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Influence of autogenous platelet concentrate on combined GTR/graft therapy in intrabony defects: a 7-year follow-up of a randomized prospective clinical split-mouth study

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Abstract

Objectives: To investigate the influence of autogenous platelet concentrate (APC) on the long-term regeneration outcome 7 years after guided tissue regeneration (GTR) in deep intrabony periodontal defects.

Material and Methods: In 25 patients, two deep contra-lateral intrabony defects were treated according to GTR (randomized split-mouth-design). In the test defects, APC was additionally applied. After 7 years, healing results were assessed clinically by a blinded examiner and compared to baseline and 12-months results. Furthermore, a tooth survival analysis was performed.

Results: After 7 years, 23 patients were available for survival analysis and 16 patients for split-mouth analysis; 84% of the test and control teeth were still *in situ*. In both groups, the median attachment level of 10.5 mm [(25/75%): test 9.0/13.0, control 10.0/12.0] at baseline was significantly ($p \leq 0.05$) reduced to 6.0 mm [test 4.0/6.8, control 5.0/7.0] after 1 year. Six years later, it had increased again to 7.0 mm in test sites [5.3/10.0] ($p \leq 0.05$) and had remained stable in control sites [5.0/7.8] ($p > 0.05$). Bleeding on Probing (BOP) had increased in both groups. During the last 6 years, only 26% of the patients received a structured supportive periodontal therapy in the clinic.

Conclusion: Within its limitations, the present study indicates that the clinical outcome of GTR therapy can be maintained over 7 years. However, the additional use of APC may even have a possibly negative influence on the long-term stability.

Key words: guided tissue regeneration; intrabony defects; long-term results; membranes; periodontal disease/surgery; platelets; tricalcium phosphate

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Conflict of interest and source of funding statement

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Guided tissue regeneration (GTR) with bioresorbable cell-occlusive membranes has become a widely accepted method in periodontal

therapy to restore at least in part the periodontal tissues in intrabony defects (Christgau et al. 1998, 2002, Cortellini & Tonetti 2000, 2004, Murphy & Gunsolley 2003, Sculean et al. 2005, 2008b). Histological studies in animals and humans (Nyman et al. 1982, Isidor et al. 1985, Caffesse et al. 1988, Sculean et al. 1999, Christgau et al. 2007) have shown the formation of new cementum, new alveolar bone and new periodontal ligament. Additionally, numerous clinical studies showed that GTR procedures result in clinical attachment gain being stable within the first years (Christgau et al. 2002, Mengel et al. 2006, Needleman et al. 2006, Sculean et al. 2007, Slotte et al. 2007). In a systematic review, Needleman et al. (2006) could calculate a mean additional attachment gain of 1.22 mm compared to open flap debridement after at least 1 year. Furthermore, Cortellini & Tonetti (2004) found in 175 patients with GTR therapy after an observation period of 2–16 years that in most of the cases clinical improvements could be maintained. Tooth survival was 96% after 10 years. Recently, other studies have confirmed these findings in smaller patient contingents (Stavropoulos & Karring 2004, Eickholz et al. 2007, Pretzl et al. 2008b, 2009, Sculean et al. 2008a, Nickles et al. 2009, Nygaard-Ostby et al. 2010).

