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Clinical outcomes following treatment of human intrabony defects with GTR/bone replacement material or access flap alone

A multicenter randomized controlled clinical trial

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Abstract

Aim: This prospective multicenter randomized controlled clinical trial was designed to compare the clinical outcomes of papilla preservation flap surgery with or without the application of a guided tissue regeneration (GTR)/bone replacement material.

Materials and Methods: One hundred and twenty-four patients with advanced chronic periodontitis were recruited in 10 centers in seven countries. All patients had at least one intrabony defect of $\geqslant 3$ mm. 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, the regenerative material was applied in the test subjects, and omitted in the controls. At baseline and 1 year following the interventions, clinical attachment levels (CALs), probing pocket depths (PPDs), recession, full-mouth plaque scores and full-mouth bleeding scores (FMBS) were assessed.

Results: One year after treatment, the test defects gained 3.3 ± 1.7 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 (3.7 ± 1.8 mm) when compared with the controls (3.2 ± 1.5 mm). A multivariate analysis indicated that the treatment, the clinical centers, baseline PPD and baseline FMBS significantly influenced CAL gains. Odds ratios (ORs) of achieving above-median CAL gains were significantly improved by the test procedure (OR = 2.6, 95% CI 1.2–5.4) and by starting with deeper PPD (OR = 1.7, 1.3–2.2) but were decreased by receiving treatment at the worst-performing clinical center (OR = 0.9, 0.76–0.99).

Conclusions: The results of this trial indicated that regenerative periodontal surgery with a GTR/bone replacement material offers an additional benefit in terms of CAL gains, PPD reductions and predictability of outcomes with respect to papilla preservation flaps alone.

Key words: bone replacement graft; guided tissue regeneration; intrabony defects; periodontal regeneration; randomized controlled clinical trial

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Considerable histologic and clinical evidence gathered over the last two decades indicate that the regeneration of periodontal tissues lost as a result of periodontitis can be achieved in humans. In particular, two clinical ap-

proaches have been routinely employed with considerable success: bone grafting (Rosen et al. 2000) and guided tissue regeneration (GTR) using barrier membranes (Cortellini & Tonetti 2000).

The evidence that these approaches offer tangible benefits, however, remains inconclusive. In fact, several randomized controlled clinical trials have indicated that previously tested regenerative approaches lead to statistically significant increases in clinical attachment levels (CALs) but the magnitude of the observed additional benefit may be modest (Needleman et al. 2002, Trombelli et al. 2002).

It has also been recognized that the morphology of the osseous defect plays an important role in the healing of the defect itself. This is true with all currently available regenerative technologies even though the literature indicates that the most significant morphological outcome predictors may be approach-specific (Tonetti et al. 1993, 1996, 2002, Cortellini & Tonetti 2000). It should also be emphasized that current regenerative approaches are able to influence only the most apical portion of the defect and in the best situations only the intrabony component of the defect. No predictable form of the therapy for the suprabony component of the defect has been reported.

In this context, considerable research emphasis has been placed on ''combination therapy'', i.e. an approach aimed at combining the positive aspects of the different regenerative principles and in order to possibly start influencing the healing of the suprabony component of the defect. Along this line considerable attention has been paid to the combination of the epithelial-exclusion characteristics of membranes with the scaffold effect provided by grafts.

Recently, a novel biomaterial combination has been proposed for use in bone regeneration: deproteinized bovine bone mineral combined with the application of a specifically designed collagen membrane. Initial experiments including human biopsies have suggested that this combination therapy results in significantly more periodontal regeneration (new cementum, new periodontal ligament and new alveolar bone) than each individual component (Camelo et al. 1998, 2001, Mellonig 2000, Nevins et al. 2003). These histological observations have been confirmed by pilot clinical investigations that reported increased CAL gains with combination therapy with respect to access flap alone (Camargo et al. 2000, Sculean et al. 2003, Stavropoulos et al. 2003). At present, however, the evidence remains limited to few cases and thus does not allow a full evaluation of the efficacy, predictability and safety of the approach.

