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Guided tissue regeneration combined with a deproteinized bovine bone mineral (Bio-Oss®) in the treatment of intrabony periodontal defects: 6-year results from a randomized-controlled clinical trial

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Abstract

Aim: To present the 6-year results of a randomized-controlled clinical trial evaluating guided tissue regeneration (GTR) combined with or without deproteinized bovine bone mineral (DBBM) in intrabony defects.

Material & Methods: In each of 45 patients, one defect was treated with GTR combined with DBBM hydrated in saline (DBBM −) or gentamicin sulphate (DBBM+) or with GTR alone. Clinical parameters were recorded pre-surgery, at 1 and 6 years postsurgery.

Results: Thirty-six patients/33 teeth were available for the 6-year control. Statistically significant clinical improvements were observed for all treatments. Clinical attachment level (CAL) gain averaged 2.5 mm (DBBM −), 4.1 mm (DBBM+), and 3.0 mm (GTR) at 1 year postsurgery, and remained stable over 5 additional years (2.3, 4.1, and 2.7 mm, respectively). Treatment did not appear to influence residual probing depths (PDs) or CAL gains at 6 years postsurgery, or the extent of PD and CAL change from 1 to 6 years, and did not associate with sites losing CAL during follow-up. No association of grafting with sites showing CAL gain $\geqslant$4 mm at the 1- or 6-year control was observed.

Conclusion: The improvements in periodontal conditions obtained after GTR treatment with or without the adjunct use of DBBM can be preserved on a long-term basis.

Key words: bioabsorbable membranes; clinical; deproteinized bovine bone; GTR; guided tissue regeneration; intrabony; long term; periodontology; xenograft

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Conflict of interest and source of funding

The authors declare they have no conflict of interests.

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There is a large body of clinical and biologic evidence (i.e. human histology, controlled clinical trials, controlled animal experiments, explainable concept) documenting that the periodontal attachment apparatus (i.e. cementum, periodontal ligament, and alveolar bone) can, under circumstances, be reestablished by means of the guided tissue regeneration (GTR) technique (for a review, see Stavropoulos 2002). Additionally, the clinical outcomes obtained following GTR application – usually including clinical attachment level (CAL) gains, reduced probing depths (PDs), and radiographic bone fill – can, under certain conditions, be preserved on a long-term basis, irrespective of the nature of the barrier material used (non-resorbable or bioresorbable) (Gottlow et al.

1992, Becker & Becker 1993, Cortellini et al. 1994, 1996, 1999, Weigel et al. 1995, De Sanctis & Zucchelli 2000, Sculean et al. 2001, Kim et al. 2002, Cortellini & Tonetti 2004, Stavropoulos & Karring 2004).

The GTR technique is often combined with bone grafts and/or bone graft substitutes placed underneath the membrane with the intention to support the barrier material and to prevent it from collapsing into the defect or onto the root (i.e. achieve space provision) and/or to enhance bone regeneration. Deproteinized bovine bone mineral (DBBM) (Bio-Oss®, Geistlich AB, Wohlhusen, Switzerland) has been widely used as such an adjunct to GTR. Indeed, histologic observations from animal studies (Yamada et al. 2002, Sakata et al. 2006) and clinical cases (Camelo et al. 1998, Mellonig 2000, Paolantonio et al. 2001, Nevins et al. 2003, Sculean et al. 2004) suggest that healing following GTR+DBBM is, at least in part, characterized by periodontal regeneration. Additionally, several publications have reported clinically successful results in the treatment of intrabony periodontal defects following this combined approach (Camelo et al. 1998, Lundgren & Slotte 1999, Camargo et al. 2000, Paolantonio 2002, Sculean et al. 2003, Stavropoulos et al. 2003a, Tonetti et al. 2004), although the results are inconsistent regarding an added effect of the combination regimen over GTR solo.

Nevertheless, in a discussion on the suitability or not of a particular biomaterial as an adjunct to GTR, an important point is regarding the longevity of the outcome/effect of the combination regimen (i.e. GTR+ biomaterial). However, the information on this issue is still very limited when it comes to this particular DBBM (Stavropoulos & Karring 2005, Sculean et al. 2007, Slotte et al. 2007).

Thus, the aim of the present study is to report the clinical and radiographic results from a randomized-controlled clinical trial (RCT) on the treatment of intrabony defects with GTR in combination with or without DBBM (Bio-Oss®), 6 years after surgery.

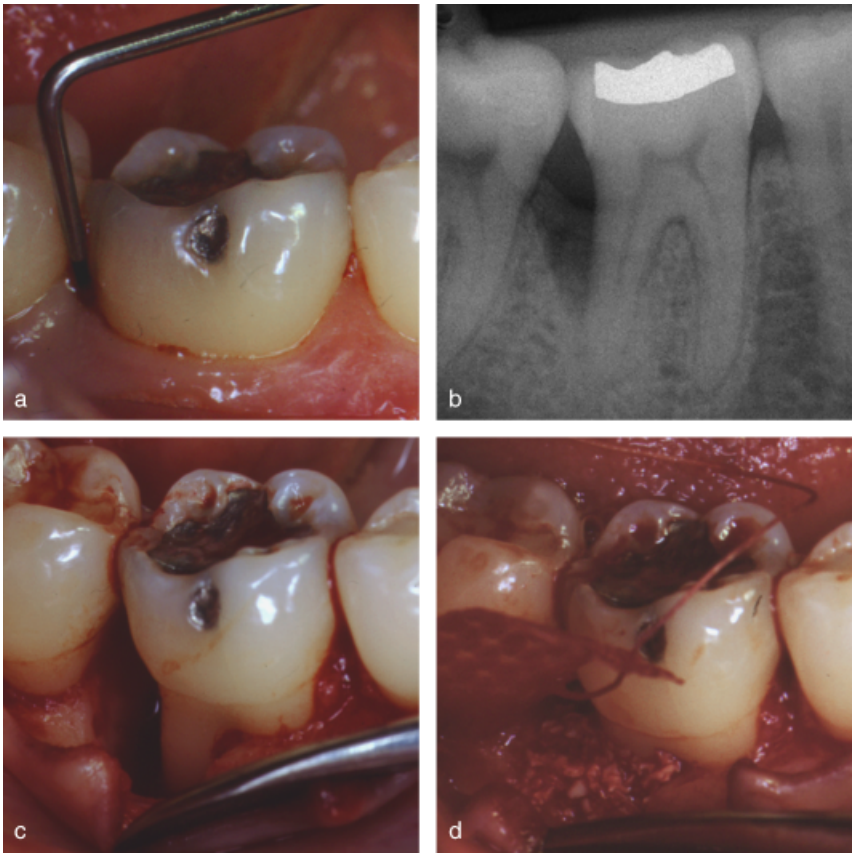

Fig. 1. (a) A deep pocket is probed on the distal side of 46 at baseline. (b) A deep defect with evidence of an intrabony component >4 mm is observed in the baseline X-ray. (c) The presence of a predominantly two-wall defect, with an intrabony component ⩾4 mm, is confirmed during surgery after removal of the granulation tissue. (d) Before the final placement and stabilization of the membrane, Bio-Oss® impregnated with saline is loosely packed into the defect without overfilling it.