A common problem of GTR therapy is the high variability and low predictability of the healing outcomes (MacNeil & Somerman 1999, Kornman & Robertson 2000). GTR membranes mainly function as a mechanical barrier to facilitate selective cell growth and to stabilize the blood clot in the defect area. Beside other factors, the healing outcome is dependent on the individual healing potential determined by the presence and amount of autogenous growth factors (MacNeil & Somerman 1999, Kornman & Robertson 2000), which influence significantly the course of soft tissue healing and tissue regeneration (Cortellini & Tonetti 2000, Kornman & Robertson 2000). In numerous histological and clinical studies the potential therapeutic influence of growth factor application on periodontal regeneration could be demonstrated (Dennisson et al. 1994, Giannobile et al. 1996, Howell et al. 1997, Gamal et al. 1998, Wikesjö et al. 1998, Gamal & Mailhot 2000, Mumford et al. 2001, Marcopoulou et al. 2003, Nevins et al. 2003, Papadopoulos et al. 2003, Dereka et al. 2006, Sarment et al. 2006, Sant'Ana et al. 2007, Shirakata et al. 2010). Especially the combinations of different growth factors were promising (Dennisson et al. 1994, Giannobile et al. 1996, Howell et al. 1997, Gamal et al. 1998, Marcopoulou et al. 2003, Sant'Ana et al. 2007).

In the past, an autogenous platelet concentrate (APC) was suggested as natural source of patient-own growth factors (Marx et al. 1998, Christgau et al. 2006a). The α-granules of the thrombocytes contain platelet-derived growth factor, transforming growth factor and insulin-like growth factor (Okuda et al. 2003, Christgau et al. 2006a). APC might have a positive influence on hard and soft tissue healing (Marx et al. 1998, Anitua 1999, Kassolis et al. 2000, Robiony et al. 2002, Maiorana et al. 2003, Kassolis & Reynolds 2005, Christgau et al. 2006b). Furthermore, several studies have investigated the potential influence of the adjunctive use of autogenous platelet concentrate in periodontal regeneration (Camargo et al. 2002, 2005, Christgau et al. 2006b, Döri et al. 2007a, Yassibag-Berkman et al. 2007). While some studies reported beneficial effects of the combined use of APC and bone grafts compared to bone grafts alone (Hanna et al. 2004, Okuda et al. 2005), other papers failed to show an additional effect (Demir et al. 2007, Döri et al. 2008b, 2009). Some further studies of Döri et al. (2007a,b, 2008a) investigated the influence of APC on GTR therapy with different materials. They could not show beneficial effects of APC. In our group, the short-term influence of APC on early wound healing and regeneration outcomes following GTR therapy with β-tricalcium phosphate (β-TCP) granules (Ceros; Mathys, Bettlach, Switzerland) and a synthetic bioresorbable membrane (Resolut XT; Gore medical, Flagstaff, AZ, USA) were investigated clinically and radiographically (Christgau et al. 2006a,b). Apart from less post-operative membrane exposures and an initially significantly higher bone density gain in the test defects after 6 months, the additional application of APC had no relevant effect on the periodontal regeneration outcomes after 6 and 12 months (Christgau et al. 2006b).

Although the data in the literature provide very heterogeneous results on regenerative treatment strategies using different growth factors, many authors have put a lot of enthusiasm into their work and concluded that it is just a matter of time until growth factors reach the state of wide therapeutic application in humans (Bosshardt & Sculean 2000). However, to date only short-term clinical data are available. No information about the long-term stability is existing yet. Therefore, the objective of the present study was to provide long-term results after the additional application of APC during GTR therapy in deep intrabony defects by (i) analysing clinical data and (ii) performing a tooth survival analysis.

Material and Methods

Study design

The present study is a 7-year follow-up of a controlled randomized prospective clinical split-mouth study investigating the additional influence of autogenous platelet concentrate (APC) on the early wound healing events and regeneration outcomes in deep intrabony periodontal defects following guided tissue regeneration (GTR) (Christgau et al. 2006b). The study design followed the requirements outlined in the CONSORT statement (Needleman et al. 2008). The study protocol was approved by the ethics committee of the Medical Faculty of the University of Regensburg in accordance with the Declarations of Helsinki (1975) and Tokyo (1983). All patients received a detailed description of the proposed treatment for informed consent.

