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Guided tissue regeneration/ deproteinized bovine bone mineral or papilla preservation flaps alone for treatment of intrabony defects. II: radiographic predictors and outcomes

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Abstract

Objectives: This study reports the secondary analysis of a randomized-controlled clinical trial designed to assess the efficacy of deproteinized bovine mineral and a collagen membrane in the treatment of intrabony defects. The specific aims of this report are (1) to analyse the radiographic bone changes 1 year after therapy and (2) to assess the association between radiographic defect angle and treatment outcomes.

Materials and Methods: Baseline and 12-month radiographs were collected from 120 patients with advanced chronic periodontitis from 10 centres in seven countries as part of a multi-centre clinical trial. All patients had at least one intrabony defect $\geqslant 3$ mm in depth. The treatment consisted of simplified or modified papilla preservation flaps to access the defect. After debridement of the area, a deproteinized bovine mineral and a collagen membrane were applied in the test subjects, and omitted in the controls. Main outcome measures were radiographic bone fill and defect resolution 1 year after surgery.

Results: One hundred and twenty pairs of radiographs were obtained, of which 110 pairs were measurable (57 tests and 53 controls). One year after treatment, radiographic resolution of the intrabony component was significantly higher in the test group (3.2 ± 1.7 mm) when compared with the controls (1.7 ± 1.9 mm). Multivariate analysis indicated that the treatment and the baseline radiographic depth of the intrabony defect significantly influenced the radiographic bone fill of the intrabony defect 1 year following treatment. The percentage of resolution of the defect was influenced by the treatment provided and the baseline plaque score. The baseline radiographic defect angle did not show a significant impact on the clinical and radiographic outcomes.

Conclusions: Regenerative periodontal surgery with a deproteinized bovine bone mineral and a collagen membrane offered additional benefits in terms of radiographic resolution of the intrabony defect and predictability of outcomes with respect to papilla preservation flaps alone.

Key words: bone fill; bone replacement graft; clinical trial; guided tissue regeneration; intrabony defects; periodontal regeneration; periodontal therapy

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Recent meta-analyses of randomized-controlled clinical trials have indicated that three regenerative approaches result in significant increases in clinical attachment levels, but the magnitude of the observed additional benefit may be modest (Needleman et al. 2002, Trombelli et al. 2002, Giannobile & Somerman 2003, Murphy & Gunsolley 2003, Reynolds et al. 2003). High heterogeneity in the studies has been found in those systematic reviews of regenerative procedures (enamel matrix derivative (EMD), Giannobile & Somerman 2003; guided tissue regeneration (GTR), Needleman et al. 2002; bone grafting materials, Trombelli et al. 2002). In order to explore possible causes of the heterogeneity found in the clinical results of GTR treatment, the authors hypothesized that the outcome of regenerative surgery could be affected by factors like frequency of the maintenance regimen, compliance with oral hygiene, cigarette smoking, defect severity, and surgical technique (Murphy & Gunsolley 2003).

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. It should also be emphasized that current regenerative approaches are able to influence only the apical portion of the defect and in the best situations the intrabony component of the defect.

Recently, a novel biomaterial combination has been proposed for use in periodontal regeneration: deproteinized bovine bone mineral combined with the application of a specifically designed collagen membrane (GTR/DBBM). Initial experiments in dogs and 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). Controlled clinical trials have also shown an added benefit in terms of clinical parameters when comparing this combination therapy with access flap alone (Sculean et al. 2003, Tonetti et al. 2004).

A correlation between radiographic changes in alveolar bone level (bone fill) occurring in intrabony defects fter periodontal access flap surgery and the corresponding pre-treatment defect angles has been described, where greater potential for bone fill was found in defects with small angles (0–45°) compared with wide angles (45–90°, Steffensen & Weber 1989). Tonetti et al. (1993a) showed that, for GTR, the wider the radiographic defect angle, the lower the regenerated probing attachment level in intrabony defects. In a retrospective three-centre study, it was shown that clinical attachment level (CAL) gain and bone fill were positively correlated to the depth of the intrabony defect and that the less favourable results of one of these three centres were attributed to the fact that this centre had treated significantly wider defects compared with the other two centres (Falk et al. 1997).