The objective of this randomized controlled clinical trial was the comparison, in a multicenter study, of the clinical outcomes obtained following treatment of intrabony defects with papilla preservation flap surgery with or without application of a bioresorbable membrane in combination with a bone replacement material.

Material and Methods

Experimental design

A parallel group, randomized, multicenter and controlled clinical trial was designed to compare the efficacy of two treatment modalities for intrabony periodontal defects. The test treatment consisted of access to the defect with papilla preservation flaps, surgical debridement, insertion of a natural bovine mineral bone replacement material into the defect and application of a collagen membrane. The same procedure was performed in the control group except for the omission of the application of the regenerative materials. A single defect was treated in each patient. Clinical outcomes were evaluated at 1 year. This investigation was performed at 10 periodontal practices constituting a practice-based research network. Centers were located in Belgium, Germany, Greece, Italy, Switzerland, the UK 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 Eastman Dental Institute, University College London.

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 interexaminer reproducibility for pocket depth, recession of the gingival margin and evaluation of defect anatomy. Intraexaminer reproducibility was evaluated as the standard deviation of the difference of triplicate measurements. All investigators reached the target of a standard deviation lower than 0.4 mm for attachment levels. Interexaminer variability was evaluated as the 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, 2002, Cortellini et al. 2001). 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) $\leqslant 25\%$ at study baseline (following completion of the initial periodontal treatment phase) (Tonetti et al. 1993, 1996). The patients were informed in detail about the possible risks and benefits and were asked to give their consent to the trial. The joint ethics committee of the Eastman Dental Institute, University College London, 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 or at the mesial aspect of the lower first molar. 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. 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 α error of 0.05 was estimated as described (Fleiss 1986), using CAL changes as the primary outcome variable. Based on previous

estimates of outcome variability (Richardson et al. 1999) and subject attrition rates observed in previous clinical trials of similar design by this group (Tonetti et al. 1998), a total of 112 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 and allocation concealment

After verification of the entry criteria, 124 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 study registrar using a custom-made program based on balanced random permuted blocks. To reduce the chance of unfavorable splits between test and control groups in terms of key prognostic factors, the randomization process balanced smoking status, and average pocket depth at the defect sites 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. To conceal assignment from the investigator until the time during the surgical procedure that will require application of the GTR/bone replacement system or its omission, the central registrar instructed the investigator to assign a previously supplied sealed envelope containing the treatment assignment to the specific subjects.

Clinical measures

Before anesthesia, the following clinical parameters were evaluated on the day of the surgical procedure and 1 year later. FMPS were recorded as the percentage of total surfaces (four aspects per tooth) that 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, Leimen, Germany). 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). CALs, 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 essentially as described (Tonetti et al. 2002). 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 using a combination of mechanical and hand instrumentation. In the test sites, deproteinized bone replacement material (Bio-Oss®, Geistlich AG, Switzerland) was applied to overfill the defect. A collagen membrane, previously adapted to the local anatomy, was positioned on top of the graft material (Bio-Gide®, Geistlich AG, Switzerland). The flaps were then replaced and sutured employing non-resorbable e-PTFE sutures (Gore-Tex™, W.L. Gore and Associates, Flagstaff, AZ, USA) as previously described (Cortellini & Tonetti 2000). The control procedure was identical to the test surgery, apart from the omission of the application of the GTR/bone replacement system.

Intrasurgical clinical measurements

The following defect morphology parameters were evaluated after debridement of the area essentially as previously 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 millimeter. 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).

Post-surgical instructions and infection control

Post-operative pain and edema were controlled with tablets of either 600 mg ibuprofen or 500 mg paracetamol. A course of doxycycline 200 mg/day was prescribed for the first post-operative week. Patients were instructed to rinse twice 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 treated area during the first 4 post-operative weeks. Smokers were asked to limit and possibly avoid smoking.

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

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, 6 and 8.

