J Clin Periodontol 2002; 29: 317–325
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Journal of Clinical Periodontology
ISSN 0303-6979

Enamel matrix proteins in the regenerative therapy of deep intrabony defects

A multicentre randomized controlled clinical trial

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Tonetti MS, Lang NP, Cortellini P, Suvan JE, Adriaens P, Dubravec D, Fonzar A, Fourmousis I, Mayfield L, Rossi R, Silvestri M, Tiedemann C, Topoll H, Vangsted T, Wallkamm B: Enamel matrix proteins in the regenerative therapy of deep intrabony defects. A multicentre randomized controlled clinical trial. J Clin Periodontol 2002; 29: 317–325. © Blackwell Munksgaard, 2002.

Abstract

Aim: This prospective multicentre randomized controlled clinical trial was designed to compare the clinical outcomes of papilla preservation flap surgery with or without the application of enamel matrix proteins (EMD).

Material and methods: 172 patients with advanced chronic periodontitis were recruited in 12 centers in 7 countries. All patients had at least one intrabony defect of ⩾3mm. Heavy smokers (⩾20 cigarettes/day) were excluded. The surgical procedures included access for root instrumentation using either the simplified or the modified papilla preservation flap in order to obtain optimal tissue adaptation and primary closure. After debridement, roots were conditioned for 2 min with a gel containing 24% EDTA. EMD was applied in the test subjects, and omitted in the controls. Postsurgically, a strict plaque control protocol was followed. At baseline and 1 year following the interventions, clinical attachment levels (CAL), pocket probing depths (PPD), recession (REC), full-mouth plaque scores and full-mouth bleeding scores were assessed. A total of 166 patients were available for the 1-year follow-up.

Results: At baseline, 86 test and 86 control patients presented with similar subject and defect characteristics. On average, the test defects gained 3.1±1.5 mm of CAL, while the control defects yielded a significantly lower CAL gain of 2.5±1.5 mm. Pocket reduction was also significantly higher in the test group (3.9±1.7 mm) when compared to the controls (3.3±1.7 mm). A multivariate analysis indicated that the treatment, the clinical centers, cigarette smoking, baseline PPD, and defect corticalisation significantly influenced CAL gains. A frequency distribution analysis of the studied outcomes indicated that EMD increased the predictability of clinically significant results (CAL gains ⩾4 mm) and decreased the probability of obtaining negligible or no gains in CAL (CAL gains <2 mm).

Conclusions: The results of this trial indicated that regenerative periodontal surgery with EMD offers an additional benefit in terms of CAL gains, PPD

reductions and predictability of outcomes with respect to papilla preservation flaps alone.

Key words: differentiation factors; intrabony defects; periodontal regeneration; periodontal surgery; randomized controlled clinical trial

Accepted for publication 19 April 2001

Considerable histologic and clinical evidence gathered over the last 2 decades indicate that the regeneration of periodontal tissues lost as a result of periodontitis can be achieved in humans. In particular, two clinical approaches have been routinely employed with considerable success: bone grafting (Rosen et al. 2000) and guided tissue regeneration using barrier membranes (Cortellini & Tonetti 2000). Nevertheless, variability in outcomes and technical difficulties have fostered a growing interest in the possibility to modulate the periodontal wound healing process using biological mediators. In this context, the use of growth factors was first suggested in the late 1980s (Lynch et al. 1989).

As the understanding of the development of the periodontal attachment apparatus progressed, the potential rôle of mediators expressed by Hertwig's root sheath in the reconstruction of the periodontal ligament was suggested (Slavkin 1976). Subsequently a series of animal experiments led to the identification of the role of enamel matrix proteins in the development of the root and the adjacent periodontal ligament (Hammarström 1997, Schonfeld & Slavkin 1977). These observations led to the development of a novel concept for the regeneration of the periodontium: the use of differentiation factors to recapitulate development during wound healing. The application of proteins derived from the enamel matrix yielded histological evidence for periodontal regeneration in monkeys (Hammarström 1997) and humans (Mellonig 1999, Sculean et al. 1999a, Yukna & Mellonig 2000). Subsequent clinical studies provided evidence of clinical attachment level gains and pocket depth reductions (Heden et al. 1999, Heijl et al. 1997, Pontoriero et al. 1999, Zetterström et al. 1997). Three clinical trials have reported improved gains in clinical attachment levels following the application of enamel matrix derivative (EMD) in the regeneration of intrabony defects with respect to access flap alone (Heijl et al. 1997, Pontoriero et al. 1999, Silvestri et al. 2000). The paucity of direct evidence evaluating the additional benefit expected from the application of EMD, however, limits the routine application of this concept.

The objective of the present clinical investigation was therefore to compare, in a multicentre randomized controlled clinical trial, the outcomes obtained following treatment of intrabony defects with papilla preservation flap surgery with or without application of enamel matrix derivative (EMD).