Cortellini & Tonetti (1999) studied 242 intrabony defects treated with GTR and found a significant difference in the CAL outcomes when they compared narrow ($<25°$) to wide ($>37°$) defects. They concluded that the radiographic defect angle could represent a useful pre-surgical parameter to determine the potential of CAL gain in intrabony defects treated with GTR (Cortellini & Tonetti 1999). The impact of the baseline radiographic angle was also analysed following regenerative periodontal therapy with enamel matrix proteins (Tsitoura et al. 2004). The authors observed increased odds ratios of obtaining CAL gain of $\geqslant 4$ mm in intrabony defects treated with EDM when the baseline radiographic defect angle was narrow ($\leqslant 22°$) as compared with wide ($\geqslant 37°$). Evidence indicates, therefore, that a correlation exists between the defect angle and the clinical outcome when periodontal access flap surgery, GTR or EDM is used in the treatment of intrabony defects. It has been suggested that different regenerative technologies may be associated with different levels of impact of defect morphology on the clinical outcomes (Cortellini & Tonetti 2005). To our knowledge, there is no study assessing the impact of the baseline radiographic angle when periodontal papilla preservation flap surgery is combined with the application of GTR/DBBM.

The specific aims of this secondary analysis (Tonetti et al. 2004) were i) to investigate whether or not the baseline radiographic angle of an intrabony defect treated with a papilla preservation flap with or without the application of GTR/DBBM was significantly associated with the treatment outcomes 1-year after treatment and ii) to analyse the radiographic bone changes 1-year after therapy.

Material and Methods

Study design

The radiographs obtained for this study, to measure the intrabony defect angles, were originally taken as part of a multicentre clinical trial that evaluated the clinical outcomes following the treatment of intrabony defects with papilla preservation flap techniques (Cortellini et al. 1995, 1999) with or without application of GTR/DBBM. The clinical and microbiological outcomes of this trial have been independently reported (Tonetti et al. 2004, Heitz-Mayfield et al. 2006).

In brief, this was a parallel group, multi-centre, randomized, and controlled clinical trial that was designed to test the efficacy of papilla preservation flaps with or without GTR/DBBM application in the treatment of intrabony defects. The control group received the same type of treatment as the test group, except for the omission of the GTR/DBBM. A single defect was treated in each patient. Clinical outcomes were evaluated at 1 year.

A calibration exercise was carried out to obtain acceptable intra- and inter-examiner reproducibility for probing pocket depth (PPD), recession of the gingival margin and evaluation of the defect anatomy, as previously described by Tonetti et al. (1998).

Clinical measures

At study baseline and 1 year after treatment, the following parameters were evaluated: full-mouth plaque score (FMPS), full-mouth bleeding score (FMBS), PPD, recession of the gingival margin (REC) and CAL.

Radiographic assessment

Routine diagnostic periapical radiographs were taken with the long cone paralleling technique using Rinn holders (Updegrave 1951). Baseline and 12-month follow-up radiographs were collected from the clinical centres and evaluated for this study. All radiographic examinations were performed with the assessor blind to the treatment assignment and unaware of the defect morphology observed during the sur-

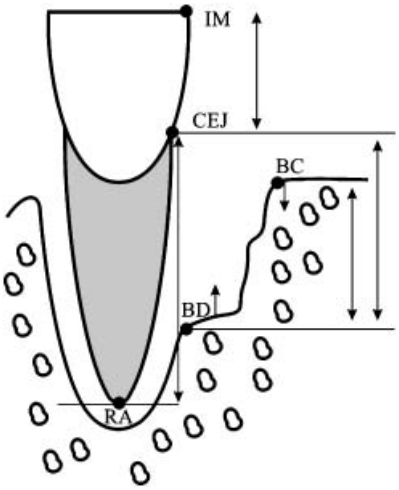

Fig. 1. Schematic drawing illustrating the anatomical landmarks and linear measurements taken from digitized radiographs. IM, incisal margin; RA, root apex; CEJ, cemento-enamel junction; BC, bone crest; BD, bottom of the defect.

gery. Radiographs were scanned with a purpose-built high definition scanner (DSR™, Diagnostic Subtraction Radiography by EMS, Electro Medical Systems, Nyon, Switzerland); images were stored in a personal computer until analysis with a customized image analysis software (DSR'', Diagnostic Subtraction Radiography by EMS, Electro Medical Systems) as previously described (Tsitoura et al. 2004).