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, USA). All calculations and analyses were

performed using SAS Version 8.2. 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 χ^2 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 center and the treatment by center interaction were incorporated as stratification factors (Fleiss 1986, Goldberg & 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 (Goldberg & 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 χ^2 test to compare distributions of outcomes at test and control sites. The odds of achieving above-median clinical outcomes 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 α 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. Sixty-two subjects were assigned to the test group and 62 to the control.

Patient retention and missing data

A total of 124 subjects were entered, randomized and treated. During the 1-year period, two subjects were lost to follow-up for treatment unrelated reasons: one test and one control patient. Two patients lost the test tooth: one tooth was extracted at patient's request due to lack of improvement in tooth mobility. The other tooth was lost due to the fact that the patient had an accident that resulted in the traumatic expulsion of the tooth. Both of these teeth were assigned to the control procedure. Complete observations were available for 120 subjects: 61 tests and 59 controls. This represented 96.8 % of entered patients. All analyses were performed using intent to treat approach last observation carried forward.

Subject and defect characteristics at baseline

Subject and defect characteristics at baseline are displayed in Table 1. No significant differences between test and control patients were observed for any of the subject or defect characteristics. Defects had deep intrabony components (5.6 ± 1.9 mm for test and 5.9 ± 2.2 mm for controls), significant suprabony components, and were associated with deep PPDs (7.8 ± 1.6 mm for test and 7.9 ± 1.5 mm for control). Similarly CAL was 9.7 ± 1.8 and 9.9 ± 2.3 mm at test and control sites, respectively.

Clinical outcomes

Table 2 describes the treatment outcomes for both GTR/bone replacement material applications in combination with papilla preservation access flaps (test) and papilla preservation access flaps alone (control). In terms of primary outcome variable, the average gain in clinical attachment was 3.3 ± 1.7 mm for the test sites and 2.5 ± 1.5 mm for the sites treated with access flap alone. The difference between test and control was statistically highly significant ($p = 0.004$). The magnitude of the observed additional benefit was 0.8 ± 1.6 mm.

One year after therapy, pocket depth reductions were 3.7 ± 1.8 mm for the test group, and 3.2 ± 1.5 for the control group ($p = 0.02$, Table 2). Between baseline and 1 year, the gingival margin receded of 0.3 ± 1.2 mm in the test sites and of 0.7 ± 0.9 mm in the controls ($p = 0.04$).

The significance of the treatment effect was also evaluated taking into account the potential sources of variability arising from the multicenter design of the study and the previously described covariates (Tonetti et al. 1993, 1995, 1996, Falk et al. 1997). Since no treatment by center interaction was observed, the main effect model was applied (Goldberg & Koury 1989). The following variables were used in the model: treatment, center effect, smoking status, antibiotic usage during initial periodontal therapy, baseline PPD, baseline FMPS, baseline FMBS, predominant defect morphology (one, two or three walls) and depth of the intrabony component of the defect (Table 3). The multivariate model was statistically significant and explained 40% of the observed variability in CAL gain.

The surgical treatment combining papilla preservation flap with the application of GTR/bone replacement material resulted in significantly greater CAL gains than the papilla preservation access flap control ($p = 0.0005$). No significant center effect was observed ($p = 0.0610$, NS). Cigarette smoking also did not have a significant effect ($p = 0.8794$). The level of oral hygiene (FMPS) did not reach statistical significance ($p = 0.1412$) while the percen-

Table 1. Patient and defect characteristics for test and control groups at baseline ($N = 124$)

Variable	Test	Control	Significance, *p*-value
subject number	62	62	–
age (years)	49.5 ± 11.3	51 ± 10.5	0.7424
gender (% females)	61.3	61.7	0.9659
smokers (%, <20 cigarettes/day)	33.9	31.7	0.7954
antibiotic during initial therapy	41.9	38.3	0.6849
FMPS (%)	11.7 ± 6.9	11.6 ± 8.2	0.9081
FMBS (%)	9.4 ± 6.7	10.6 ± 7.2	0.3286
PPD (mm)	7.8 ± 1.6	7.9 ± 1.5	0.7387
CAL (mm)	9.7 ± 1.8	9.9 ± 2.3	0.6091
CEJ-BD (mm)	10.1 ± 2.4	10.4 ± 2.7	0.6344
intrabony component (mm)	5.6 ± 1.9	5.9 ± 2.2	0.4978
predominantly one wall (%)	22.6	23.3	
predominantly two walls (%)	50	53.3	0.7886*
predominantly three walls (%)	27.4	23.4	

*Defect wall morphology (Mantel–Haenszel χ^2).