Patient selection

All patients had one pair of contralaterally located deep interproximal, intrabony periodontal defects with a probing pocket depth (PPD) of at least 6 mm and radiographic evidence of angular bone loss of at least 4 mm at baseline. None of the teeth showed a furcation involvement. Full mouth

supra- and subgingival scaling and root planing as well as splinting of extremely mobile teeth had to be successfully completed at least 4 to 6 weeks before surgery. None of the patients had systemic diseases with a possibly negative influence on the healing outcome.

Clinical therapeutic procedure

The APC was prepared as described previously (Christgau et al. 2006b). Briefly, the APCs were prepared at the Division of Transfusion Medicine at the University Clinic of Regensburg using an apheresis technique. For clinical use, 2.5 ml platelet concentrate was reactivated with 0.5 ml of a sterile 10% calcium chloride solution. All surgical interventions were performed by one experienced surgeon (M.C.) according to the principles of GTR and have been described elsewhere (Christgau et al. 2006b). The treatment allocation (test and control) was performed by using a computer generated randomization table. After thorough defect debridement, the selected test site was treated in the following way: root surfaces were conditioned using 24% EDTA gel (Prefgel; Straumann, Freiburg, Germany) for 2 min. Following rinsing with sterile 0.9% NaCl solution, reactivated APC (Christgau et al. 2006b) was applied to the root surfaces. Then the defect was filled with β-TCP granules (Ceros, Mathys) which were soaked with APC and covered with a synthetic bioresorbable GTR membrane (Resolut XT; Gore medical). Finally the wound was closed tension-free by a coronally re-positioned flap. Control sites were treated in the same way without application of APC. The β-TCP granules were soaked with blood taken from the defect region. The post-operative procedures have been described previously (Christgau et al. 2006b).

Within the first post-operative year, all patients were included in a strict supportive periodontal therapy (SPT) programme in the Department of Operative Dentistry and Periodontology of the University of Regensburg with visits every 3 months. Afterwards, the further participation in the SPT programme was recommended, but the great majority of the patients returned to the referring dentist. The SPT of the other patients was carried out twice a year in the undergraduate programme in the dental school.

Clinical examination

Clinical examination was performed by a blinded examiner, who had no knowledge about the treatment modality used in the individual defects. The clinical parameters were recorded immediately before as well as 1 and 7 years after surgery: Approximal Plaque Index (API) (Lange et al. 1977), Papillary Bleeding Index (PBI) (Saxer & Mühlemann 1975) and Bleeding on Probing (BOP). The following clinical parameters were recorded with a pressure calibrated probe: the gingival recession (REC) as the distance between the cemento-enamel junction (CEJ) or the margin of a restauration to the gingival margin, the PPD as the distance from the gingival margin to the fundus of the periodontal pocket and the clinical attachment level (CAL) as the distance from the CEJ or the margin of a restauration to the fundus of the periodontal pocket. CAL change was chosen as the primary outcome variable. Furthermore, the Vertical relative Attachment Gain (V-rAG) was calculated as the percentage of the CAL gain related to the osseous defect depths (Christgau et al. 2006b).

Compliance

For evaluating the compliance of the patients, the frequencies of participation in SPT were calculated. A patient who complied with at least one visit per year at the Department of Operative Dentistry and Periodontology of the University of Regensburg was classified to have regular SPT (Eickholz et al. 2007, Pretzl et al. 2009). Patients who failed at a maximum of 1 year during the whole 7 year period were termed to have irregular SPT. A patient failing more often was classified to have no SPT in the University Clinic.

Data analysis

In this study, the single patient was regarded as the evaluation unit. As previously discussed (Christgau et al. 1997), clinical measurements were expressed as median values (with 25 and 75 percentiles) and were statistically evaluated using a non-parametric test. Taking into account the paired nature of the data, the Wilcoxon-Signed-Rank test was used on a significance level of $\alpha = 0.05$. (Woolson 1987, Page et al. 1995).