Material and Methods

Experimental design

A parallel group, randomized, multicentre and controlled clinical trial was designed to test the efficacy of two treatment modalities in intrabony periodontal defects. The test treatment consisted of access of the defect with papilla preservation flaps, surgical debridement, root conditioning and application of enamel matrix derivative (EMD) to the debrided root surface. The same procedure was performed in the control group except for the omission of enamel matrix derivative. A single defect was treated in each patient. Patient outcomes were evaluated during the healing period, while clinical outcomes were evaluated at 1 year and radiographic assessment was performed after 18 months. The study design is outlined in Fig. 1. This investigation was performed at 2 university and 10 periodontal practices constituting a practice based research network. Centers were located in Belgium, Germany, Greece, Italy, The Netherlands, Switzerland and the USA. In each center, the examiner and the therapist were identical. To limit assessment bias, clinicians did not have previous measurements available to them and used a pressure sensitive probe. Each clinical center was connected with and supervised by a central monitoring facility at the University of Berne, Switzerland.

Investigators' meeting and calibration

An investigator meeting was performed as previously described (Tonetti et al. 1998). In brief, a calibration exercise was performed to obtain acceptable intra- and inter-examiner reproducibility for pocket depth, recession of the gingival margin, and evaluation of defect anatomy. Intra-examiner reproducibility was evaluated as standard deviation of the difference of triplicate measurements. All investigators reached the target of a standard deviation lower than

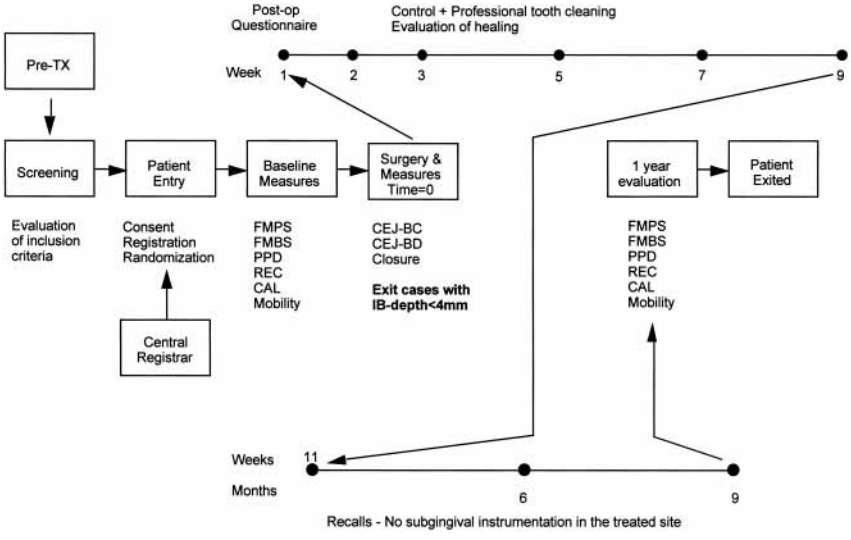

Fig. 1. Schematic illustration of the study outline. See text for abbreviations.

0.4 mm for attachment levels. Inter-examiner variability was evaluated as standard deviation of the difference from the gold standard represented by the first author. The computed value for attachment level was less than 0.5 mm for all clinicians.

Subject population

Inclusion and exclusion criteria were as previously reported (Tonetti et al. 1998). In brief, patients younger than 21 years, with uncontrolled or poorly controlled diabetes, unstable or life threatening conditions, requiring antibiotic prophylaxis, or heavy smokers (more than 20 cigarettes/day) were excluded (Tonetti et al. 1995). Only patients with a diagnosis of severe periodontitis previously treated by oral hygiene instructions and scaling and root planing were invited to participate. These subjects had to present with full-mouth plaque scores (FMPS) and/or full-mouth bleeding scores (FMBS) $<25\%$ at study baseline (following completion of the initial periodontal treatment phase) (Tonetti et al. 1993, Tonetti et al. 1996). The patients were informed in detail about the possible risks and benefits and were asked to give their consent to the trial. The Ethical Board of the University of Berne, Switzerland had previously approved the study protocol.

Entry criterion was the presence of a deep intrabony defect ($\geqslant$3 mm), located in the interproximal area, in anterior and premolar teeth. Defects extending into a furcation were not included. Depth of the intrabony component of the defect and absence of furcation involvement were preliminarily evaluated during the screening phase but had to be confirmed during surgery. Inclusion of defects involving the mesial aspect of the lower first molar was individually evaluated for access and thickness of the alveolar ridge (ability to preserve the papilla). The presence of a 2–3 mm band of keratinized gingiva to allow surgical manipulation, flap adaptation and suturing according to the protocol was also required.

The size of the required sample to detect a true difference of 0.5 mm between test and control with 90% power and with an alpha error of 0.05 was estimated as described (Fleiss 1986) using clinical attachment level changes as the primary outcome variable. Based on previous estimates of outcome variability (Heijl et al. 1997) and subject attrition rates observed in previous clinical trials of similar design by this group (Tonetti et al. 1998), a total of 150 subjects with complete data were required.

Pre-treatment

Control of periodontal infection in the dentition was achieved prior to the experimental phase by an initial treatment consisting of patient motivation, oral hygiene instructions and scaling and root-planing. When indicated, clinicians supplemented mechanical debridement with antiseptics.