FMPS, full-mouth plaque scores; FMBS, full-mouth bleeding scores; PPD, probing pocket depth; CAL, clinical attachment level; CEJ_BD, distance from the cemento-enamel junction (CEJ) to the bottom of the defect.

Table 2. Clinical outcomes at 1 year

Outcome variable	Test, $N = 61$ (GTR/bone mineral)	Control, $N = 59$ (papilla preservation flap)	Significance, p-value
gain in CAL	3.3 ± 1.7	2.5 ± 1.5	0.004
decrease in PPD	3.7 ± 1.8	3.2 ± 1.5	0.02
increase in REC	0.3 ± 1.2	0.7 ± 0.9	0.04

CAL, clinical attachment level; PPD, probing pocket depth; REC, recession of the gingival margin.

Table 3. Multivariate analysis of CAL gain

Parameter	Estimate	Significance, p-value
treatment effect	0.8 ± 0.3	0.0005
center effect (worst versus best)	-1.9 ± 1	0.06, NS
smoking (yes versus no)	-0.05 ± 0.3	0.8794, NS
baseline FMPS	0.04 ± 0.02	0.1412, NS
baseline FMBS	-0.1 ± 0.04	0.0109
baseline PPD (mm)	0.5 ± 0.1	<0.0001
defect morphology (one wall versus three walls)	-0.5 ± 0.04	0.2610, NS
depth of intrabony component	-0.01 ± 0.09	0.8751, NS

Significance of model $p < 0.0001$, adjusted $R^2 = 0.40$.
CAL, clinical attachment level; FMPS, full-mouth plaque scores; FMBS, full-mouth bleeding scores; PPD, probing pocket depth.

Table 4. Frequency distribution of CAL gain

	Changes in CAL (mm)			
	loss	0–1	2–3	$\geqslant 4$
test (%)	–	11.3	50	38.7
control (%)	–	23.3	55	21.7

Mantel–Haenszel χ^2, $p = 0.0181$.
CAL, clinical attachment level.

tage of sites displaying bleeding on probing at baseline (FMBS) had a significant negative impact on the outcome ($p = 0.0109$). Among the considered defect characteristics, the initial pocket depth was a highly significant covariate ($p < 0.0001$) while the predominant defect morphology in terms of residual bony walls or the depth of the intrabony component did not have a significant impact ($p = 0.2610$ and 0.8751, respectively).

The frequency distributions of various CAL gains at test and control sites are depicted in Table 4. Highly significant ($p = 0.0181$, Mantel–Haenszel χ^2) differences are evident. CAL loss was not observed at any test or control site. The test treatment resulted in higher frequency of sites gaining 4 mm or more CAL and in lower frequencies of sites gaining 0–1 mm. The majority of sites in both the test and the control groups, however, gained 2–3 mm of CAL.

The probability of obtaining CAL gains above the median of 3 mm is displayed in Table 5. A logistic regression analysis with backwards elimination of non-significant factors ($p = 0.1$) evaluated the impact of gender, smoking status, treatment modality, oral hygiene (FMPS at baseline), bleeding on probing (FMBS at baseline), PPD, depth of the intrabony component of the defect, CEJ-BD, predominant defect morphology in terms of residual bony walls, bleeding tendency of the defect and corticalization of the bony walls of the defect (Tonetti et al. 2002). Data indicated that odds of achieving above-average outcomes were significantly increased by using the test treatment and by having deeper baseline PPD but were decreased by receiving treatment in the center with the worst overall performance.