The following anatomical landmarks (Fig. 1) of the intrabony defect were identified on the scanned radiographs based on criteria set by Bjorn et al. (1969) and by Schei et al. (1959):

1. The cemento-enamel junction of the tooth with the intrabony defect (CEJ).
2. The most coronal position of the alveolar bone crest of the intrabony defect when it touches the root surface of the adjacent tooth before treatment, the top of the crest (BC).
3. The most apical extension of the intrabony destruction where the periodontal ligament space still retained its normal width before treatment, the bottom of the defect (BD).

If restorations were present, the apical margin of the restoration was used to replace the CEJ as a fixed reference point.

The following linear measurements were performed with the calibrated image analysis system: CEJ to bottom of the defect (CEJ-BD) and CEJ to most coronal extent of the inter-dental alveolar crest (CEJ-BC). The depth of the intrabony defect at baseline was measured subtracting those parameters: (CEJ-BD) − (CEJ-BC) = intrabony depth.

Radiographic bone changes

Pre- and post-treatment radiograph pairs were analysed in order to measure i) the presence of crestal bone resorption (BC change), ii) the extent of bone fill of the intrabony component of the defect (BF), and iii) the extent of defect resolution (BF − BC change).

As the radiographic pairs were not identical, the vertical distortion between the baseline radiograph and the 12-month radiograph was estimated as previously described (Tonetti et al. 1993b). In order to estimate this distortion, an anatomically non-variable distance as the root length (distance from the CEJ to the root apex (CEJ-RA)) was measured on both radiographs and a correction factor was calculated as follows:

$$\frac{\text{CEJ-RA (baseline)}}{\text{CEJ-RA (1 year)}} = \text{Correction factor.}$$

In case it was not possible to measure the root length, the crown length was assessed (distance from the incisal margin of the crown to the CEJ).

The radiographic bone fill (BF) after 1 year was calculated after applying the correction factor as follows:

$$\text{CEJ-BD (baseline)} - [\text{CEJ-BD (1 year)} \times \text{correction factor}] = \text{Bone fill}$$

In the same way, the BC change was assessed as follows:

$$\text{CEJ-BC (baseline)} - [\text{CEJ-BC (1 year)} \times \text{correction factor}] = \text{Bone crest change.}$$

If the results were negative, this meant that a process of bone resorption had occurred.

The radiographic defect resolution (DR) was evaluated subtracting BC change from the BF of the defect:

$$\text{Bone fill (BF)} - \text{bone crest (BC) change} = \text{Defect resolution (DR)}.$$

Finally, the percentage of DR was calculated as follows:

$$\frac{\text{Defect resolution}}{\text{Depth of intrabony (baseline)}} \times 100 = \%\text{ of defect resolution.}$$

Measurement of the baseline defect angle

The radiographic defect angle was defined by the two lines that represent the root surface of the involved tooth and the bone defect surface, essentially as previously described (Steffensen & Weber 1989, Tonetti et al. 1993a, Tsitoura et al. 2004).

Calibration

The same examiner (Examiner A, A.L.) carried out all measurements of the radiographic defect angle, CEJ-BC and CEJ-BD. Examiner A was trained and calibrated in the measurement of the radiographs by another examiner (Examiner B, M.S.T.), who represented the ''gold standard''. Intra-examiner reproducibility was evaluated as the standard error of the mean difference of the duplicate measurements. This was 0.86° for Examiner A and 0.55° for Examiner B in terms of radiographic defect angle and 0.13 and 0.07 mm for the CEJ-BD linear measurement. Inter-examiner agreement was evaluated as the standard error of the mean difference of the measurements performed by Examiner A and those performed by Examiner B in 22 defects. This was 0.85° for the angle and 0.14 mm for CEJ-BD. Ninety per centof all the measurements carried out by the two examiners were within ± 5° for the angle and 1 mm for CEJ-BD.

Data management and statistical analysis

Data were entered into an excel database and proofed for entry errors. The database was subsequently locked, imported into SAS (Statistical Application Software, Version 8.2. SAS Institute, Cary, NC, USA) format and analysed. After verification of the normality assumptions, numerical data were summarized as means and standard deviations; categorical data were summarized as frequency distributions. Significance of differences between test and control in terms of continuous parameters was evaluated with the unpaired *t*-test; significance of differences in categorical variables was assessed with χ^2 statistics.