Randomization

After verification of the entry criteria, 172 subjects gave informed consent and were enrolled into the study. All subjects were assigned a patient number, and were randomly assigned to one of the two treatment regimens. Assignment was performed by a central randomization facility using a custom-made program based on balanced random permuted blocks. Furthermore, to reduce the chance of unfavorable splits between test and control group in terms of key prognostic factors, the randomization process balanced smoking status, average pocket depth at the defect sites and the number of deep defects (PPD $>$8 mm) in the test and control groups. Except for the above-mentioned prognostic variables, no patient or defect characteristics were available to the central randomization registrar.

Clinical measures

Before anesthesia, the following clinical parameters were evaluated on the day of the surgical procedure and 1-year later. Full mouth plaque scores (FMPS) were recorded as the percentage of total surfaces (4 aspects per tooth) which revealed the presence of plaque (O'Leary et al. 1972). Bleeding on probing from the bottom of the pocket was assessed dichotomously at a force of 0.3 N with a manual pressure sensitive probe (Brodontic® probe equipped with a PCP-UNC 15 tip, Hu-friedy). Full mouth bleeding scores (FMBS) were then calculated.

Probing pocket depth (PPD) and recession of the gingival margin (REC) were recorded to the nearest millimeter with a manual pressure sensitive probe by trained investigators at the deepest location of the selected interdental site. All measurements were taken with a pressure sensitive manual periodontal probe at 0.3 N (Brodontic® probe equipped with a PCP-UNC 15 tip, Hu-friedy). Clinical attachment levels (CAL), calculated as the sum of PPD and REC, were the primary outcome variable.

Surgical procedures

Test and control defects were accessed using papilla preservation flaps. The simplified papilla preservation flap was used to gain access to the root surface and the marginal alveolar bone in areas where the interproximal space had a mesio-distal width of 2 mm or less as measured at the level of the interproximal soft tissue (Cortellini et al. 1999). The modified papilla preservation technique was used in areas with a mesio-distal width of the interproximal space greater than 2 mm (Cortellini et al. 1995). The exposed defects were carefully scaled and root planed to remove residual mineralized deposits, but not necessarily the root cementum. A combination of sonic, ultrasonic and/or hand instrumentation was used for this purpose. Root surfaces at both test and control sites were conditioned with a neutral pH EDTA gel (PrepHgel®, Biora AB, Sweden) for 2 min (Blomlof et al. 1996, Blomlof & Lindskog 1995). In the test sites, enamel matrix derivative (EMD, Emdogain®, Biora AB, Sweden) gel was applied on the root surface and to overfill the defect. The flaps were then replaced and sutured employing non-resorbable e-PTFE sutures (Gore-Tex™, W.L. Gore and Associates, Flagstaff, AZ) as previously described (Cortellini & Tonetti 2000). The control procedure was identical to the test surgery, apart from the omission of the EMD application.

Intrasurgical clinical measurements

The following defect morphology parameters were evaluated after debridement of the area essentially as described (Cortellini et al. 1993): (i) distance from the cemento-enamel junction (CEJ) to the bottom of the defect (CEJ-BD); (ii) distance from the CEJ to the most coronal extension of the interdental bone crest (CEJ-BC) to the nearest mm. These measurements were performed at the deepest interdental point of the defect (i.e., the deepest point of the site

defined by the interdental line angles of the affected tooth). The intrabony component of the defect (INFRA) was calculated as INFRA=(CEJ-BD) – (CEJ-BC).

The duration of the surgical procedure was timed and the number of teeth involved in the surgical procedure was recorded.

Post-surgical instructions and infection control

Post-operative pain and edema were controlled with tablets of either 600 mg ibuprofen or 500 mg acetaminophen. Patients were instructed to rinse 2× daily with 0.12% chlorhexidine and to use modified oral hygiene procedures in the treated area for the first 4 post-operative weeks. They were instructed to start gentle wiping of the operated dento-gingival area with a post-surgical toothbrush (Vitis Surgical, Dentaid SA, Barcelona, Spain) soaked in a 0.12% chlorhexidine solution from the third post-operative day. No interdental cleaning was allowed in the first four post-operative weeks. Smokers were asked to limit and possibly avoid smoking.

Post-surgical controls and professional tooth cleaning (weeks 1 to 6)

Sutures were removed after 1 week. Post-surgical controls and professional tooth cleaning consisting of supragingival prophylaxis with a rubber cup and 0.2% chlorhexidine gel (Plak-Out gel, Hawe-Neos, Switzerland), were performed at weeks: 1, 2, 3, 4 and 6. At these time points presence of edema, hematoma, suppuration, flap dehiscence, and patient complaints were dichotomously recorded.

Maintenance care (months 3, 6 and 9)

All patients were maintained in supportive care programs and they received full mouth professional prophylaxis and calculus removal at 3, 6 and 9 months as previously detailed (Tonetti et al. 1998).