Discussion

Human histological case reports have indicated that the combined application of anorganic bone replacement material with a collagen membrane can result in periodontal regeneration (Camelo et al. 1998, 2001, Nevins et al. 2003). Data from this multicenter randomized controlled clinical trial indicate that application of the tested combination therapy resulted in significant improvements in CAL, PPD and REC compared with papilla preservation access flaps alone (Table 2).

The absolute value of the observed added benefit was relatively small (0.8 ± 0.3 mm, Table 3), but in line with outcomes previously reported by this group in similarly designed randomized controlled multicenter studies after GTR treatment with bio-resorbable polymeric membranes (Tonetti et al. 1998, Cortellini et al. 2001) where adjusted added benefits of 0.8 and 0.9 mm above the papilla preservation access flap controls were observed. Furthermore the added benefit observed in this study agrees well with a current meta-analysis of the literature that provides an estimate of 0.95 ± 0.47 mm of added benefit after application of collagen membranes to intrabony defects (Murphy & Gunsolley 2003).

The current study evaluated the combined efficacy of bovine bone replacement material and a collagen membrane with respect to papilla preservation access flap controls. It is therefore not possible to ascertain whether or not the observed effect would have been observed following the application of a single component of the combination therapy. Given the results of recent meta-analyses of the benefits of the two mono-therapies, i.e. grafting and GTR membranes, however, it can be observed that the magnitude of the added benefit reported in this study after combination therapy was not different from those reported following application of collagen membranes or bone replacement grafts alone (Trombelli et al. 2002, Murphy & Gunsolley 2003). In fact, the literature pertaining directly to the regenerative material used in this study presents conflicting results on this aspect. Paolantonio (2002) described a benefit when combining natural bone mineral and a membrane versus only membrane, but another recent study did not find an added benefit following the combined application of the two materials (Stavropoulos et al. 2003). A recent meta-analysis has explored the added benefit of placing a variety of bone replacement grafts (primarily demineralized freeze-dried bone allograft) under GTR membranes in the treatment of intrabony defects. The included studies failed to demonstrate the presence of a synergistic effect arising from the simultaneous application of the grafting and GTR principles to promote periodontal regeneration (Murphy & Gunsolley 2003). On the other hand, outcomes of human histological studies have indicated that

Table 5. Logistic regression analysis of factors significantly affecting the probability of obtaining CAL gains above the median ($\geqslant 3$ mm)

Parameter	Odds ratio	95% CI	Significance, *p*-value
treatment (test versus control)	2.6	1.2–5.4	0.0117
center (worst versus best)	0.9	0.76–0.99	0.0460
baseline PPD (mm)	1.7	1.3–2.2	<0.0001

CAL, clinical attachment level; PPD, probing pocket depth.

the combination of a collagen membrane with deproteinized bone replacement material resulted in better coronal extension of the portion of the wound that healed with complete periodontal regeneration (Camelo et al. 2001). In an animal study Yamada et al. (2002) elucidated the effect of the bone substitute material by comparing application of a collagen membrane (GTR) with collagen membrane plus bone substitute material. The data demonstrated a comparable amount of new cementum but significantly more regenerated bone by using the combination of membrane plus bone substitute material. At present, such observations provide the principle rationale for the combined application of graft and membrane into the test treatment.

The multivariate analysis of the factors affecting the primary outcome variable of the study, CAL gain at 1 year (Table 3), provided further insight into the data. In this study, defect morphology parameters, cigarette smoking and center variability did not reach statistical significance. This was not in agreement with the results of our previous multicenter studies and may support the hypothesis that the employed treatments were less influenced by these factors than those tested in previous studies. Further investigations are necessary to explore these hypotheses.

Among the measured variables, the percentage of sites with bleeding on probing at baseline was significantly associated with reduced CAL gains. This finding was in agreement with those of previous investigations (Tonetti et al. 1993, 1995, 1996) and should be interpreted as an indication of high levels of persistent periodontal infection after completion of the initial phase of therapy in this population (data not shown). It is of interest that FMBS had a significant effect in spite of the rigorous post-operative protocol and the plaque control regimen enforced during the study that included a 1-week course of antibiotic in the post-operative period.