Material & Methods

The study population and the 1-year results of the RCT have been described in detail previously (Stavropoulos et al. 2003a). The present paper reports primarily on the 3 GTR groups of the original RCT, because only limited information could be obtained regarding the fourth group treated solely with open flap debridement (only six out of 15 patients attended the 6-year control); thus, only average values of the clinical and radiographic parameters from this latter group are presented, while no further analysis was performed on this group. Briefly, 60 patients (age range 26–62 years) presenting at the Department of Periodontology and Oral Gerontology, School of Dentistry, University of Aarhus, Denmark, and seeking treatment for advanced periodontitis were included in the study (recruitment and active treatment period: September 1998–May 1999).

Approximately 2 months after the initial periodontal treatment, which consisted of repeated oral hygiene instruction/reinforcement and scaling and root planing under local anaesthesia, the defects/teeth included in the study presented the following characteristics: (a) PD ⩾7 mm and radiographic evidence of an intrabony component (IC) ⩾4 mm (Fig. 1a and b), which did not include a furcation involvement, (b) the site had not been treated surgically within the last year before initiation of the study, and (c) systemic or local antibiotics had not been used within the last 6 months before treatment. All patients gave a signed written consent after having received vocal and written information about the purpose and the possible risks/side-effects of the treatment. The study protocol had previously been approved by the Science Ethics Committee for the district of Aarhus.

A detailed description of the treatment protocol, including surgical technique, antimicrobial regime, and the post-operative control schedule, is provided in the paper by Stavropoulos et al. (2003a). Briefly, during the surgical procedures, and first following site debridement, root scaling and planing, and

confirmation of defect depth and configuration (i.e. primarily one – or two – wall) (Fig. 1c), treatment allocation was performed at random – by choosing a sealed envelope out of a bunch of 60 identical envelopes – among 1 of the following four options: (1) GTR alone (GTR group) with a bioresorbable barrier membrane (Resolut XTs, W.L. Gore & Associates, Flagstaff, AZ, USA), (2) GTR in combination with Bio-Oss® soaked in sterile saline (DBBM− group) (Fig. 1d), (3) GTR in combination with Bio-Oss® soaked in gentamicin sulphate 2 mg/ml (Garamycin, Schering-Plough A/S, Farum, Denmark) (DBBM+group), and (4) open flap debridement alone (OFD group). One year after surgery, a control examination was carried out and the patients were transferred to their own private general dentist either for additional general treatment, if needed, or for maintenance based on an individualized recall programme. All patients received a written invitation (per post; two attempts) for another control of the treated sites 6 years after treatment.

Just before anaesthesia on the day of surgery (baseline), after 1 and after 6 years, the following clinical parameters were recorded at each treated site (both from the buccal and the palatal/lingual aspect) to the closest millimetre, by means of a periodontal probe with 0.5-mm tip and 1-mm marked increments (Hu-Friedy LL 20: Hu-Friedy Mfg. Co. Inc., Chicago, IL, USA): (a) PD: the distance from the gingival margin to the level of probe–tip penetration; (b) gingival recession (REC): the distance from the cemento-enamel junction (CEJ) to the gingival margin – in case the CEJ was difficult to distinguish or absent, the margin of a restoration or a crown was used as the coronal reference point; and (c) CAL: PD+REC. In addition, the presence/absence of plaque (PI) and the presence/absence of bleeding on probing (BoP) were assessed. The site (buccal or oral) of the interproximal defect with the deepest PD value at baseline was chosen as the site of analysis. In case the baseline PD values did not differ, the site (buccal or oral) with the deepest PD after 1 year was chosen as the site of analysis.

Information about the patients' smoking habits was collected at both the 1- and the 6-year controls visits. The frequency of regular control dental visits since the 1-year examination and the kind of periodontal treatment administered during these visits were also recorded according to information provided by the patients. Furthermore, in case the treated tooth was not present at the 6-year control, the reason and timing of extraction was recorded according to the information provided by the patients. Finally, peri-apical radiographs were taken under standardized and reproducible conditions at baseline, and at the 1- and 6-year controls. The method of acquiring and evaluating the radiographs has been described in detail elsewhere (Stavropoulos et al. 2003a). Briefly, by means of an image analysis program (PorDios, Institute of Orthodontic Computer Science Ltd., Aarhus, Denmark), the following parameters were estimated on the images: (a) the distance from the CEJ (or the margin of a restoration) to the bottom of the intrabony defect (BD), representing the radiographic bone level (RBL), (b) the distance from CEJ to the bone crest (BC), and (c) the distance from BC to BD, representing the IC of the lesion. The BD was defined as the most coronal point where the periodontal ligament space showed subjectively a continuous regular width. In order to avoid including in the analysis radiographs that presented a large dimensional distortion from the real anatomical features due to their projection geometry, patients whose baseline radiographs had an RBL 1.5 mm smaller than the corresponding CAL were excluded. Additionally, radiographs where the artificial CEJ of a restoration was not distinguishable or had been altered during the observation period and/or images where the periodontal ligament space could not be identified were also excluded.

A single investigator (A. S.) performed all the surgeries and made the recordings at baseline and after 6 years, while another previously calibrated periodontist (data not presented) made all the recordings during the 1-year control. The first investigator, when collecting baseline data, did not know into which treatment group the individual patient would be allocated, and when collecting the 6-year data, did not have access to patient files, while the second investigator collecting the 12-month data did not know what treatment had been administered. The radiographic evaluation was performed by a single experienced investigator (A. S.), masked regarding treatment group and time of exposure. Evaluation of this method of radiographic analysis has previously shown high intra-examiner reproducibility regarding CEJ–BD and BC–BD distance estimation (i.e. for RBL and IC, respectively) (Stavropoulos et al. 2003a).

Significance of differences for PI and BoP between baseline, 1-, and 6-year registrations was evaluated using McNemar's test. Significance of differences between baseline, 1- and 6-year clinical data was evaluated with Student's *t*-test for paired observations. Patients declaring that they smoked regularly (at least five cigarettes on a daily basis) at both the 1- and the 6-year controls were classified as habitual smokers. The presence of plaque at the treated site in both the 1- and the 6-year controls was acknowledged as evidence of poor oral hygiene. Treated sites that bled after probing in both the 1- and the 6-year controls were classified as showing frequent BoP. Patients receiving a dental control and/or professional prophylaxis after the 1-year control at least three times annually (on a regular basis) were classified as being frequently controlled.