Data management and statistical analysis

Data were entered in a microcomputer and proofed for entry errors. The resulting database was locked and loaded in SAS format (Statistical Application Software, SAS Institute, Cary, NC). All calculations and analyses were performed using SAS Version 6.12. Data are expressed as means±SD. Unbalances in the test and control groups arising from the randomization process were evaluated using the unpaired *t*-test for continuous variables and the chi-square test for categorical variables. The significance of the treatment effects on the dependent variables CAL changes and PPD changes was estimated by constructing generalized linear models using the SAS GLM procedure. The clinical centre and the treatment by centre interaction were incorporated as stratification factors (Fleiss 1986, Golgberg & Koury 1989). In case of a non-significant treatment-by-center interaction, the interaction term was removed from the analysis and the main effect model was applied (Golgberg & Koury 1989). Final models were selected by elimination of non-significant factors. Model diagnostics included distribution of errors and analysis of residuals. Data were also analyzed as frequency distributions employing the Mantel-Haenszel chi-square test to compare distributions of outcomes at test and control sites. The odds of achieving a highly significant clinical outcome with the test treatment (CAL gains of $\geqslant$3 mm) were evaluated by constructing a logistical model. The final model was selected with a backward elimination procedure that allowed factors to remain in the model whenever their significance was $p=0.1$. For all other analyses the alpha error was set at 0.05.

Results

Randomization

The patient and defect characteristics of the test and control groups resulting from the randomization process yielded no significant differences between any of the patient associated variables. Both test and control groups were assigned 86 subjects with 37% smokers (<20 cigarettes/day) in each of the groups. The mean pocket probing depths after randomization were 7.70±1.36 mm for the test and 7.69±1.36 mm for the control group, respectively.

Patient retention and missing data

A total of 172 subjects were entered and randomized. 3 subjects withdrew informed consent before surgery. 169 subjects received treatment (85 test and 84 controls). During the 1-year period, 3 subjects were lost to follow-up: 2 test and 1 control patient. Complete observations were available for 166 subjects: 83 tests and 83 controls. This represented 96.5% of entered patients. All subsequent analyses were performed on this population with the exception of intent to treat analysis of the primary outcomes (CAL gain, PPD reductions, REC changes) in which missing data were handled as no differences between the baseline and the 1-year follow-up.

Subject characteristics at baseline

The mean age of the patients was 48±9 years for both test and control group. Females represented 54.2% of the test group and 60.2% of the control group. 22.9% of the test and 19.3% of the control group had received previous periodontal therapy before initiation of the study. At baseline, 36.1% of the test and 41.0% of the control group were smokers of less than 20 cigarettes per day. No significant differences between test and control patients were observed for any of the subject characteristics at baseline (Table 1).

Oral hygiene

Baseline FMPS and FMBS are displayed in Table 1. At 1 year, the FMPS was 14±10% for test and 13±10% for control treated patients ($p=0.58$, *t*-test). Similarly, the FMBS was 10±8% for test and 11±7% for control subjects ($p=0.63$, *t*-test).

Defect characteristics at baseline

Mean pocket probing depths (PPD) at the defect sites were 8±1.5 mm for test and 7.7±1.5 mm for control sites (Table 1). Mean clinical attachment levels (CAL) were 9.4±2.1 mm and 9.1±2.0 mm at test and control defect sites, respectively. The mean distances from the CEJ to the bottom of the defect were 10.3±2.4 mm for test and 10.0±2.2 mm for control defects with an intrabony component of 5.8±2.1 at the test and 5.4±2.0 mm at the control sites. No significant differences were observed for any of the defect characteristics between test and control sites at baseline.

Clinical outcomes

Table 2 describes the treatment outcomes for both EMD applications in

Table 1. Patient and defect characteristics for test and control groups at baseline (n=166*)

Variable	Test	Control	Significance p-value
subject no.	83	83	–
age (years)	48±9	48±9	0.684
gender (% females)	54.2	60.2	0.434
smokers (%, <20 cigarettes/day)	36.1	41	0.525
FMPS (%)	11±6	11±7	0.176
FMBS (%)	13±6	13±6	0.728
previous periodontal therapy (%)	22.9	19.3	0.569
PPD (mm)	8±1.5	7.7±1.5	0.817
CAL (mm)	9.4±2.1	9.1±2	0.585
CEJ-BD (mm)	10.3±2.4	10±2.2	0.411
intrabony component (mm)	5.8±2.1	5.4±2	0.592
predominantly 1 wall (%)	33.8	25.6	0.150**
predominantly 2 walls (%)	42.5	41.1	0.150**
predominantly 3 walls (%)	23.7	33.3	0.150**

* 3 subjects who withdrew consent before treatment and 3 subjects lost to follow-up have been excluded from the analysis.
** Defect wall morphology (Mantel-Haenszel χ^2).

Table 2. Clinical outcomes at 1 year

Outcome variable	Test (n=83) (EMD)	Control (n=83) (access flap)	Significance p-value
gain in CAL	3.1±1.5	2.5±1.5	0.01
decrease in PPD	3.9±1.7	3.3±1.7	0.02
increase in REC	0.8±1.2	0.8±1.2	0.86

combination with papilla preservation access flaps (test) and papilla preservation access flaps alone (control). As for the primary outcome variable, the average gain in clinical attachment was 3.1±1.5 mm for the test sites and 2.5±1.5 mm for the sites treated with access flap alone. This additional benefit at the test sites was statistically highly significant (p=0.01).