Table 1. Patient characteristics at baseline (means ± SD), $N = 110$

Variable	Test	Control	Significance, *p*-value
Subject number	57	53	–
Age (years)	49.5 ± 11.3	51 ± 10.5	0.7424
Gender (% females)	61.4	66.0	0.6137
Smokers (%, <20 cigarettes/day)	36.8	34	0.7524
Antibiotics during initial therapy (%)	40.4	39.6	0.9379
Baseline FMPS (%)	11.6 ± 6.7	11.6 ± 8.5	0.9848
Baseline FMBS (%)	9.7 ± 6.8	11.3 ± 7.1	0.2197

FMPS, full-mouth plaque scores; FMBS, full-mouth bleeding scores.

Table 2. Defect characteristics at baseline, $N = 110$

Variable	Test	Control	Significance, *p*-value
PPD (mm)	7.8 ± 1.6	8 ± 1.5	0.5666
Recession (mm)	2.1 ± 1.6	1.9 ± 1.4	0.5244
CAL (mm)	9.9 ± 1.9	9.9 ± 2.2	0.9865
Radiographic defect angle	25.2 ± 6.5	25.9 ± 7.4	0.6328
Radiographic CEJ-BD distance (mm)	9.7 ± 2.4	10.1 ± 2.8	0.4858
Radiographic CEJ-BC distance (mm)	4.4 ± 1.8	4.6 ± 1.9	0.5588
Interdental width (mm)	3.2 ± 1.4	3.5 ± 2.0	0.3691
Predominantly one-wall defect (%)	24.6	23.1	0.8882*
Predominantly two-wall defect (%)	50.9	51.9	
Predominantly three-wall defect (%)	24.6	23.1	

*Defect wall morphology and corticalization of bony walls (Mantel–Haenszel χ^2).
PPD, probing pocket depth; CAL, clinical attachment level; CEJ-BD, cemento-enamel junction to bottom of the defect; CEJ-BC, cemento-enamel junction to most coronal extent of the inter-dental alveolar crest.

Table 3. One-year radiographic and clinical outcomes, $N = 110$

Variable	Test	Control	Significance, *p*-value
Correction factor	1 ± 0.1	1 ± 0.1	0.4049
Radiographic bone fill (mm)	3.2 ± 2.0	1.8 ± 1.9	0.0002
Radiographic bone crest change (mm)	0.06 ± 0.92	0.04 ± 0.97	0.9380
Radiographic resolution of intrabony (mm)	3.2 ± 1.7	1.7 ± 1.9	<0.0001
Percentage of radiographic resolution of intrabony component	59 ± 24	30 ± 32	<0.0001
One-year PPD (mm)	4.1 ± 1.1	4.8 ± 1.5	0.0050
One-year recession (mm)	2.4 ± 1.6	2.6 ± 1.6	0.3722
One-year CAL (mm)	6.5 ± 1.6	7.5 ± 2.1	0.0060
CAL gain (mm)	3.5 ± 1.8	2.5 ± 1.4	0.0019

BF, radiographic bone fill; DBC, radiographic bone crest change; BF-DBC, radiographic intrabony resolution.
PPD, probing pocket depth; CAL, clinical attachment level.

The correlation matrix between the various clinical and radiographic measurements of the defect was evaluated with the Spearman correlation coefficient. Multivariate models predicting CAL gains, radiographic BF and radiographic DR at 1 year on the basis of baseline clinical and radiographic parameters were constructed using the GLM SAS procedure and the Logistic SAS procedure with treatment, centre effect, and smoking status as classification variables, as described previously (Tonetti et al. 2002).

Results

Subject accountability

A total of 122 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. Complete observations were available for 120 subjects: 61 tests and 59 controls. Ten subjects were withdrawn because of poor quality of one or both X-rays (four in the test group and six in the control group). This represents 9.9% of the enrolled population. Fifty three subjects were treated with papilla preservation flap alone (control group) and 57 with the papilla flap combined with GTR/DBBM (test group). All results presented in this paper refer to these latter 110 subjects.

Subject and defect characteristics at baseline

Subject and defect characteristics at baseline are displayed in Tables 1 and 2. No significant differences between test and control patients were observed for any of the subject or defect characteristics.

Mean baseline PPD was 7.8 ± 1.6 mm for the test and 8 ± 1.5 mm for the control defect sites. Mean baseline recession for the test group was 2 ± 1.5 and 1.9 ± 1.5 mm for the control group. Mean baseline CAL was 9.9 ± 1.9 and 9.9 ± 2.1 mm at the test and the control defect sites, respectively.