Generalized linear models were constructed to evaluate the influence of GTR treatment group on the following outcome variables: CAL gain, PD, RBL gain, and IC at the 6-year control, as well as on the change of CAL and PD from 1 to 6 years after treatment, correcting for smoking habits (smoking/no smoking), oral hygiene (good/bad), BoP (frequent/infrequent), and frequency of dental controls (frequent/infrequent). The tooth loss observed in the present group of patients at the 6-year control was most likely due to progressing/advanced CAL loss. Therefore, a composite outcome variable including ''sites with CAL loss between the 1- and 6-year controls or tooth extraction'' was established and its association with GTR treatment group, smoking, and infrequent dental controls was evaluated with χ^2 statistics. The threshold to characterize sites losing attachment was set to (a) CAL loss $\geqslant 1$ mm, (b) CAL loss $\geqslant 2$ mm, and CAL loss $\geqslant 4$ mm (i.e. three separate analyses were performed). A similar evaluation regarding the possible association of poor oral hygiene and frequent BoP with sites experiencing CAL loss $\geqslant 1$, 2, or 4 mm was also performed. Obviously, the latter evaluation did use the composite outcome variable including tooth loss, as no information regarding the level of oral hygiene and presence/absence of BoP could be obtained in the absence of the teeth. Finally, in order to evaluate the effect of grafting on the treatment

outcome in terms of CAL, the two DBBM groups were pooled together, and the association of grafting with sites showing CAL gain $\geqslant 4$ mm at the 1- or at the 6-year controls or CAL loss (using the above-mentioned composite variable; 3 separate analyses for CAL loss $\geqslant 1$, $\geqslant 2$, or $\geqslant 4$ mm) between the 1- and the 6-year controls was examined with χ^2 statistics. The level of significance was set at $p < 0.05$. All calculations were performed using the SPSS for Windows (version 13.0.0) software package (SPSS Inc., Chicago, IL, USA).

Results

Post-operative healing occurred without significant problems and the patients did not report any complications/adverse events other than medium-size swelling and pain. Membrane exposure was a common event in all GTR groups, because a total of 61.4% of all membranes became exposed to the oral environment. In most of these cases, the exposure presented as an ''opening'' (separation) of the interdental papillae occurring 2–3 weeks postsurgery, and was not associated with signs of excessive inflammation. None of the exposed membranes was removed and the exposed portion of the membranes was resorbed after an additional time of approximately 2 weeks. An analysis performed in the previous paper by Stavropoulos et al. (2003a) showed no significant difference in the frequencies of membrane exposure between groups.

In total, 42 patients (36 belonging to the GTR groups and six to the OFD group) responded to the invitation and participated at the 6-year control examination (total drop-out in the three GTR groups and in the OFD group was 20% and 60%, respectively); two patients belonging in the GTR groups did not come due to general health problems, but informed over the telephone that they had not experienced any problems with the treated teeth. From those 36 patients of the GTR groups, only three (8.3%) had lost the treated tooth. Two teeth, both maxillary second premolars, belonged to the BDDM+group (extracted 2–3 years and 5 years after surgery, respectively) and were lost according to the patients due to ''persistent periodontitis''. Both teeth showed a PD of 6 mm at the 1-year control; also, both teeth were endodontically treated by a general dentist at some point after GTR surgery. The third lost tooth, a mandibular second pre-molar, belonged to the DBBM− group (extracted 5 years after surgery), and presented a PD of 4 mm at the 1-year examination and a clear reason for extraction could not be established. From the six patients belonging to the OFD group, one has lost the treated tooth (a maxillary premolar) 2–3 years after treatment due to ''persistent periodontitis''. All patients conveyed that regular maintenance care (i.e. professional tooth cleaning) was the only periodontal treatment administered to the teeth included in the project.

As already mentioned, the statistical analysis considered only the 3 GTR groups; the OFD group was excluded due to the high drop-out rate (60%) observed at the 6-year control. Regarding PI and BoP, no significant differences were observed between the GTR groups at any observation period. GTR treatment, with or without DBBM, resulted in significant clinical improvements (i.e. CAL gain and PD reduction) 1 year after surgery, which were basically preserved during the following 5-year observation period (Table 1). The GLMs showed no differences among the three GTR groups regarding CAL gain and PD at the 6-year control (Table 2), as well as on the change of CAL and PD from 1 to 6 years after treatment (Table 3). In general, PD had increased to a minor extent (range: 0.4–1 mm) from the 1- to the 6-year controls visit, but the average amount of residual PD was not significantly different between the two observation periods. This increase in PD was mainly due to REC reduction rather than CAL loss. In a few patients, part of the CAL gain originally obtained after 1 year from treatment was lost during the following 5-year observation period (Table 4), but no association of treatment group, grafting, smoking, poor oral hygiene,

Table 1. Average PI and BoP (in %) and average PD and CAL [in mm (± SD)] at baseline; average PI and BoP (in %) and average PD and CAL gain [in mm (± SD)] at the 1- and 6-year controls, from those patients present at the 6-year control

	GTR	DBBM−	DBBM+	OFD
Clinical data				
N	12	10	11	5
Baseline				
PI	0.00	0.00	0.00	0.00
BoP	58.3	60.0	81.8	60.0
PD	8.8 (± 1.7)	8.5 (± 1.1)	9.3 (± 1.2)	7.8 (± 0.5)
CAL	9.8 (± 2.1)	9.6 (± 1.6)	10.3 (± 1.6)	8.4 (± 1.1)
1 year				
PI	50.0*	40.0	27.3	40.0
BoP	67.3	50.0	54.5	80.0
PD	4.8 (± 1.0)†	4.6 (± 1.0)†	4.2 (± 1.0)†	6.2 (± 1.6)
CAL gain	3.0 (± 2.0)†	2.5 (± 2.5)†	4.1 (± 1.8)†	− 0.2 (± 2.8)
6 years				
PI	58.3‡	30.0	27.3	40.0
BoP	75.0	40.0	63.6	100.0
PD	5.8 (± 1.9)§	4.9 (± 1.3)§	4.6 (± 1.2)§	7.6 (± 2.1)
CAL gain	2.4 (± 2.1)§	2.3 (± 2.1)§	4.1 (± 1.6)§	− 1.2 (± 2.4)
Radiological data				
Baseline				
N	8	8	7	4
RBL	9.78 (± 1.82)	10.5 (± 3.15)	10.2 (± 2.94)	8.95 (± 1.45)
IC	5.94 (± 1.51)	5.00 (± 2.33)	5.65 (± 2.34)	6.13 (± 1.62)
1 year				
RBL gain	3.25 (± 2.12)†	2.79 (± 0.73)†	4.17 (± 2.15)†	1.48 (± 0.87)
IC reduction	3.56 (± 2.11)†	2.41 (± 0.98)†	3.52 (± 2.37)†	0.79 (± 1.22)
6 years				
RBL gain	3.64 (± 2.44)§	3.73 (± 2.37)§	4.41 (± 2.42)§	− 0.15 (± 0.63)
IC reduction	4.22 (± 2.00)§	3.82 (± 2.12)§	3.93 (± 2.45)§	0.20 (± 0.12)

*p baseline *versus* 1 year: $p = 0.03$.
†p baseline *versus* 1 year: $\leqslant 0.01$.
‡p baseline *versus* 5 years: = 0.02, analysed with McNemar test.
§p baseline *versus* 6 years: $\leqslant 0.01$, analysed with the Students' *t*-test for paired observations.
BoP, bleeding on probing; CAL, clinical attachment level; SD, standard deviation; RBL, radiographic bone level; IC, intrabony component; PD, probing depth; GTR, guided tissue regeneration; DBBM, deproteinized bovine bone mineral.