One year after therapy, pocket depth reductions were 3.9±1.7 mm for the test group, and 3.3±1.7 for the control group (Table 2). This difference was statistically significant (p=0.02). Between baseline and 1 year, the gingival margin receded of 0.8±1.2 mm in both the test and the control sites with no statistically significant differences (p=0.86).

Essentially identical results were observed when an intent to treat analysis was used to handle the missing data: CAL gains of 3±1.6 mm were observed for test subjects while controls resulted in CAL gains of 2.4±1.6 mm (p=0.006). PPD reductions of 3.8±1.8 mm and 3.1±1.8 mm were detected for test and control, respectively (p=0.01). Changes in recession of the gingival margin were 0.8±1.2 mm for test and 0.7±1.2 mm for control (p=0.75).

The significance of the treatment effect was also evaluated taking into account the potential sources of variability arising from the multicentre design of the study and the previously described covariates (Falk et al. 1997, Tonetti et al. 1993, Tonetti et al. 1995, Tonetti et al. 1996). Since no treatment by center interaction was observed the main effect model was applied (Golgberg & Koury 1989). The following variables were used in the model: treatment, center effect, smoking status, baseline PPD and defect corticalisation (Table 3). The multivariate model was statistically significant and explained 42% of the observed variability in CAL gain.

The surgical treatment combining access flap with the application of EMD resulted in significantly greater CAL gains than the access flap control (p=0.0293). A highly significant center effect was also observed (p=0.0001). The difference between the center that obtained the largest CAL gains and the one with the smallest was 2.6±0.6 mm. Also, non-smokers had better treatment outcomes than smokers did (p=0.0882). On average, non-smokers had 0.4±0.2 mm higher CAL gains than smokers did. Among the considered defect characteristics, the initial pocket depth was a highly significant covariate (p<0.0001). Furthermore, markedly corticalized and very cancellous bleeding intrabony defects had significantly (p=0.0132) lower CAL gains than defects with a regular cribriform bony lining.

The frequency distribution of various CAL gains at test and control sites are depicted in Table 4. Highly significant (p=0.01) differences yielded by the Mantel-Haenszel χ^2 test are evident. Almost double as high proportions of sites with clinically relevant attachment level gains of 4 mm or more (38.3%) were evidenced in the test sites when compared with access flap surgery alone (20.5%). On the other hand, sites with no or very small gains were more frequent in the control (17.9%) than in the test sites (9.9%). Likewise, the few sites, which lost attachment as a result

Table 3. Multivariate analysis of CAL gain

Parameter	Estimate	Significance
treatment effect	0.5±0.2	p=0.0293
centre effect (worst versus best)	−2.6±0.6	p=0.0001
smoking (yes versus no)	−0.4±0.2	p=0.0882
baseline PPD (mm)	0.4±0.1	p=0.0001
defect corticalisation*	0.8±0.3	p=0.0132

Significance of model p<0.0001, adjusted r-Square=0.42.
* See text for explanation.

Table 4. Frequency distribution of CAL gain

	Changes in CAL (mm)				
	loss	0–1	2–3	4–5	⩾6
test (EMD)	1.2%	9.9%	50.6%	34.5%	3.8%
control	2.6%	17.9%	59.0%	17.9%	2.6%

Mantel-Haenszel χ^2 p=0.01.

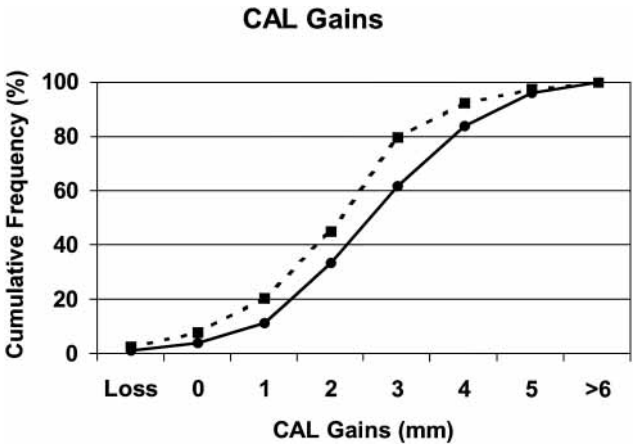

Fig. 2A. Cumulative frequency diagram illustrating CAL changes in test (filled circles) and control (filled squares) sites.

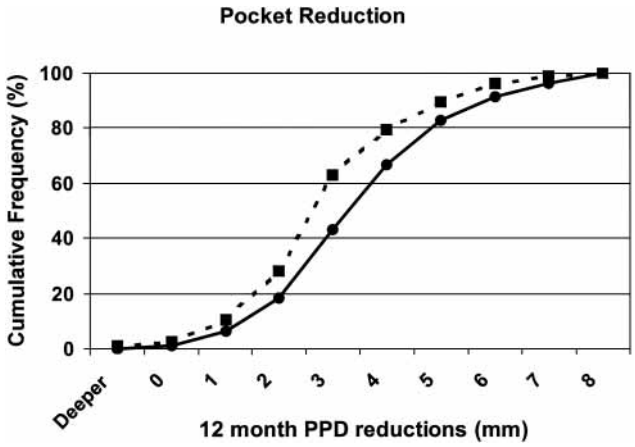

Fig. 2B. Comulative frequency diagram illustrating PPD reductions in test (filled circles) and control (filled squares) sites.

of surgical therapy, were less frequent in the test sites (1.2%). The controls showed more than twice as high proportions (2.6%) of sites with attachment loss.