The mean radiographic distance from the CEJ-BD was 9.7 ± 2.3 mm for the test and 10 ± 2.7 mm for the control defects. The mean radiographic distance from the CEJ-BC was 4.3 ± 1.8 for the test and 4.5 ± 1.9 for the control defects. The mean baseline radiographic defect angle was similar in both groups: 25.2° in the test and 25.9° in the control group. A predominantly two-wall defect was found in 50% of the test group and 51% in the control group.

Surgical parameters

A modified papilla preservation flap was carried out in 66% sites of the test group, and in 62% of the sites of the control group. Primary closure was achieved in 89% sites of the test group and 96% of the control group.

Clinical and radiographic outcomes at 1 year

The clinical and radiographic outcomes of papilla preservation flap with or without GTR/DBBM are described in Table 3. FMPS for the test group (11.5 ± 8.3) and the control group (11.5 ± 7.3) were similar at 1 year ($p = 0.9889$). FMBS for the control group (11.3 ± 7.1) was slightly higher than in the test group (8.8 ± 8.3) at 1 year, but the difference did not reach statistical significance ($p = 0.2275$).

Table 4. Multivariate analysis of CAL gain

Parameter	Estimate ± SE	Significance, *p*-value
Treatment effect (test *versus* control)	0.97 ± 0.29	0.0011
Center effect (best *versus* worst)	1.25 ± 0.70	0.0805
Smoking (no *versus* yes)	0.16 ± 0.31	0.6077
Gender (female *versus* male)	0.11 ± 0.29	0.7131
Baseline FMPS (%)	0.04 ± 0.02	0.1423
Baseline FMBS (%)	− 0.08 ± 0.04	0.0595
Baseline PPD (mm)	0.57 ± 0.1	< 0.0001
Radiographic defect angle (each degree)	0.005 ± 0.02	0.8138

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

Table 5. Multivariate analysis of radiographic bone fill, $N = 110$

Parameter	Estimate ± SE	Significance, *p*-value
Treatment effect (test *versus* control)	1.44 ± 0.36	0.0001
Center effect (best *versus* worst)	1.69 ± 0.88	0.0583
Smoking (no *versus* yes)	0.41 ± 0.39	0.2910
Gender (female *versus* male)	− 0.26 ± 0.37	0.4760
Baseline FMPS (%)	0.009 ± 0.03	0.7868
Baseline FMBS (%)	− 0.014 ± 0.05	0.7984
Baseline PPD (mm)	− 0.09 ± 0.12	0.4457
Baseline radiographic intrabony defect (mm)	0.43 ± 0.10	0.0001
Baseline radiographic defect angle (each degree)	− 0.01 ± 0.02	0.6179

Significance of model $p = 0.0002$, adjusted $R^2 = 0.37$.
FMPS, full-mouth plaque scores; FMBS, full-mouth bleeding scores; PPD, probing pocket depth.

Different multivariate models were constructed to assess potential sources of variability in terms of CAL gain, radiographic BF and radiographic DR.

A multivariate analysis was carried out to predict changes in clinical attachment level 1 year after both therapies. In the model only variables known or measurable before surgery were used in order to evaluate their potential utility as predictors of the surgical outcome. The multivariate model (Table 4) was highly statistically significant ($p < 0.0001$) and explained 40% of the observed variability in CAL gain. The surgical treatment combining papilla preservation flap with the application of GTR/DBBM resulted in significantly greater CAL gains than the papilla preservation access flap control ($p = 0.0011$). No significant centre effect was observed ($p = 0.0805$, NS). Cigarette smoking also did not have a significant effect ($p = 0.6077$, NS). The level of oral hygiene (FMPS) did not reach statistical significance ($p = 0.1423$, NS), while the percentage of sites displaying bleeding on probing at baseline (FMBS) had a close to significant negative impact on the outcome ($p = 0.0595$, NS). Among the considered defect characteristics, the initial pocket depth was a highly significant covariate ($p < 0.0001$) while the radiographic defect angle was not ($p = 0.8138$).

A logistic model was similarly constructed categorizing the defect angles comparing the lower (narrow angles $< 20^\circ$) and upper (wide angles $\geqslant 29^\circ$) quartiles. This multivariate model was statistically significant and explained 40% of the observed variability in CAL gain. In this model the variables that significantly affected CAL gains were treatment provided and baseline PPD ($p = 0.001$ and < 0.0001, respectively). The group angle variable comparing narrow *versus* wide defects did not reach significance ($p = 0.5784$).