Table 2. Significance levels (p), estimates of differences (Est.) and lower and upper limits of 95% confidence interval for PD and CAL gain at the 6-year control, corrected for smoking, poor oral hygiene (OH), frequent BoP, and frequent dental controls

	df	Sum of squares	Mean square	F-value	p
PD at 6 years					
Model	6	27.3	4.55	2.24	0.71
Error	26	52.9	2.03		
Total	32	80.2			
R^2	0.34				

	Est.	T for H_0	95% confidence interval		p
			lower	upper	
Group					
GTR *versus* DBBM−	−0.49	−0.67	−2.01	1.02	0.51
GTR *versus* DBBM+	−0.49	−0.67	−1.99	1.01	0.50
DBBM− *versus* DBBM+	0.001	0.001	−1.34	1.34	0.99
Smoking	0.39	0.72	−0.71	1.49	0.48
Poor OH	0.95	1.48	−0.38	2.28	0.15
Frequent BoP	1.10	1.97	−0.05	2.24	0.60
Frequent controls	−0.002	−0.003	−1.22	1.22	0.99

	df	Sum of squares	Mean square	F-value	p
CAL gain at 6 years					
Model	6	51.43	8.57	2.58	0.04
Error	26	86.4	3.32		
Total	32	137.8			
R^2	0.37				

	Est.	T for H_0	95% confidence interval		p
			lower	upper	
Group					
GTR *versus* DBBM−	−0.52	−0.56	−2.46	1.41	0.58
GTR *versus* DBBM+	0.99	1.06	−0.93	2.90	0.30
DBBM− *versus* DBBM+	1.51	1.81	−0.20	3.22	0.08
Smoking	−0.46	−0.67	−1.87	0.95	0.51
Poor OH	−0.84	−1.02	−2.54	0.85	0.32
Frequent BoP	−1.56	−2.19	−3.02	0.09	0.04
Frequent controls	0.28	0.36	−1.29	1.84	0.72

BoP, bleeding on probing; PD, probing depth; CAL, clinical attachment level; DBBM, deproteinized bovine bone mineral; GTR, guided tissue regeneration.

frequent BoP, and infrequent dental controls with sites that showed CAL loss during follow-up was observed (Table 4). From the 33 sites included in the present evaluation, 51.5% and 45.5% presented at least 4 mm CAL gain 1 and 6 years after treatment. At 1 year post-op, CAL loss (compared with baseline) was observed in only two sites (1 and 2 mm of CAL loss), both belonging to the DBBM− group (Table 5). From those two ''loser'' sites, the former exhibited the same amount of CAL loss after 6 years, while the latter showed a CAL gain of 1 mm compared with baseline. The second site presenting 2 mm CAL loss (compared with baseline) at the 6-year control belonged to the GTR group (Table 5). No association of grafting with sites showing CAL gain $\geqslant$4 mm at the 1- or at the 6-year controls was observed ($p = 0.48$ and 1.00, respectively). A power calculation on the per-protocol study population using CAL gain as the primary outcome variable showed that the study had a power of 77% (an error probability set at 0.05).

The analysis of the eligible radiographs showed a statistically significant effect of all three GTR treatment modalities in terms of RBL gain and IC reduction 1 year after treatment, which was preserved during the rest of the observation period (Table 1). No differences were observed among the three GTR groups in terms of RBL gain and IC at the 6-year control (Table 6). In most of the cases in the DBBM− and DBBM+ groups, the grafted defect space was easily discernible on the 1-year radiographs due to its more radio-opaque appearance compared with the adjacent (pristine) alveolar bone (Fig. 2). On the 6-year radiographs, however, the original defect space was only barely distinguishable, i.e. the occupying tissues resembled closely the neighbouring pristine bone, except on a few occasions, where limited change in the radiographic appearance of the site since the 1-year examination was observed (Fig. 3).

Discussion

The results from this study show that the clinical improvements (i.e. PD reduction, CAL gain, radiographic defect resolution) observed 1 year after treatment of intrabony periodontal defects with GTR combined with or without DBBM implantation can basically be maintained over 5 additional years. These observations are in line with previous reports on the long-term stability of treatment outcomes following GTR therapy in intrabony defects using various types of bioresorbable (Sculean et al. 2001, Kim et al. 2002, Stavropoulos & Karring 2004) and non-resorbable (Gottlow et al. 1992, Becker & Becker 1993, Cortellini et al. 1994, 1996, 1999, Weigel et al. 1995) barrier devices, without the adjunct use of bone grafts or substitutes. Moreover, the observations herein support and expand previous reports on the long-term outcome of the GTR+DBBM combination regimen (Stavropoulos & Karring 2005, Sculean et al. 2007, Slotte et al. 2007).

Slotte et al. (2007) treated one deep intrabony defect (mean baseline PD = 10.0 mm and CAL = 11.9 mm) in each of 24 patients using DBBM and a bioresorbable collagen or PLA/citric-acid–ester copolymer membrane. One year after surgery, treatment resulted in a significant mean PD reduction and CAL gain amounting to 5.2 and 4.2 mm, respectively. These clinical improvements were on average preserved almost unchanged for 4 additional years (5.3 and 4.3 mm, respectively). In this study,

Table 3. Significance levels (p), estimates of differences (Est.) and lower and upper limits of 95% confidence interval for PD and CAL gain changes from the 1- to the 6-year controls, corrected for smoking, poor oral hygiene (OH), frequent BoP, and frequent dental controls

	df	Sum of squares	Mean square	F-value	p
CAL gain change					
Model	6	17.8	2.98	0.85	0.54
Error	26	90.7	3.49		
Total	32	111.0			
R^2	0.16				

	Est.	T for H_0	95% confidence interval		p
			lower	upper	
Group					
GTR *versus* DBBM−	0.24	0.25	−1.73	2.22	0.80
GTR *versus* DBBM+	0.37	0.39	−1.59	2.34	0.69
DBBM− *versus* DBBM+	0.13	0.15	−1.63	1.88	0.88
Smoking	−0.22	−0.31	−1.66	1.23	0.76
poor OH	0.28	0.33	−1.46	2.01	0.75
Frequent BoP	−1.39	−1.91	−2.89	0.10	0.07
Frequent controls	0.34	0.44	−1.26	1.94	0.66

	df	Sum of squares	Mean square	F-value	p
PD change					
Model	6	11.8	1.98	0.92	0.49
Error	26	56.0	2.15		
Total	32	67.88			
R^2	0.17				

	Est.	T for H_0	95% confidence interval		p
			lower	upper	
Group					
GTR *versus* DBBM−	0.47	0.62	−1.09	2.02	0.54
GTR *versus* DBBM+	0.19	0.25	−1.35	1.73	0.80
DBBM− *versus* DBBM+	−0.28	−0.41	−1.66	1.10	0.68
smoking	−0.25	−0.45	−1.38	0.89	0.66
poor OH	−0.13	0.19	−1.49	1.24	0.85
Frequent BoP	−1.00	−1.74	−2.18	0.18	0.93
Frequent controls	0.06	0.10	−1.19	1.32	0.92