The cumulative frequencies for CAL gains and PPD reductions are also visualized in Figs. 2A, B, respectively. Clearly, the application of EMD (Emdogain®) in combination with access flaps resulted in shifts of the cumulative frequencies towards higher CAL gains (Fig. 2A) and increased PPD reductions (Fig. 2B) when compared with access flaps alone.

Finally, the factors affecting the treatment outcomes in the test group alone were analyzed using a logistic regression model. A clinically relevant CAL gain of 3 mm or more by an application of EMD in combination with access flap surgery was significantly affected by the center, the smoking status, the corticalisation and the number of bony walls limiting the intrabony defect. However, FMPS, depth of the intrabony component of the defect and periosteal incisions did not significantly influence the treatment outcomes. In essence, the chance of gaining 3 mm CAL in a particular center may be reduced by 27%. Smoking may impinge on the treatment outcome with a 74% reduction in odds. Defects with either a dense cortical or very cancellous, bleeding walls displayed an 86% reduced chance over a normally corticalized, cribriform defect. Intrabony defects with 3 walls had a 269% higher chance than 1-wall intrabony defects to gain 3 mm CAL or more (Table 5).

Discussion

The results of this investigation indicated that the use of EMD combined with papilla preservation flaps provide significant additional benefit in the regenerative treatment of intrabony defects. In the multivariate analysis, EMD resulted in an additional mean benefit of 0.5±0.2 mm in clinical attachment gain after correcting for the known covariates. Furthermore a significantly greater reduction in PPD was observed in the test subjects (Table 2). No differences in recession of the gingival margin were observed comparing test and control. Thanks to the high percentage of patients retained into the study until the 1-year follow-up appointment (96.5%), these results were essentially the same when an intent to treat analysis was performed.

The average CAL gain observed in the test group was 3.1±1.5 mm. Previous clinical studies have reported average CAL gains ranging from 2.1 to 4.6 mm (Heden et al. 1999, Heijl et al. 1997, Parashis & Tsiklakis 2000, Pontoriero et al. 1999, Sculean et al. 1999b, Silvestri et al. 2000, Zetterström et al. 1997). A summary of these reports using weighted means indicates that CAL gains of 3.6±1.7 mm were observed in 375 defects in 8 studies (95% C.I. 3.4–3.9 mm). It should however be considered that these reports include both controlled randomized clinical trials and case series. A focus on controlled clinical trials comparing EMD to access flap controls indicates that the observed CAL gains were 2.7+1.4 mm in only 54 defects in 3 studies (95% C.I. 2.1–3.3 mm) (Heijl et al. 1997, Pontoriero et al. 1999, Silvestri et al. 2000). The average CAL gains observed in this controlled, randomized clinical trial are in agreement with the reports of the previous controlled studies but are significantly smaller than those observed in case series. This is not unexpected: since it is widely accepted that the unconscious bias associated with uncontrolled study design leads to an overestimation of the clinical outcomes.

The observed, uncorrected additional benefit of EMD application of 0.6 mm detected in the present study is identical to the one reported by Heijl et al. (1997) following application of EMD. The similarity with the results of the study

Table 5. Logistic regression analysis of factors significantly affecting the probability of obtaining CAL gains ⩾3 mm using EMD

Parameter	Odds ratio	95% C.I.
centre	0.73	0.57–0.9
smoking	0.26	0.1–0.9
defect corticalisation*	0.14	0.03–0.5
no. bony walls (3 walls versus 1 wall)	2.69	1.1–7.5
periosteal incision to close the flap	0.25	0.05–1.1, NS
FMPS	0.91	0.82–1.0, NS

* See text for explanation.

by Heijl et al. (1997) is remarkable given the extensive differences in design between the two investigations: parallel group versus split-mouth design. The average magnitude of the observed CAL gain was inferior to that previously reported by this group using bioresorbable barrier membranes (Cortellini et al. 2001, Tonetti et al. 1998). In those studies an average benefit of 0.8 and 0.9 mm was observed using Resolut and Guidor membranes respectively. Although a proper comparison cannot be made across studies, the data seem to suggest that application of resorbable barrier membranes may result in a 25 to 30% greater average added benefit with respect to control.

These data clearly support the concept that the application of EMD is effective. Averages, however, are not conducive to a discussion of the clinical significance of a treatment modality. Frequency distributions of the outcomes were presented for this purpose (Table 4, Figs. 1, 2A, 2B). These analyses indicated that EMD treatment is able to significantly shift the distribution of the outcomes towards higher values of CAL gain. In particular, the test treatment resulted in twice as many cases that gained 4 mm of CAL or more than the control. Conversely, the control treatment resulted in clinically insignificant CAL gains (less than 2 mm) twice as often as the test.