Results

All initially recruited 25 patients received the intended treatment and completed the 1 year observation period (baseline to 12 months) (Fig. 1). After 7 years, two patients had terminated their participation without explaining their reasons. Nineteen of the residual 23 patients could be scheduled for clinical examination, four patients were only available for an oral interview by telephone. For pair-wise split-mouth analysis only 16 patients were suitable due to tooth extractions. A survival analysis could be performed in 23 patients (Fig. 1).

SPT

The calculation for SPT frequencies was carried out on basis of the dental charts of 23 patients; 26% of the patients had participated regularly in SPT in the clinic, 17% participated irregularly in SPT and 57% received no SPT at the University clinic. Tooth extraction was not associated with the frequencies in supportive periodontal therapy (Table 1).

Tooth survival analysis

A tooth survival analysis could be performed in 23 patients. After 7 years, eight teeth in six patients had been extracted by the referring dentists because of prosthodontic reasons, four teeth in each group. Accordingly, 84% of the teeth were still *in situ*. The frequency distribution of different baseline parameters in relation to the tooth loss was calculated (Table 2). No association of tooth loss could be found with regard to tooth type, CAL at baseline or defect categories.

Clinical healing results

Concerning the time course, the power between baseline and the 7-year outcome was calculated as

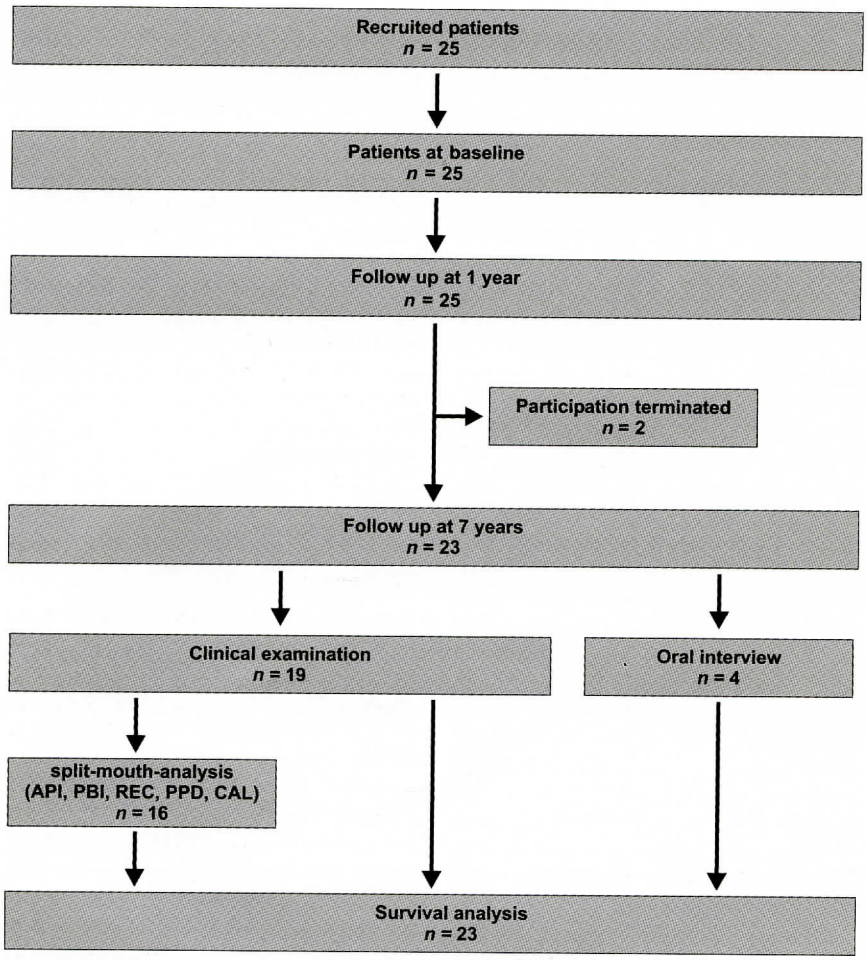

Fig. 1. Flow Chart of the study outline.