BoP, bleeding on probing; PD, probing depth; CAL, clinical attachment level; DBBM, deproteinized bovine bone mineral; GTR, guided tissue regeneration.

a significant radiographic defect resolution was also reported for both time-points (i.e. 1 and 5 years) after surgery. However, lack of standardization of exposure parameters makes any rational interpretation of the presented information difficult. Similarly, Sculean et al. (2007) reported that the significant PD reduction and CAL gain (5.4 and 4.0 mm, respectively, on average) observed 1 year after treatment of deep intrabony defects (mean baseline PD = 9.1 mm and CAL = 10.4 mm) with a bioresorbable collagen membrane+DBBM implantation (10 patients) were basically preserved over an additional 4 years (4.8 and 3.7 mm, respectively, on average). The paper of Stavropoulos & Karring (2005) presented the 5-year data of the 11 patients belonging to the DBBM+group of the current report.

In the DBBM and GTR groups of the present study, a minimal, statistically and clinically insignificant mean CAL loss (0.2 and 0.3 mm, respectively) was observed between 1 and 6 years post-op; in the DBBM+ group, the average CAL remained unchanged but some sites did lose attachment during the follow-up period also in this group. In general, however, and after taking into account the three teeth most likely lost due to further CAL loss, the vast majority of treated sites – 48%, 75%, or 84%, depending on whether the threshold to characterize sites losing attachment was set at 1, 2, or 4 mm, respectively – did not experience CAL loss during follow-up. Moreover, 45.5% of the sites showed at least 4 mm CAL gain 6 years after treatment; the corresponding value at 1 year post-op was 51.5%. Analogous results have been reported in previous publications on the long-term outcome after GTR treatment (Sculean et al. 2001, 2007, Cortellini & Tonetti 2004, Stavropoulos & Karring 2004). For example, in the study of Sculean et al. (2007) mentioned earlier, an average CAL loss of 0.3 mm from 1 to 5 years post-op GTR+DBBM was observed, but this was attributable to only two (out of 10) sites; 50% of the sites in this study showed a CAL gain of 4 mm or more compared with baseline at the 5-year control. Likewise, Stavropoulos & Karring (2004) reported that from an average of 3.8 mm CAL gain obtained 1 year after GTR treatment with PLA/citric acid ester copolymer bioresorbable membranes, only 0.2 mm were lost 6–7 years after surgery, and that this CAL loss could be attributed to only four (out of 25) sites. In that patient group, CAL gain $\geqslant$4 mm at the 6- to 7-year controls was observed in 36% of the sites. In a recent retrospective analysis of a large material including 175 deep intrabony defects treated by means of GTR with or without various bone grafts and/or substitutes, it was found that only one third of all treated sites had lost more than 1 mm in CAL over a 15-year period (Cortellini & Tonetti 2004). Thus, it is reasonable to conclude that the clinical improvements obtained after GTR therapy can be preserved for a rather long period of time in the majority of the cases/sites.

Partial loss of the CAL gain obtained 1 year after GTR treatment has been previously associated with smoking, poor oral hygiene (Cortellini et al. 1996, Cortellini & Tonetti 2004), and lack of compliance with a supportive periodontal programme (Cortellini et al. 1994, Weigel et al. 1995). In contrast to these observations, smoking, poor oral hygiene, frequent BoP, and infrequent dental controls did not seem to play a role in the stability of CAL gain in the present study. This discrepancy is difficult to explain, but may be due to the rather limited total number of sites in the present study. However, it must also be kept in mind that classification of sites

Table 4. Classification of ''sites with CAL loss between the 1- and 6-year controls or tooth extraction'', according to group, smoking habits, level of oral hygiene, frequency of BoP, and frequency of dental controls

	CAL loss ⩾1 mm or extraction			CAL loss ⩾2 mm or extraction			CAL loss ⩾4 mm or extraction		
	no	yes	p^*	no	yes	p^*	no	yes	p^*
Group									
GTR	8	4		10	2		10	2	
DBBM−	6	5		7	4		10	1	
DBBM+	7	6	0.77	10	3	0.54	10	3	0.66
Grafting									
Yes	13	11		17	7		20	4	
No	8	4	0.47	10	2	0.41	10	2	1.00
Smoking									
Yes	12	9		14	7		17	4	
No	9	6	0.86	13	2	0.17	13	2	0.65
Dental controls									
Frequent	9	8		12	5		14	3	
Infrequent	12	7	0.53	15	4	0.56	16	3	0.88
Total	21	15		27	9		30	6	
%		41.6%			25%			16%	
BoP									
Frequent	7	6		9	4		10	3	
Infrequent	14	6	0.35	18	2	0.13	20	0	0.06
Oral hygiene									
Good	15	10		20	5		23	2	
Bad	6	2	0.44	7	1	0.63	7	1	0.70
Total†	21	12		27	6				
%		36.4%			18.2%				

*Analysed with Pearsons' χ^2 test.
†Obviously, the parameters ''Frequency of BoP'' and ''Level of Oral Hygiene'' could only be evaluated for the teeth present at the 6-year control (i.e. 33 sites).
BoP, bleeding on probing; CAL, clinical attachment level; DBBM, deproteinized bovine bone mineral; GTR, guided tissue regeneration.

Table 5. Number of lost teeth and classification of sites showing CAL loss or CAL gain at the 1- and 6-year controls as compared with baseline CAL values

	Lost teeth	CAL loss	0⩽CAL gain <2	2⩽CAL gain <4	4⩽CAL gain <6	6⩽CAL gain
1 year						
Group						
GTR	0	0	2	5	4	1
DBBM−	0	2	1	2	5	0
DBBM+	0	0	0	4	4	3
Total	0	2	3	11	13	4
%	0	6.1	9.1	33.3	39.4	12.1
6 years						
Group						
GTR	0	1	2	4	5	0
DBBM−	1	1	2	4	3	0
DBBM+	2	0	0	4	5	2
Total	3	2	4	12	13	2
%	8.3	6.1	12.1	36.4	39.4	6.1

% of lost teeth was calculated on the total number of patients present at the 6-year control.
% of CAL loss or gain was calculated on the total number of treated teeth available for evaluation at the 6-year control.
BoP, bleeding on probing; CAL, clinical attachment level; DBBM, deproteinized bovine bone mineral; GTR, guided tissue regeneration.

showing poor oral hygiene and frequent BoP in the present study was based on findings obtained at only two time-points and with a large time interval (5 years) between examinations/registrations, which in turn may only poorly reflect the true situation during most of the follow-up period.