The results of this investigation indicated that a substantial degree of variability in outcomes was observed. The multivariate analysis showed that there was a substantial center effect. Significant center effects following regenerative therapy with GTR membranes have been observed in two previous randomized controlled clinical trials from this group (Cortellini et al. 2001, Tonetti et al. 1998). Of interest is the observation that the magnitude of the center effect observed following application of EMD and different GTR membranes was similar (2.6 mm in the present study compared with 2.1 mm and 1.7 mm with Guidor and Resolut membranes, respectively). Therefore, the application of this novel regenerative principle did not reduce or eliminate the previously reported center effect. The observed center variability is clinically relevant and could be dependent on differences in the enrolled patients in terms of social background, type of periodontal disease, response to therapy, persistence of specific pathogens and differences in technical ability, clinical organization and experience of the different clinicians. It confirms the relevance of patients and clinician-associated factors in the outcomes of periodontal surgical therapy.

Among the measured variables, cigarette smoking, baseline pockets depths and defect corticalisation were associated with reduced expected amounts of CAL gains. Other factors found to be relevant in previous investigations of GTR therapy in intrabony defects (Tonetti et al. 1993, Tonetti et al. 1995, Tonetti et al. 1996), such as FMPS, FMBS, and depth of the intrabony component of the defect, were not significant. The post-operative protocol and the rigid plaque control regimen enforced during the study could explain, at least in part, the lack of significance of FMPS and FMBS on the clinical outcomes. In two recent studies on GTR essentially the same results were observed (Cortellini et al. Submitted, Tonetti et al. 1998). The lack of significance of the baseline intrabony component of the defect (Tonetti et al. 1993, Tonetti et al. 1996) is probably due to the impact of pocket depth in the statistical model: pocket depth and depth of the intrabony component of the defect are highly correlated.

Of interest was the impact of defect corticalisation on the healing outcome. In this study, defects with dense and cortical or cancellous and bleeding walls displayed lower amounts of CAL gain at one year as compared to the defects with a regular cribriform bony lining. The present observations clearly indicate that the quality of the bony lining of the defect has a significant impact on CAL gains. Interestingly both highly corticalized and highly cancellous defects negatively impacted the outcome. The significance of cancellous and bleeding walls was somehow unexpected. In interpreting these observations, care should be taken not to limit the discussion to the availability of capillary loops for the wound healing period: differences in the disease status of a cancellous/bleeding defect may also contribute to the observation. Nevertheless, clinicians have long paid considerable attention to the characteristics of the bony lining of the defect. Anecdotal evidence has suggested: (i) the use of intra-marrow penetration of the defect walls with small burs during surgery, in order to obtain a bleeding defect and/or (ii) the treatment of acute lesions. Cited rationale for this practice has included better clot formation and improved angiogenesis. In conjunction with the use of grafting material Sanders et al. (1983) have reported a 10% increase in the frequency of successful results in cases where intra-marrow penetration was performed. Based on the present data, however, it is unclear whether or not modification of defect corticalisation has the potential to positively affect clinical outcomes.

The following conclusions can be drawn from this investigation.

(I) Application of EMD in conjunction with papilla preservation flaps offered a significant added benefit in terms of CAL gains and PPD reductions in the surgical management of intrabony defects.

(II) Application of EMD doubled the probability of obtaining a highly significant clinical outcome (CAL gain $\geq$4 mm) and halved the probability of obtaining clinically insignificant results (CAL gains $<$2 mm). Half of the patients, however, displayed CAL gains of 2–3 mm following both the test and the control procedure.

(III) The probability of obtaining CAL gains of 3 mm or more following application of EMD was improved in non-smokers, in subjects treated in specific clinical centers, in defects with a normal, cribriform bony lining and with a predominantly 3-wall anatomy. These characteristics can assist in establishing case prognosis.

Acknowledgements

This study was partly supported by the Clinical Research Foundation (CRF) for the Promotion of Oral Health, University of Berne, the Accademia Toscana di Ricerca Odontostomatologica (ATRO), Firenze, Italy, Switzerland, the Periodontal Research Fund of the Department of Periodontology of the Eastman Dental Institute, University of London, UK and a research grant from Biora AB, Malmo, Sweden. Authors wish to express their gratitude to Dentaid SA, Spain, for kindly providing the oral hygiene aids used in the post-operative phase.

Zusammenfassung

Schmelzmatrixproteine in der regenerativen Therapie von tiefen intraalveolären Defekten. Eine multizentrische randomisierte klinisch kontrollierte Studie