Table 1. Frequency distribution of patients with lost teeth according to the frequencies in supportive periodontal therapy (SPT)

	n	%
Regular SPT	3	50
Irregular SPT	1	16.7
No SPT	2	33.3
Total	6	100

n, number of patients; SPT, supportive periodontal therapy.

93.5%. However, considering CAL changes between baseline and 7 years, the power dropped to 25.8% when calculating differences between test and control sites.

For the split-mouth analysis, the clinical data of 16 patients were available. The results regarding the clinical evaluation are summarized in Tables 3–6.

The median full mouth API increased from 10.0% at baseline to 13.5% after 1 year and 14.5% after 7 years. On the other hand, the median full mouth PBI started with 12.0% at baseline and decreased to 10.0% after 1 year and 9.0% after 7 years. These differences were not significantly different.

At baseline, the median PBI at the surgical sites was 1.0 in both test and control sites, decreased to 0.0 at the 1 year visit and remained stable after 7 years. The median BOP at baseline was negative in the test and positive in the control group. After 1 year, the median BOP was negative in both groups after 1 year and positive after 7 years.

Both defect groups revealed a median baseline PPD of 10.0 mm, which decreased during the first year by 6.5 mm (test) and 6.0 mm (control) to 3.0 mm (test) and 4.0 mm (control). After 7 years, median PPD had increased again in the test site by 2.0mm to 5.0mm and had remained stable in the control sites. The 7 year data differed significantly between the test and control defects. The median REC started at baseline with 0.5 mm (test) and 1.0 mm (control) and increased to 3.0 mm after 1 and 7 years in both groups. At baseline, the median CAL was 10.5 mm in both groups and decreased within the first 12 months by 5.0mm to 6.0mm. At the 7 year visit, the median CAL had increased by 2.0mm to 7.0mm in the test sites and remained stable in the control group. The vertical relative attachment gain after 1 year was 75.0% in the test and 66.7% in the control group and decreased to 50.0% in the test and 64.6% in the control group after 7 years. The changes in PPD and CAL as well as in the vertical relative attachment gain between 1 and 7 years differed significantly between test and control sites.

Discussion

The present study investigated the long-term healing outcomes 7 years after GTR in combination with an autogenous platelet concentrate. To the best of our knowledge, this is the first long-term evaluation on this topic. The present study design allowed the assessment of the isolated effect of APC on the periodontal regeneration.

Compliance and SPT

It is known from the literature, that regular supportive periodontal therapy is essential for long-term therapeutic success in periodontal therapy (Axelsson & Lindhe 1981, Axelsson et al. 2004, Leininger et al. 2010). In the present study, between the 1-year and the 7-year examination, only 26% of the patients participated regularly in SPT; 17% participated irregularly in SPT and 57% received no SPT in the University Clinic during this time period. It is a rather small number of patients who could be classified as compliant. According to the literature, in the year 1983, only 16% of the patients complied with the recommended maintenance schedules (Wilson et al. 1984). In the recent years, much higher frequencies could be calculated. In the study of Pretzl et al. (2009) 9 of 11 patients (82%) participated regularly in SPT during 10 years. From the

Table 2. Frequency distribution of tooth loss in test and control sites according to the tooth type, clinical attachment loss (CAL) at baseline and defect categories

	Test sites		Control sites	
	n	%	*n*	%
Tooth type				
Incisors, canines	2	50	1	25
Premolars	2	50	0	0
Molars	0	0	3	75
Total	4	100	4	100
CAL at baseline				
9 mm	0	0	0	0
10 mm	1	25	1	25
11 mm	0	0	0	0
12 mm	1	25	1	25
13 mm	2	50	1	25
14 mm	0	0	1	25
15 mm	0	0	0	0
Total	4	100	4	100
Defect categories				
1-wall	0	0	0	0
2-wall	1	25	1	25
3-wall	0	0	3	75
Combined 1-/2-wall	0	0	0	0
Combined 1-/3-wall	1	25	0	0
Combined 2-/3-wall	1	25	0	0
Combined 1-/2-/3-wall	1	25	0	0
Total	4	100	4	100

n, number of defects; CAL, clinical attachment loss.