The magnitudes of clinical and radiographical improvements observed in the DBBM groups of the current study are comparable to those reported by others after regenerative treatment of intrabony defects using bioresorbable membranes combined with this particular DBBM product (Camargo et al. 2000, Sculean et al. 2003, Tonetti et al. 2004, Stavropoulos et al. 2004b), while some papers reported an even larger CAL gain (up to 5.5 mm) (Camelo et al. 1998, Lundgren & Slotte 1999). Despite such positive outcomes, however, direct evaluation of the GTR+DBBM combination regimen *versus* GTR solo in intrabony defects seems to yield contradictory results. Although another randomized-controlled study (Paolantonio 2002) yielded a significantly larger CAL gain after GTR+DBBM as compared with GTR solo (5.1 *versus* 4.0 mm, respectively), no significant differences were observed in the clinical and radiographic improvements between the groups in the present material (Stavropoulos et al. 2003a). Likewise, a recently published systematic review evaluating a variety of bone grafts and/or substitutes (including DBBM) as adjuncts to GTR has also failed to find any added clinical benefit of the combination regimen over that achieved by the use of only a membrane (Murphy & Gunsolley 2003).

As already mentioned, the aim of combining the GTR technique with a bone graft and/or a substitute placed underneath the membrane is that the material supports the barrier and prevents its collapse, thus avoiding a compromised healing result due to reduced/limited space for tissue ingrowth, and/or it directly promotes bone regeneration, hence enhancing periodontal regeneration. In an experimental study in dogs, Yamada et al. (2002) surgically induced two-wall intrabony defects and treaded them with either a bioresorbable collagen membrane+DBBM or with GTR solo. After 2 months of healing, the authors reported that although the amount of new attachment (i.e. new cementum with inserting collagen fibres) was similar in the two groups, bone formation – hence, also periodontal regeneration – occurred up to a statistically significant higher level in the sites receiving the combination approach. Thus, despite the lack of difference in the clinical outcome of GTR+DBBM *versus* GTR solo observed in the current study, the possibility that a more favourable healing outcome in terms of periodontal regeneration was

Table 6. Significance levels (p), estimates of differences (Est.) and lower and upper limits of 95% confidence interval for RBL gain and IC at the 6-year control, corrected for smoking, poor oral hygiene (OH), frequent BoP, and frequent dental controls

	df	Sum of squares	Mean square	F-value	p
RBL gain at 6 years					
Model	6	7.39	1.23	0.18	0.98
Error	16	111.5	6.97		
Total	22	118.9			
R^2	0.06				

	Est.	T for H_0	95% confidence interval		p
			lower	upper	
Group					
GTR *versus* DBBM−	0.21	0.13	−3.22	3.64	0.89
GTR *versus* DBBM+	1.45	0.77	−2.53	5.43	0.45
DBBM− *versus* DBBM+	1.24	0.79	−2.06	4.54	0.44
Smoking	0.68	0.53	−2.06	3.43	0.60
Poor OH	1.20	0.69	−2.51	4.19	0.50
Frequent BoP	−0.91	−0.66	−3.84	2.01	0.52
Frequent controls	0.28	0.20	−2.62	3.17	0.84

	df	Sum of squares	Mean square	F-value	p
IC at 6 years					
Model	6	5.72	0.95	0.70	0.65
Error	16	21.6	1.35		
Total	32	27.4			
R^2	0.21				

	Est.	T for H_0	95% confidence interval		p
			lower	upper	
Group					
GTR *versus* DBBM−	0.13	0.19	−1.38	1.65	0.85
GTR *versus* DBBM+	0.70	0.85	−1.05	2.45	0.41
DBBM− *versus* DBBM+	0.56	0.82	−0.89	2.02	0.42
Smoking	−0.30	−0.53	−1.51	0.91	0.61
Poor OH	0.67	0.86	−0.97	2.30	0.40
Frequent BoP	0.45	0.75	−0.84	1.74	0.47
Frequent controls	0.72	1.19	−0.56	1.99	0.25

BoP, bleeding on probing; DBBM, deproteinized bovine bone mineral; GTR, guided tissue regeneration; IC, intrabony component; RBL, radiographic bone level.

indeed achieved with the combined approach cannot be ruled out. Nevertheless, the possibility that the defects in the present study (although being predominantly of the one- or two-wall type) did not have a configuration and dimensions that really involved a risk for membrane collapse cannot be excluded; this could in fact explain why an added effect of grafting was not observed. Indeed, along the same line, a recent systematic review of preclinical models involving combinations of barrier membranes and grafting materials concluded that additional benefits of combination treatments over the use of membranes alone were detected only in non-contained two-wall intrabony or supraalveolar defects (Sculean et al. 2008b). On the other hand, results from controlled experimental studies using a variety of pre-clinical models and species have questioned the potential of DBBM to enhance bone regeneration when used as an adjunct to GTR (Stavropoulos et al. 2001, 2003c, Araujo et al. 2002, Carmagnola et al. 2002, 2003). In this context, it is acknowledged that significant biological differences exist between alveolar bone regeneration in the presence and absence of teeth (Polimeni et al. 2004), and thus the above concerns regarding the bone-forming potential in GTR+DBBM-treated periodontal defects may not be valid.

To date, there is no information on the basis of histological evidence regarding the long-term outcome of GTR+DBBM in periodontal defects. Nevertheless, DBBM particles have been observed inside augmented/implanted bone sites after rather long periods of time – 1.5 years in rats (Stavropoulos et al. 2004a) and up to 6 years in humans (Schlegel & Donath 1998) – and in a single-case histology of a periodontal site 5 years after implantation (Sculean et al. 2008a). Thus, there is no reason not to expect that DBBM particles would remain present for quite a long time also in GTR-treated periodontal sites. In fact, in the above-mentioned paper of Slotte et al. (2007), it was reported that DBBM was still distinguishable in the 5-year radiographs in the majority of the cases, similar to what observed in some 6-year radiographs in this study. Nevertheless, the results of the present study, where the clinical improvements obtained after surgery were basically preserved for at least 5 additional years and no association of treatment group and/or grafting with sites losing CAL was observed, suggest that the mere presence of DBBM particles in the regenerated and/or repaired periodontal tissues may have no consequence per se on the stability of the improved clinical conditions.

In the DBBM+ group, the graft particles were impregnated with gentamicin sulphate 2 mg/ml before implantation. Preliminary data from an experimental study, published a few months earlier than the time the RCT was initiated, had suggested that gentamicin might promote early vascularization of bone grafts (Holck et al. 1998). Recognizing the importance of angiogenesis in bone – (Rhinelander & Wilson 1982) and periodontal regeneration (Wikesjo & Selvig 1999), it was thought that impregnation of DBBM with gentamicin might enhance wound healing in intrabony periodontal defects. Nevertheless, the lack of significant differences observed between the DBBM+and the DBBM− groups did not lend support to such a hypothesis. Additionally, in an experimental study in rats no added effect of gentamicin on bone formation produced by DBBM and GTR was observed (Stavropoulos et al. 2003b). On the other hand, the possibility that the trend for better clinical improvements observed in the BDDM+ group