Ziel: Diese prospektive multizentrische randomisierte klinisch kontrollierte Studie wurde geplant, um die klinischen Ergebnisse der Papillenschonungstechnik bei der Lappenoperation mit und ohne zusätzliche Applikation von Schmelzmatrixproteinen (EMD) zu vergleichen.
Material und Methoden: 172 Patienten mit fortgeschrittener chronischer Parodontitis wurden in 12 Zentren in 7 Ländern hinzugezogen. Alle Patienten hatten mindestens einen intraalveolären Defekt ⩾3 mm. Starke Raucher (⩾20 Zigaretten/Tag) wurden ausgeschlossen. Die chirurgischen Prozeduren schlossen den Zugang für die instrumentelle Wurzeloberflächenbearbeitung entweder unter Nutzung einer einfachen oder der modifizierten Papillenschonungstechnik und Lappenbildung ein, um eine optimale Gewebeadaptation und einen primären Nahtjyverschluss zu erzielen. Nach der Wurzelreinigung und -glättung wurden die Wurzeln für 2 Minuten mit einem Gel, das 24% EDTA enthielt, konditioniert. EMD wurde bei den Testpersonen verwendet, bei den Kontrollen nicht. Postoperativ wurde eine strenge Plaquekontrolle durchgeführt. Zur Basis und 1 Jahr nach der Intervention wurden die klinischen Stützgewebeniveaus (CAL), die Sondierungstiefen (PPD), die Rezessionen (REC), die vollständigen Plaquewerte und die vollständigen Blutungswerte aufgezeichnet. Insgesamt standen noch 166 Patienten nach 1 Jahr zur Verfügung.
Ergebnisse: Zur Basis zeigten die 86 Test- und 86 Kontrollpersonen ähnliche Persönlichkeits- und Defektcharakteristiken. Im Durchschnitt gewannen die Testdefekte 3.1±1.5 mm CAL, während die Kontrolldefekte einen signifikant geringeren CAL Gewinn von 2.5±1.5 mm aufwiesen. Die Reduktion der Sondierungstiefen war auch signifikant höher in der Testgruppe (3.9±1.7 mm), wenn mit den Kontrollen verglichen wurde (3.3±1.7 mm). Eine multivariate Analyze zeigte, dass die Behandlung, das klinische Zentrum, das Zigarettenrauchen, die anfängliche PPD und die Defektkortikalisation den CAL Gewinn signifikant beeinflusste. Eine Häufigkeitsanalyse der klinischen Ergebnisse zeigte, dass EMD die Vorhersagbarkeit von klinischen signifikanten Ergebnissen (CAL Gewinn ⩾4 mm) vergrößerte und die Wahrscheinlichkeit von unwesentlichen oder keinen Gewinn von CAL (CAL Gewinn <2 mm) verringerte.
Schlussfolgerungen: Die Ergebnisse dieser Studie zeigten, dass die regenerative parodontale Chirurgie mit EMD einen zusätzlichen Nutzen in Form des CAL Gewinns, der PPD Reduktion und der Vorhersagbarkeit der Ergebnisse in Beziehung zur Lappenoperation mit Papillenschonungstechnik allein erbringt.

Résumé

Protéines de la matrice amélaire dans le traitement de régénération des lésions intra-osseuses profondes. Un essai clinique contrôle, multicentrique et au hasard

But: Cette étude clinique contrôlée, au hasard, multicentrique et prospective a été effectuée pour comparer les effets cliniques de la chirurgie par lambeau de préservation de la papille avec ou sans l'application des protéines dérivées de la matrice amélaire (EMD).
Matériaux et méthodes: 172 patients avec parodontite chronique avancée ont été recrutés parmi 12 centres de 7 pays. Tous les patients avaient au moins une lésion osseuse ⩾3 mm. Les grands fumeurs (⩾20 cigarettes (jour) ont été exclus. Les procédures chirurgicales comprenaient l'accès pour l'instrumentation radiculaire en utilisant soit le lambeau de préservation papillaire simplifié ou modifié afin d'obtenir l'adaptation tissulaire optimale en fin d'opération. Après le nettoyage, les surfaces radiculaires ont été conditionnées pendant 2 min avec un gel contenant 24% d'EDTA, EMD appliqué chez les sujets tests et omis chez les contrôles. Après la chirurgie, un contrôle de plaque dentaire strict a été imposé. Lors de l'examen initial et une année après la chirurgie, les niveaux d'attache clinique (CAL), les profondeurs de poche au sondage (PPD), la récession (REC), les scores de plaque dentaire et les scores de saignement de l'ensemble de la bouche ont été relevés. En tout, 166 patients ont été revus après une année.
Résultats: Lors de l'examen initial, les 86 patients tests et les 86 contrôles présentaient des lésions semblables. En moyenne, les lésions tests gagnaient 3.1±1.5 mm de CAL et les contrôles que 2.5±1.5 mm. La réduction des poches était également plus importante dans le groupe test (3.9±1.7 mm) comparée aux contrôles (3.3±1.7 mm). Une analyse multivariée a indiqué que le traitement, les centres cliniques, le tabagisme, le PPD lors de l'examen initial et la corticalisation de la lésion influençaient significativement les gains CAL. L'analyse de répartition de fréquence des données étudiées a indiqué que l'EMD augmentait la prévision des résultats cliniques (gain CAL ⩾4 mm) et diminuait la probabilité de n'obtenir aucun gain ou des gains négligeables de CAL (gain de CAL <2 mm).
Conclusions: Les résultats de cet essai ont indiqué que la chirurgie parodontale régénérative avec l'EMD offrait un bénéfice supplémentaire en terme de gain CAL, de réduction PPD et de prévision des données par rapport à une chirurgie par lambeau préservant la papille.

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