same group, Eickholz et al. (2007) reported only 6 of 50 patients (12%) terminating SPT. Nevertheless, due to the split-mouth design, test as well as control defects benefit from SPT in the same way. However, the main influence factors for the healing outcomes seem to be site specific rather than patient specific (Cortellini et al. 1996b).

In the present study, after the 1-year examination, the majority of the patients returned to their referring dentists. This has to be seen in the geographical context of Regensburg. The catchment area of the University Clinic of Regensburg is rather wide spread. Therefore, the patients have to accept long ways and high additional costs to reach the University Clinic. Thus, most of them preferred their local dentists and the compliance in participating regularly in SPT at the University Clinic declined. Returning to the referring dentists did not necessarily mean that professional maintenance was insufficient, but could not be controlled by the investigators. However, in this context it was interesting, that the parameters describing the quality of oral hygiene (API, PBI) remain stable during this time period.

Clinical healing results

One of the objectives of the present study was to evaluate the long-term stability of the treatment outcomes following GTR therapy in deep intrabony periodontal defects. Several studies in a meta-analysis have revealed favourable clinical results 12 months after GTR therapy with bioresorbable membranes in intrabony defects (Murphy & Gunsolley 2003). The mean clinical attachment gain ranged from 1.5 to 4.6 mm (Cortellini et al. 1996a, Mayfield et al. 1998). Follow-up studies dealing with results 5 years post-operatively, showed slightly reduced mean clinical attachment gains in a range

Table 3. Median value (with 25/75-percentiles) of the papillary bleeding index (PBI) at the surgical sites, the bleeding on probing (BOP) at the surgical sites, the gingival recession (REC), probing pocket depth (PPD) and the clinical attachment loss (CAL) at baseline and after 1 and 7 years

	Test sites (n = 16)					Control sites (n = 16)				
	PBI	BOP	REC [mm]	PPD [mm]	CAL [mm]	PBI	BOP	REC [mm]	PPD [mm]	CAL [mm]
Baseline										
Median	1.0¶	0.0¶	0.5¶, §	10.0¶, §	10.5¶, §	1.0	1.0	1.0¶, §	10.0¶, §	10.5¶, §
25%	0.0	0.0	0.0	9.0	9.0	0.0	0.0	0.0	9.0	10.0
75%	1.0	1.0	3.0	10.0	13.0	2.0	1.0	2.0	10.0	12.0
1 year										
median	0.0¶	0.0¶, **, *	3.0¶	3.0¶, **	6.0¶, **	0.0	0.0**, *	3.0¶	4.0¶	6.0¶
25%	0.0	0.0	0.3	3.0	4.0	0.0	0.0	1.3	3.0	5.0
75%	0.8	0.0	4.8	4.0	6.8	1.0	1.0	3.0	4.0	7.0
7 years										
median	0.0	1.0**	3.0§	5.0§, **, *	7.0§, **	0.0	1.0**	3.0§	4.0§, *	6.0§
25%	0.0	0.0	1.0	4.0	5.3	0.0	0.3	1.0	3.0	5.0
75%	0.8	1.0	4.8	7.0	10.0	0.0	1.0	4.0	4.8	7.8

n, number of split-mouth defects; BOP: 0 = negative; 1 = positive.
*Statistically significant difference between test and control ($p \leq 0.05$).
¶Statistically significant difference between baseline and 1 year ($p \leq 0.05$).
§Statistically significant difference between baseline and 7 years ($p \leq 0.05$).
**Statistically significant difference between 1 and 7 years ($p \leq 0.05$).