These data clearly support the concept that the application of GTR/bone replacement material was effective. In order to appreciate the clinical significance of the data frequency distribution analyses were performed (Tables 4 and 5). Data indicated that the probability of obtaining CAL gains greater than the observed median of 3 mm was significantly increased by application of the test therapy (odds ratio (OR) = 2.6, 95% CI 1.2–5.4).

The following conclusions can be drawn from this investigation:

(I) Application of GTR/bone replacement material 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 GTR/bone replacement material increased the odds of obtaining CAL gains $\geqslant 3$ mm in intrabony defects. Half of the patients, however, displayed CAL gains of 2–3 mm following both the test and the control procedure.

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References

Camargo, P. M., Levkovic, V., Weinlaender, M., Nedic, M., Vasilic, M., Wolinsky, L. E. & Kenney, E. B. (2000) A controlled re-entry study on the effectiveness of bovine porous bone mineral used in combination with a collagen membrane of porcine origin in the treatment of intrabony defects in humans. *Journal of Clinical Periodontology* **27**, 889–896.

Camelo, M., Nevins, M. L., Lynch, S. E., Schenk, R. K., Simion, M. & Nevins, M. (2001) Periodontal regeneration with an autogenous bone-Bio-Oss composite graft and a Bio-Gide membrane. *International Journal of Periodontics and Restorative Dentistry* **21**, 109–119.

Camelo, M., Nevins, M. L., Schenk, R. K., Simion, M., Rasperini, G., Lynch, S. E. & Nevins, M. (1998) Clinical, radiographic, and histologic evaluation of human periodontal defects treated with Bio-Oss and Bio-Gide. *International Journal of Periodontics and Restorative Dentistry* **18**, 321–331.

Cortellini, P., Pini-Prato, G. P. & Tonetti, M. S. (1993) Periodontal regeneration of human infrabony defects. I. Clinical measures. *Journal of Periodontology* **64**, 254–260.

Cortellini, P., Pini-Prato, G. & Tonetti, M. (1995) The modified papilla preservation technique. A new surgical approach for interproxymal regenerative procedures. *Journal of Periodontology* **66**, 261–266.

Cortellini, P., Pini Prato, G. & Tonetti, M. (1999) The simplified papilla preservation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. *International Journal of Periodontics and Restorative Dentistry* **19**, 589–599.

Cortellini, P. & Tonetti, M. (2000) Focus on intrabony defects: guided tissue regeneration. *Periodontology 2000* **22**, 104–132.

Cortellini, P., Tonetti, M., Lang, N., Suvan, J., Zucchelli, G., Vangsted, T., Silvestri, M., Rossi, R., McClain, P., Fonzar, A., Dubravec, D. & Adriaens, P. (2001) The simplified papilla preservation flap in the regenerative treatment of deep intrabony defects. A multicentre randomized controlled clinical trial evaluating clinical outcomes and postoperative morbidity. *Journal of Periodontology* **72**, 1702–1712.

Falk, H., Laurell, L., Ravald, N., Teiwik, A. & Persson, R. (1997) Guided tissue regeneration therapy of 203 consecutively treated intrabony defects using a bioabsorbable matrix barrier. Clinical and radiographic findings. *Journal of Periodontology* **68**, 571–581.

Fleiss, J. (1986) Analysis of data from multicenter trials. *Controlled Clinical Trials* **7**, 267–275.

Goldberg, J. & Koury, K. (1989) Design and analysis of multicenter trials. In: *Statistical Methodology in the Pharmaceutical Sciences*, eds. Berry, D., pp. 201–237. New York: Marcel Dekker Inc.

Mellonig, J. T. (2000) Human histologic evaluation of a bovine-derived bone xenograft in the treatment of periodontal osseous defects. *International Journal of Periodontics and Restorative Dentistry* **20**, 19–29.

Murphy, K. G. & Gunsolley, M. (2003) Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A systematic review. *Annals of Periodontology*, **8**, 266–302.

Needleman, I., Tucker, R., Giedrys-Leeper, E. & Worthington, H. (2002) A systematic review of guided tissue regeneration for periodontal infrabony defects. *Journal of Periodontal Research* **37**, 380–388.