A logistic regression analysis with backwards elimination of non-significant factors ($p = 0.1$) evaluated the impact of centre effect, gender, smoking status, treatment modality, oral hygiene (FMPS at baseline), bleeding on probing (FMBS at baseline), baseline PPD, and radiographic defect angle (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 centre with the worst overall performance.

A multivariate analysis (Table 5) adding the radiographic depth of the intrabony defect to the above-mentioned variables in Table 4 was carried out to predict changes in radiographic BF 1 year after treatment. The multivariate model was highly statistically significant ($p = 0.0002$) and explained 37% of the observed variability in radiographic BF. The surgical treatment combining papilla preservation flap with the application of GTR/DBBM resulted in significantly greater radiographic BF than the papilla preservation access flap control ($p = 0.0001$). Centre effect was close to significance ($p = 0.0583$, NS). Cigarette smoking also did not have a significant effect ($p = 0.2910$, NS). The level of oral hygiene (FMPS) and percentage of sites displaying bleeding on probing at baseline (FMBS) did not reach statistical significance ($p = 0.7868$, 0.7984, respectively). Among the considered defect characteristics, the baseline PPD did not show an impact in the 1 year radiographic BF ($p = 0.4457$, NS). The initial radiographic intrabony defect was a highly significant covariate ($p = 0.0001$) while the radiographic defect angle did not reach statistical significance ($p = 0.6179$).

A multivariate analysis (Table 6) incorporating the above-mentioned variables was carried out to predict changes in radiographic BC at 1 year. The multivariate model was not statistically significant ($p = 0.1361$).

A multivariate analysis considering the above-mentioned variables was carried out to predict changes in radiographic DR 1 year after both therapies. The multivariate model was highly statistically significant ($p < 0.0001$) and explained 45% of the observed variability in radiographic DR . The surgical treatment combining papilla preservation flap with the application of GTR/DBBM resulted in highly significant greater radiographic DR than the papilla preservation access flap control ($p < 0.0001$). A significant centre effect was observed ($p = 0.0278$). Cigarette smoking did not have a significant effect ($p = 0.0949$, NS). The level of oral hygiene (FMPS) and percentage of sites displaying bleeding on probing at baseline (FMBS) were not significant ($p = 0.1622$, 0.3360, respectively). Among the considered defect characteristics, the baseline PPD did not show a significant impact on the 1 year radiographic DR ($p = 0.3810$, NS). The initial radiographic intrabony defect was a highly significant covariate ($p < 0.0001$), while the radiographic defect angle did not reach statistical significance ($p = 0.1352$).

Table 6. Multivariate analysis of radiographic bone crest change, $N = 110$

Parameter	Estimate ± SE	Significance, *p*-value
Treatment effect (control *versus*. test)	− 0.08 ± 0.18	0.6603
Center effect (best *versus*. worst)	− 0.87 ± 0.51	0.0964
Smoking (no *versus*. yes)	− 0.16 ± 0.2	0.4154
Gender (female *versus*. male)	− 0.13 ± 0.19	0.4772
Baseline FMPS (%)	− 0.03 ± 0.01	0.0628
Baseline FMBS (%)	0.03 ± 0.02	0.2505
Baseline PPD (mm)	− 0.0008 ± 0.06	0.9893
Baseline radiographic intrabony defect (mm)	− 0.04 ± 0.05	0.4500
Baseline radiographic defect angle (each degree)	0.02 ± 0.01	0.1122

Significance of model $p = 0.1361$, adjusted $R^2 = 0.21$.
FMPS, full-mouth plaque scores; FMBS, full-mouth bleeding scores; PPD, probing pocket depth.

Table 7. Multivariate analysis of percentage of radiographic defect resolution

Parameter	Estimate ± SE	Significance, *p*-value
Treatment effect (test *versus* control.)	26 ± 5	<0.0001
Center effect (best *versus* worst)	25 ± 13	0.0746
Smoking (no *versus* yes)	7 ± 6	0.2522
Gender (female *versus* male)	2 ± 5	0.7340
Baseline FMPS (%)	1 ± 0.5	0.0444
Baseline FMBS (%)	0.7 ± 0.8	0.3866
Baseline PPD (mm)	3 ± 2	0.1185
Baseline radiographic intrabony defect (mm)	1 ± 1	0.3004
Baseline radiographic defect angle (each degree)	0.6 ± 0.4	0.1560

Significance of model $p = 0.0008$, adjusted $R^2 = 0.34$.
FMPS, full-mouth plaque scores; FMBS, full-mouth bleeding scores; PPD, probing pocket depth.

