiographic assessments, bone level gain at EMDOGAIN® treated sites was both predictable and clinically relevant during the entire study period, while control sites showed unchanged bone levels. Based on the definitions of adverse events used in this clinical trial there were no serious adverse events and only 4 non-serious adverse events recorded during the course of the trial. This relative lack of adverse events in a long-term trial with EMDOGAIN® supports the concept that the device can be used safely in periodontal surgical procedures.

At 8 months, the figures for clinical attachment level gain, pocket depth reduction as well as the figures for radiographic bone gain or loss, whether expressed in mm or in percent of initial bone loss, showed a significant difference for all outcome variables in favor of EMDOGAIN®-treated sites. At 16 months, the pocket depth reduction was maintained in EMDOGAIN® treated sites as well as in the control sites. The clinical attachment level gain had increased somewhat in both test and control sites. However, the radiographic bone gain in EMDOGAIN® treated sites showed a marked increase compared to the control sites, which showed an unchanged level (or a further loss) of radiographic bone. At 36 months, the figures (expressed as means from observations in 26 patients) for clinical attachment level gain and pocket depth reduction still show significant differences in favor of the EMDOGAIN® treated sites. The mean radiographic bone gain at 36 months was 36% of initial bone loss in EMDOGAIN® treated sites compared to an unchanged bone level in the control sites. The 36% radiographic bone gain in the test sites corresponded to a 66% defect fill in the radiographic analysis. Furthermore, 74% of the test sites regained more than 20% of the initial bone loss, while none of the control sites reached a gain of 20% of the initial bone loss. These differences in clinical outcome between test and control sites are clearly in favor of EMDOGAIN®-treatment and must be considered clinically relevant.

If radiographic bone gain or loss was considered as a function of time (Fig. 7), it could be demonstrated that the control sites showed a mean additional loss of radiographic bone at each of the examination intervals, i.e., 8, 16 and 36 months. The test sites, on the other hand, show an increasing gain of bone over time. This is an interesting observation, which may illustrate the potential for continued and marked alveolar bone gain over long periods of time, if the attachment apparatus is reformed with adequate functional capacity.

The magnitude of the difference in probing attachment gain between test and control sites at 16 months and 36 months follow-up were 0.6 mm and 0.5 mm, respectively, which demonstrate a statistically significant difference between groups at the 0.01 level. To what extent such improvements also constitute a clinically significant difference is a matter of discussion and to the best of our knowledge no guidelines have been issued that specifically deal with this question. Recently, however, the American Dental Association (ADA) published proposed guidelines for acceptance of products for professional, non-surgical treatment of adult periodontitis (Imrey et al. 1994). Although these guidelines deal with determining the efficacy of products to supplement scaling and root planing in non-surgical treatment of periodontitis, they include a general discussion of clinical significance and implementation of clinical significance criteria for new products intended for periodontal treatment. ADA proposes to interpret clinical significance as a minimal level of effectiveness which should be considered to provide meaningful (i.e., clinically relevant) benefit. Since no biological rationale for an absolute criterion of clinical significance (such as x mm of clinical attachment gain) can be given, the absence of such a rationale a minimally clinically significant effect may best be expressed as a fraction of the effect of the control procedure alone. Thus, a minimally clinically significant effect for products like EMDOGAIN® to be used in conjunction with surgery would be best expressed as a fraction of the effect of the surgical procedure alone on the improvement of periodontal attachment levels/bone levels. In the present study, the additional improvement in the EMDOGAIN® treated sites is 35% for clinical attachment levels and 31% for radiographic bone at 16 months, and 30% for clinical attachment levels and 36% for radiographic bone at 36 months. It is suggested that fractions of this magnitude, one third over and above the effect of the control treatment, constitute clinically significant improvement over the surgical procedure used as a control.