Nevins, M. L., Camelo, M., Lynch, S. E., Schenk, R. K. & Nevins, M. (2003) Evaluation of periodontal regeneration following grafting intrabony defects with bio-oss collagen: a human histologic report. *International Journal of Periodontics and Restorative Dentistry* **23**, 9–17.

O'Leary, T. J., Drake, R. B. & Naylor, J. E. (1972) The plaque control record. *Journal of Periodontology* **43**, 38.

Paolantonio, M. (2002) Combined periodontal regenerative technique in human intrabony defects by collagen membranes and anorganic bovine bone. A controlled clinical study. *Journal of Periodontology* **73**, 158–166.

Richardson, C. R., Mellonig, J. T., Brunsvold, M. A., McDonnell, H. T. & Cochran, D. L. (1999) Clinical evaluation of Bio-Oss: a bovine-derived xenograft for the treatment of periodontal osseous defects in humans. *Journal of Clinical Periodontology* **26**, 421–428.

Rosen, P., Reynolds, M. & Bowers, G. (2000) The treatment of intrabony defects with bone grafts. *Periodontology 2000* **22**, 88–103.

Sculean, A., Berakdar, M., Chiantella, G. C., Donos, N., Arweiler, N. B. & Brecx, M. (2003) Healing of intrabony defects following treatment with a bovine-derived xenograft and collagen membrane. A controlled clinical study. *Journal of Clinical Periodontology* **30**, 73–80.

Stavropoulos, A., Karring, E. S., Kostopoulos, L. & Karring, T. (2003) Deproteinized bovine bone and gentamicin as an adjunct to GTR in the treatment of intrabony defects: a randomized controlled clinical study. *Journal of Clinical Periodontology* **30**, 486–495.

Tonetti, M., Cortellini, P., Suvan, J., Adriaens, P., Baldi, C., Dubravec, D., Fonzar, A., Fourmousis, I., Magnani, C., Muller-Campanile, V., Patroni, S., Sanz Alonso, M., Vangsted, T., Zabalegui, I., Pini-Prato, G. & Lang, N. (1998) Evaluation of generalizability of the added benefit of GTR in the treatment of deep intrabony defects. A phase iv multicenter randomized controlled clinical trial. *Journal of Periodontology* **69**, 1183–1192.

Tonetti, M., Pini-Prato, G. & Cortellini, P. (1993) Periodontal regeneration of human infrabony defects. IV. Determinants of the healing response. *Journal of Periodontology* **64**, 934–940.

Tonetti, M., Pini-Prato, G. & Cortellini, P. (1995) Effect of cigarette smoking on periodontal healing following GTR in infrabony defects. A preliminary retrospective study. *Journal of Clinical Periodontology* **22**, 229–234.

Tonetti, M., Pini-Prato, G. & Cortellini, P. (1996) Factors affecting the healing response of intrabony defects following guided tissue regeneration and access flap surgery. *Journal of Clinical Periodontology* **23**, 548–556.

Tonetti, M. S., Lang, N. P., Cortellini, P., Suvan, J. E., Adriaens, P., Dubravec, D., Fonzar, A., Fourmousis, I., Mayfield, L., Rossi, R., Silvestri, M., Tiedemann, C., Topoll, H., Vangsted, T. & Wallkamm, B. (2002) Enamel matrix proteins in the regenerative therapy of deep intrabony defects. *Journal of Clinical Periodontology* **29**, 317–325.

Trombelli, L., Heitz-Mayfield, L. J., Needleman, I., Moles, D. & Scabbia, A. (2002) A systematic review of graft materials and biological agents for periodontal intraosseous defects. *Journal of Clinical Periodontology* **29** (Suppl. 3), 117–135, discussion 160–162.

Yamada, S., Shima, N., Kitamura, H. & Sugito, H. (2002) Effect of porous xenographic bone graft with collagen barrier membrane on periodontal regeneration. *International Journal of Periodontics and Restorative Dentistry* **22**, 389–397.

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