Another multivariate analysis was performed in order to assess the impact of the previous model variables on the percentage of radiographic DR (Table 7). The model was significant ($p = 0.0008$) and could explain 34% of the observed variability in percentage of DR . Again, the test treatment showed a highly significant association with the percentage of radiographic DR ($p < 0.0001$). The baseline level of oral hygiene (FMPS) reached a statistically significant effect on the percentage of radiographic DR ($p = 0.0444$). The rest of the variables were not significant in the model, although centre effect approached significance ($p = 0.0746$).

A linear regression analysis was performed in order to assess the association between CAL gain and radiographic BF . The analysis was statistically significant ($p = 0.0005$), with an estimate of 0.8120, 95% CI 0.68–0.93. The R^2 value was 0.60. This means that 60% of radiographic BF variability was explained by CAL gains.

Discussion

Data from the secondary analyses of this multi-centre randomized-controlled clinical trial indicate that application of the GTR/DBBG resulted in significant improvements in radiographic BF, radiographic DR, and percentage of DR compared with application of papilla preservation access flap techniques alone. The absolute value of the observed added benefit in terms of radiographic BF and radiographic DR was 1.4 ± 0.3 mm. GTR/DBBG resulted in 26 ± 5% greater percentage of radiographic DR. These observations indicate the efficacy of the combined treatment in providing radiographic resolution of the intrabony defects. No significant differences were found in terms of BC change between the test and control groups; keeping in mind that the study was not powered to detect changes in this variable, this may be interpreted as a lack of ability of the GTR/DBBM to prevent the limited amounts of resorption of the BC of the defect observed following elevation of papilla preservation flaps. On the other hand, the minimal amount of crestal bone resorption observed following simplified papilla preservation access flap underscores the importance of primary intention healing on stability of the position of the alveolar crest adjacent to the defect.

The multivariate analysis of the factors (treatment, centre, smoking, gender, FMBS, FMPS, baseline PPD, radiographic intrabony, and radiographic baseline angle) affecting radiographic BF, radiographic DR, and percentage of DR at 1 year provided clarification of the present results. In this study, cigarette smoking did not reach statistical significance. The fact that heavy smokers were excluded from the study may have provided an exclusion bias in this result. Further investigations are necessary to explore the effect of smoking on radiographic BF following treatment of intrabony defects with regenerative procedures. Centre effect was not significant in terms of BF, BC change, and percentage of DR. However, in terms of radiographic DR it was significant. This may be explained as a result of the centre variability (close to significant) in radiographic BF and BC change, as these variables form the radiographic DR variable. This means that the operator's skills and experience may have a clinical impact. The radiographic outcomes reported in this study are similar to previous reports using different regenerative materials (Cortellini et al. 1993a, b, Heijl et al. 1997, Klein et al. 2001, Eickholz et al. 2004, Francetti et al. 2004). A roughly 60% radiographic DR was achieved in this study and the above-mentioned studies. However, it should be taken into account that radiographs tend to underestimate clinical values. As reported by Cortellini et al. (1993b) radiographic measurements underestimated the extent of bone gain at 1 year re-entry following treatment of intrabony defects with a non-resorbable membrane. In that study, the mean BF at re-entry was 73 ± 31% and the corresponding radiographic BF was 59 ± 37%. These radiographic data are very similar to the results reported in this study.

The radiographic outcomes after GTR/DBBM should also be interpreted with caution, as these grafts in the radiographs are hardly distinguishable from the host bone, and grafts appearing on radiographs may not necessarily be incorporated in bone (Carmagnola et al. 2003). This could introduce some bias in the radiographic examination process and overestimation of radiographic bone levels in the test group.

A limitation in the radiographic analysis has to be taken into account, ase the pairs of radiographs (pre-treatment and 1 year) were not identical. The

calculation of a correction factor assessing the level of distortion between pre- and post- treatment radiographs may have helped to minimize errors. The correction factor for test and control groups was close to one (Table 4); this means that the projection of pre- and post-operative radiographs was similar in both groups.