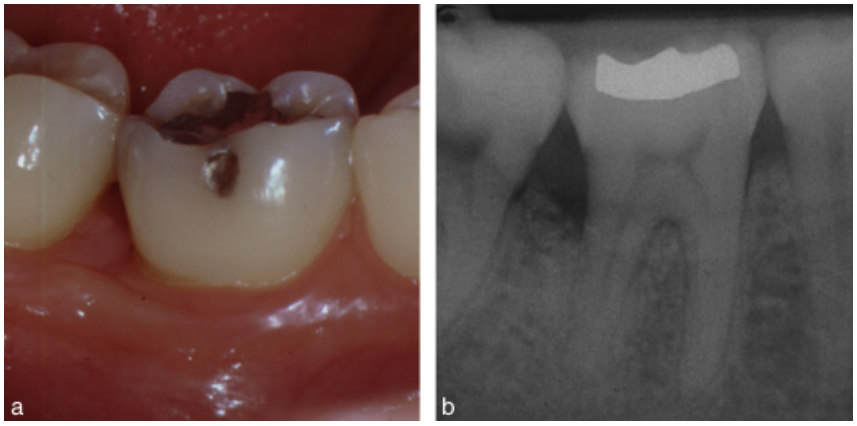

Fig. 2. (a) The clinical result 1 year after treatment. (b) Resolution of the defect with radiographic bone-level gain and slight crestal resorption is observed in the X-ray 1 year after treatment.

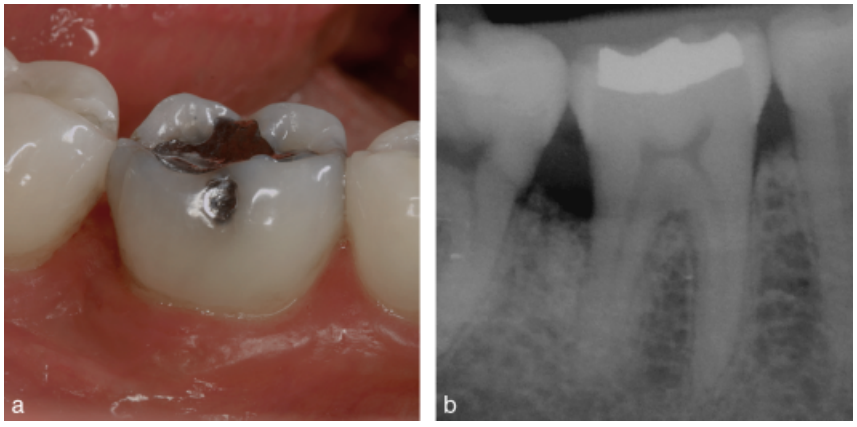

Fig. 3. (a) The clinical result 6 years after treatment. Note the slight improvement in the soft tissue conditions when compared with the 1-year result. (b) Radiographically stable conditions are observed 5 years after treatment.

after 1 and 6 years would have reached statistical significance if a larger number of patients had originally been included and/or a larger number of patients had attended the 6-year control cannot be definitely ruled out. In fact, a power calculation based on the per-protocol study population, using CAL gain at 6 years as the primary outcome variable, showed a power of 77% (an error probability set at 0.05).

In the present study, only a small number of patients treated with OFD attended the 6-year follow-up control. The reason for this high drop-out (60%) cannot be identified, but one might think that only patients experiencing some problems with the treated tooth were interested in attending. In fact, an average CAL loss of 1.2 mm compared with baseline values was observed in those five patients; nevertheless, the information on the OFD group was deemed limited (Oxford Centre for Evidence Based Medicine 2009), and no further statistical evaluation was performed. On the other hand, the lower drop-out rate observed in the GTR groups could be due to the awareness of those patients that a ''special'' (i.e. regenerative) treatment was administered.

In conclusion, the findings of the present study suggest that the improvements in periodontal conditions obtained after GTR treatment in intrabony defects, with or without the adjunct use of DBBM, can be preserved on a long-term basis.

References

Araujo, M. G., Sonohara, M., Hayacibara, R., Cardaropoli, G. & Lindhe, J. (2002) Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. *Journal of Clinical Periodontology* **29**, 1122–1131.

Becker, W. & Becker, B. E. (1993) Treatment of mandibular 3-wall intrabony defects by flap debridement and expanded polytetrafluoroethylene barrier membranes. Long-term evaluation of 32 treated patients. *Journal of Periodontology* **64**, 1138–1144.

Camargo, P. M., Lekovic, V., Weinlaender, M., Nedic, M., Vasilic, N., 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., 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.

Carmagnola, D., Adriaens, P. & Berglundh, T. (2003) Healing of human extraction sockets filled with Bio-Oss. *Clinical Oral Implants Research* **14**, 137–143.

Carmagnola, D., Berglundh, T. & Lindhe, J. (2002) The effect of a fibrin glue on the integration of Bio-Oss with bone tissue. A experimental study in labrador dogs. *Journal of Clinical Periodontology* **29**, 377–383.

Cortellini, P., Paolo, G., Prato, P. & Tonetti, M. S. (1996) Long-term stability of clinical attachment following guided tissue regeneration and conventional therapy. *Journal of Clinical Periodontology* **23**, 106–111.

Cortellini, P., Pini-Prato, G. & Tonetti, M. (1994) Periodontal regeneration of human infrabony defects (V). Effect of oral hygiene on long-term stability. *Journal of Clinical Periodontology* **21**, 606–610.

Cortellini, P., Stalpers, G., Pini, P. G. & Tonetti, M. S. (1999) Long-term clinical outcomes of abutments treated with guided tissue regeneration. *Journal of Prosthetic Dentistry* **81**, 305–311.

Cortellini, P. & Tonetti, M. S. (2004) Long-term tooth survival following regenerative treatment of intrabony defects. *Journal of Periodontology* **75**, 672–678.

De Sanctis, M. & Zucchelli, G. (2000) Interleukin-1 gene polymorphisms and long-term stability following guided tissue regeneration therapy. *Journal of Periodontology* **71**, 606–613.

Gottlow, J., Nyman, S. & Karring, T. (1992) Maintenance of new attachment gained through guided tissue regeneration. *Journal of Clinical Periodontology* **19**, 315–317.

Holck, D. E., Dutton, J. J., Proia, A., Khawly, J., Mittra, R., Dev, S. & Imami, N. (1998) Rate of vascularization of coralline hydroxyapatite spherical implants pretreated with saline/gentamicin, rTGF-beta 2, and autogenous plasma. *Ophthalmic Plastic and Reconstructive Surgery* **14**, 73–80.

Kim, T. S., Holle, R., Hausmann, E. & Eickholz, P. (2002) Long-term results of guided tissue regeneration therapy with non- resorbable and bioabsorbable barriers. II. A case series of infrabony defects. *Journal of Periodontology* **73**, 450–459.

Lundgren, D. & Slotte, C. (1999) Reconstruction of anatomically complicated periodontal defects using a bioresorbable GTR barrier supported by bone mineral. A 6-month follow-up study of 6 cases. *Journal of Clinical Periodontology* **26**, 56–62.

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, J. C. (2003) Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A systematic review. *Annals of Periodontology* **8**, 266–302.

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.

Oxford Centre for Evidence Based Medicine (2009) Levels of Evidence – March 2009. Available at http://www.cebm.net/index.aspx?o=1025 (accessed 14 October 2009).