The single most difficult problem in determining effectiveness in regeneration studies is the interpretation of results. If the clinical outcome in a 1-wall test site showing true regeneration, characterized by the reformation of normal cementum with an associated periodontal ligament and new alveolar bone, is compared to that of a control site showing a healing response characterized by downgrowth of a junc-
tional epithelium to the pre-surgical level and reformation of a dense connective tissue in the gingiva, clinical measurements will only demonstrate a small difference in regard to change in probing clinical attachment level in spite of a marked difference in functional (histological) attachment. However, in 3-wall (and to some degree in 2-wall or combined 3-wall intrabony defects), healing will often result in a significant regrowth of bone, although often without the reformation of cementum and an associated functional periodontal ligament. This type of periodontal healing will affect probing assessments as well as radiographic evaluation and show a gain which may be regarded in a site showing true periodontal regeneration. Clinical probing attachment level measurements are determined by the extent of probe tip penetration into the gingival sulcus/periodontal pocket (Magnarson & Listgarten 1980, Caton et al. 1981, Fowler et al. 1982). Regardless of the level of histological attachment, a major determinant of the level to which the probe will penetrate is the status of the gingival/periodontal tissues, i.e., degree of inflammation and relative content of fibers and cells, providing other parameters such as examiner, type of probe, probe pressure etc., are standardized (Van der Velden 1979). This means that probing clinical attachment level measurements may not be a suitable primary outcome parameter in regenerative periodontal surgery.

Thus, it is not surprising that the individual data regarding clinical attachment gain (Fig. 1) showed smaller differences compared to individual data for radiographic bone gain (Fig. 2). 18 or 58% of the test sites and 8 or 25% of the control sites showed more than a 2 mm gain of clinical attachment at 16 months, while 23 or 74% of the test sites and none of the control sites had a radiographic bone gain of more than 20% of the initial bone loss. A most identical observations were made at 36 months, when more than 2 mm gain of clinical attachment was found in 56% of the test sites versus 33% of the control sites, and bone gain exceeding 20% of the initial bone loss was found in 74% of the test sites and none of the control sites. This is to be expected in a well-maintained study population in which the gingival tissues are relatively free of inflammation, i.e., local scores for plaque and bleeding on probing less than 10% (Lang et al. 1996).

Since effectiveness was thoroughly established in suitable animal experiments (Hammarstrom 1997) and since the present trial resulted in gain in clinical attachment and radiographic bone a case for effectiveness of EMDO
gain \textsuperscript{a} in regenerative periodontal treatment can be made. If animal study results are accepted as an indicator of the type of healing obtained in humans, it follows that the healing in EMDO
gain \textsuperscript{a} treated sites in the present clinical trial would be one of regeneration and not of repair.

In the last several years, studies have been performed to systematically identify factors which could enhance predictability and maximize treatment outcome in regenerative periodontal surgery (Preber & Bergstrom 1986, 1990, Tonetti et al. 1993, Grossi et al. 1995, Rydel 1996). In the present clinical trial, however, no effort was made to exclude any patients with smoking habits, various systemic disorders, etc.

The patients included were typical of the patients routinely encountered in the office of a practicing periodontist. Furthermore, patients included in the present clinical trial had been subjected to systematic periodontal treatment including repeated mechanical debridement and therapy supplemented with antimicrobial as well as surgical procedures in the experimental areas. These therapeutic regimens had been provided over long periods of time, which was often several years. The overall assessment of these patients is that they presented with a periodontal disease that was not responsive to conservative periodontal therapy. Furthermore, this group of subjects probably represents a cohort, which normally is not included in regenerative periodontal studies.

Many regenerative periodontal studies are focused on 3-wall defects and buccal mandibular molar class II furcation defects. The 3-wall defects have many attributes such as unique anatomic features which appear to have great impact on the possibility to perform successful and predictable treatment in patients with good oral hygiene (Poison & Hjell 1978). Likewise, buccal mandibular molar class II furcation defects have often shown favorable treatment results (Pontoriero et al. 1987, 1988). 1- and 2-wall intrabony periodontal defects as investigated in the present clinical trial are, however, not routinely included in periodontal regeneration studies. The primary reason is that they usually do not respond well to regenerative therapy (Revert et al. 1985, Proestakis et al. 1992 a, b). The favorable results obtained in the present clinical trial underlines the therapeutic prospects in regenerative periodontal therapy for EMDO
gain \textsuperscript{a}.

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