An interesting finding of the present investigation was the lack of significance of the baseline radiographic angle of intrabony defects in the clinical and radiographic outcomes following treatment with papilla preservation flaps with or without the application of GTR/DBBM. This was not in agreement with the results of previous studies with different regenerative materials, and may support the hypothesis that the combined treatment of GTR/DBBM was less influenced by the baseline radiographic angle of the defect than those tested in previous studies (Tonetti et al. 1993a, Cortellini & Tonetti 1999, Klein et al. 2001, Eickholz et al. 2004, Tsitoura et al. 2004). Tonetti et al. (1993a, b) showed that the radiographic angle of an intrabony defect treated with guided tissue regeneration has a significant impact on the clinical outcomes of the therapy. In another study, Cortellini and Tonetti (1999) reported 1.5 mm more clinical attachment level gain in narrow defects ($\leqslant 25^\circ$) than wide defects ($\geqslant 37^\circ$), following GTR treatment. Their study concluded that the radiographic defect angle could be used by the clinician as a useful presurgical parameter to determine the potential of clinical attachment level gain in intrabony defects treated with guided tissue regeneration. Klein et al. (2001) showed that the baseline radiographic angle did not have a significant impact in terms of CAL gain; however, the baseline angle showed a statistically significant effect on the radiographic BF. The results of their study are in agreement with the outcomes of our study in terms of clinical parameters but not of the radiographic ones. However, it has to be taken into account that the cut-off values utilized by Klein et al. (2001) were different from those used in the present study. The authors compared $<26^\circ$ *versus* $\geqslant 26^\circ$ in a multivariate model. Models reported in this study tested the effect of the angle at each degree or using the inter-tertile ranges ($<20^\circ$, 20 to $<29^\circ$, and $\geqslant 29^\circ$). It should also to emphasized that the sample size of their study was relatively small (29 patients and 39 intrabony defects) and there was no control group. Similar results were reported in a similar study by the same group (Eickholz et al. 2004). Again the baseline radiographic defect angle had no significant impact in terms of CAL gain 24 months following GTR treatment with resorbable or non-resorbable membranes. Radiographic outcomes measured as BF were significantly associated with the baseline angle. Limitations of this study were a relatively small sample size (32 patients/50 defects) and lack of a control group. When using enamel matrix proteins, Tsitoura et al. (2004) reported that the baseline radiographic angle of intrabony defects treated with papilla preservation flaps and EMD application was significantly associated with the CAL changes observed 1 year later. Moreover, the probability of obtaining CAL gains of $\geqslant 4$ mm was 2.5 times higher when the radiographic defect angle was $\leqslant 22^\circ$ than when it was $\geqslant 36^\circ$. No analysis of radiographic outcomes was performed in that study. The differences in baseline radiographic defect angle between the present study and the previous ones by Tsitoura et al. (2004) and Cortellini & Tonetti (1999) should also be underlined: the present study treated narrower defects. Such a difference may arise from the implication in clinical practice of the previous studies, and thus a more careful selection of the cases. Several hypotheses have been postulated to explain the impaired outcomes of regenerative procedures in wide intrabony defects in comparison with narrow defects. One is the collapse of the membranes in wider defects. This in turn will result in a decrease of the space available for regeneration and in a possible interference with the blood clot stabilization (Egelberg 1987). The use of a bone graft in order to support the membrane might overcome this problem as suggested in the clinical and radiographic data of our study. Wider defects may present a special healing challenge: more tissue is lost in a wide defect (in particular more mineralized tissue), and the superficial component of a wide defect may be more exposed to the adverse effects of the oral environment (Tonetti et al. 1993a, b).

When evaluating results of periodontal regenerative therapy, care should be exercised in interpreting such results. While improvement in clinical parameters can result in actual gain in attachment, it should be remembered that placement of a bone graft into a defect may impede penetration of the periodontal probe without necessarily having induced any gain in clinical attachment. Nevertheless, human histological case reports have indicated that the combined application of GTR/DBBM can result in true periodontal attachment in terms of new cementum and functionally oriented periodontal ligament fibres (Camelo et al. 1998, 2001, Nevins et al. 2003). Furthermore, Stavropoulos & Karring (2005) reported clinical and radiographic stability of intrabony defects treated with DBBM and a synthetic resorbable membrane, with a follow-up of up to 4 years.

The combined use of GTR/DBBM with papilla preservation flaps showed additional benefits in terms of CAL gain (Tonetti et al. 2004) as well as radiographic DR compared with papilla preservation flaps alone. The present study failed to show a significant association between the radiographic defect angle of an intrabony defect at baseline and the changes in CAL or radiographic outcomes after treating the intrabony defect with GTR/DBBM regenerative surgery.

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