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.

Paolantonio, M., Scarano, A., di, P. G., Tumini, V., d'Archivio, D. & Piattelli, A. (2001) Periodontal healing in humans using anorganic bovine bone and bovine peritoneum-derived collagen membrane: a clinical and histologic case report. *International Journal of Periodontics and Restorative Dentistry* **21**, 505–515.

Polimeni, G., Koo, K. T., Qahash, M., Xiropaidis, A. V., Albandar, J. M. & Wikesjo, U. M. (2004) Prognostic factors for alveolar regeneration: bone formation at teeth and titanium implants. *Journal of Clinical Periodontology* **31**, 927–932.

Rhinelander, F. W. & Wilson, J. W. (1982) Blood supply to developing, mature and healing bone. In: Sumner-Smith, G. (ed). *Bone in Clinical Orthopaedics*, pp. 81–158. Philadelphia, USA: W. B. Saunders.

Sakata, J., Abe, H., Ohazama, A., Okubo, K., Nagashima, C., Suzuki, M. & Hasegawa, K. (2006) Effects of combined treatment with porous bovine inorganic bone grafts and bilayer porcine collagen membrane on refractory one-wall intrabony defects. *International Journal of Periodontics and Restorative Dentistry* **26**, 161–169.

Schlegel, A. K. & Donath, K. (1998) BIO-OSS – a resorbable bone substitute? *Journal of Long Term Effects of Medical Implants* **8**, 201–209.

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.

Sculean, A., Chiantella, G. C., Arweiler, N. B., Becker, J., Schwarz, F. & Stavropoulos, A. (2008a) Five-year clinical and histologic results following treatment of human intrabony defects with an enamel matrix derivative combined with a natural bone mineral. *International Journal of Periodontics and Restorative Dentistry* **28**, 153–161.

Sculean, A., Donos, N., Miliauskaite, A., Arweiler, N. & Brecx, M. (2001) Treatment of intrabony defects with enamel matrix proteins or bioabsorbable membranes. A 4-year follow-up split-mouth study. *Journal of Periodontology* **72**, 1695–1701.

Sculean, A., Nikolidakis, D. & Schwarz, F. (2008b) Regeneration of periodontal tissues: combinations of barrier membranes and grafting materials – biological foundation and preclinical evidence: a systematic review. *Journal of Clinical Periodontology* **35**, 106–116.

Sculean, A., Schwarz, F., Chiantella, G. C., Donos, N., Arweiler, N. B., Brecx, M. & Becker, J. (2007) Five-year results of a prospective, randomized, controlled study evaluating treatment of intra-bony defects with a natural bone mineral and GTR. *Journal of Clinical Periodontology* **34**, 72–77.

Sculean, A., Stavropoulos, A., Windisch, P., Keglevich, T., Karring, T. & Gera, I. (2004) Healing of human intrabony defects following regenerative periodontal therapy with a bovine-derived xenograft and guided tissue regeneration. *Clinical Oral Investigations* **8**, 70–74.

Slotte, C., Asklow, B. & Lundgren, D. (2007) Surgical guided tissue regeneration treatment of advanced periodontal defects: a 5-year follow-up study. *Journal of Clinical Periodontology* **34**, 977–984.

Stavropoulos, A. (2002) Guided tissue regeneration in combination with deproteinized bovine bone and gentamicin. PhD Thesis, p. 163, Aarhus, University of Aarhus, Denmark.

Stavropoulos, A., Karring, E. S., Kostopoulos, L. & Karring, T. (2003a) 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.

Stavropoulos, A. & Karring, T. (2004) Long-term stability of periodontal conditions achieved following guided tissue regeneration with bioresorbable membranes: case series results after 6-7 years. *Journal of Clinical Periodontology* **31**, 939–944.

Stavropoulos, A. & Karring, T. (2005) Five-year results of guided tissue regeneration in combination with deproteinized bovine bone (Bio-Oss) in the treatment of intrabony periodontal defects: a case series report. *Clinical Oral Investigations* **9**, 271–277.

Stavropoulos, A., Kostopoulos, L., Mardas, N., Nyengaard, J. R. & Karring, T. (2001) Deproteinized bovine bone used as an adjunct to guided bone augmentation: an experimental study in the rat. *Clinical Implant Dentistry and Related Research* **3**, 156–165.

Stavropoulos, A., Kostopoulos, L., Mardas, N., Nyengaard, J. R. & Karring, T. (2003b) Gentamicin used as an adjunct to GTR. *Journal of Clinical Periodontology* **30**, 455–462.

Stavropoulos, A., Kostopoulos, L., Nyengaard, J. R. & Karring, T. (2003c) Deproteinized bovine bone (Bio-Oss®) and bioactive glass (Biogran®) arrest bone formation when used as an adjunct to guided tissue regeneration (GTR). An experimental study in the rat. *Journal of Clinical Periodontology* **30**, 636–643.

Stavropoulos, A., Kostopoulos, L., Nyengaard, J. R. & Karring, T. (2004a) Fate of bone formed by guided tissue regeneration with or without grafting of Bio-Oss or Biogran. An experimental study in the rat. *Journal of Clinical Periodontology* **31**, 30–39.

Stavropoulos, A., Sculean, A. & Karring, T. (2004b) GTR treatment of intrabony defects with PLA/PGA copolymer or collagen bioresorbable membranes in combination with deproteinized bovine bone (Bio-Oss). *Clinical Oral Investigations* **8**, 226–232.

Tonetti, M. S., Cortellini, P., Lang, N. P., Suvan, J. E., Adriaens, P., Dubravec, D., Fonzar, A., Fourmousis, I., Rasperini, G., Rossi, R., Silvestri, M., Topoll, H., Wallkamm, B. & Zybutz, M. (2004) Clinical outcomes following treatment of human intrabony defects with GTR/bone replacement material or access flap alone. A multicenter randomized controlled clinical trial. *Journal of Clinical Periodontology* **31**, 770–776.

Weigel, C., Bragger, U., Hammerle, C. H., Mombelli, A. & Lang, N. P. (1995) Maintenance of new attachment 1 and 4 years following guided tissue regeneration (GTR). *Journal of Clinical Periodontology* **22**, 661–669.

Wikesjo, U. M. & Selvig, K. A. (1999) Periodontal wound healing and regeneration. *Periodontology 2000* **19**, 21–39.

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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Clinical Relevance

Scientific rationale for the study: Limited information exists on the long-term clinical outcomes of GTR combined with DBBM implantation in the treatment of periodontal intrabony defects.

Principal findings: The clinical improvements (i.e. PD reduction, CAL gain, radiographic defect resolution) observed 1 year after treatment remained basically unchanged over 5 additional years.

Only relatively few sites lost part of the CAL gain during follow-up, and almost half of the sites showed $\geqslant 4$ mm CAL gain 6 years after treatment. No inter-group differences in disease recurrence were observed at 6 years postsurgery.

Practical implications: GTR combined with DBBM in the treatment of intrabony defects results in improved clinical conditions that can be maintained on a long-term basis.