Table 4. Median value (with 25/75-percentiles) of the changes in gingival recession (ΔREC), probing pocket depth (ΔPPD), clinical attachment loss (ΔCAL) and vertical relative attachment gain (V-rAG) after 1 and 7 years

	Test sites (n = 16)				Control sites (n = 16)			
	ΔREC [mm]	ΔPPD [mm]	ΔCAL [mm]	V-rAG [%]	ΔREC [mm]	ΔPPD [mm]	ΔCAL [mm]	V-rAG [%]
BL – 1 year								
median	−1.0	6.5¶, §	5.0¶, §	75.0¶, §	−1.0	6.0§	5.0§	66.7§
25%	−2.0	5.3	4.3	67.9	−2.0	6.0	4.0	51.4
75%	0.0	7.0	6.0	95.8	0.0	7.0	6.0	87.5
BL – 7 years								
median	−1.0**	4.5¶, **, *	3.5¶, **	50.0¶, **	−1.0**	5.5**, *	5.0**	64.6**
25%	−3.0	3.0	2.0	33.3	−2.0	5.0	3.3	38.1
75%	−1.0	5.0	4.4	88.3	−1.0	6.0	6.0	95.8
1–7 years								
median	−1.0**	−2.0§, **, *	−2.0§, **, *	−25.0§, **, *	0.0**	−0.5§, **, *	0.0§,**,*	0.0§,**,*
25%	−1.8	−3.8	−3.8	−50.0	−1.0	−1.0	−2.0	−25.0
75%	0.8	−0.3	0.0	0.0	0.0	1.0	0.8	9.4

n, number of split-mouth defects; BL, baseline.
*Statistically significant difference between test and control ($p \leq 0.05$).
¶Statistically significant difference between (BL – 1 year) and (BL – 7 years) ($p \leq 0.05$).
§Statistically significant difference between (BL – 1 year) and (1 – 7 years) ($p \leq 0.05$).
**Statistically significant difference between (BL – 7 years) and (1 – 7 years) ($p \leq 0.05$).

Table 5. Frequency distribution of clinical attachment level changes in the different observation periods

	Test sites (n = 16)			Control sites (n = 16)		
	BL-1a	BL-7a	1a-7a	BL-1a	BL-7a	1a-7a
≤ −2 mm	–	–	9	–	1	5
0 ± 1 mm	–	2	7	–	–	9
2–3 mm	1	6	–	2	3	2
4–5 mm	10	6	–	9	7	–
≥ 6 mm	5	2	–	5	5	–
	16	16	16	16	16	16

n, number of split-mouth defects; BL, Baseline; 1a, 1 year observation point; 7a, 7 years observation point.

Table 6. Frequency distribution of residual pocket depths at the different time points

	Test sites (n = 16)			Control sites (n = 16)		
	BL	1a	7a	BL	1a	7a
≤ 2 mm	–	3	–	–	1	–
2–4 mm	–	11	6	–	12	12
5–6 mm	–	2	5	–	3	2
7–8 mm	1	–	3	2	–	1
9–10 mm	13	–	1	11	–	–
11–12mm	1	–	1	2	–	–
>12 mm	1	–	–	1	–	1
	16	16	16	16	16	16

n, number of split-mouth defects; BL, Baseline; 1a, 1 year observation point; 7a, 7 years observation point.

between 2.2 and 3.0 mm (Eickholz et al. 2004, Mengel et al. 2006). Recent studies have been published, which presented long-term results of GTR therapy with bioresorbable membranes in intrabony defects (Stavropoulos & Karring 2004, Pretzl et al. 2008b, 2009, Sculean et al. 2008a, Nygaard-Ostby et al. 2010). After up to 10 years, mean clinical attachment gains were 2.4–3.8 mm (Pretzl et al. 2009, Nygaard-Ostby et al. 2010). In the present study, 7 years after therapeutic treatment, a median attachment gain of 3.5 mm (test sites) and 5.0 mm (control sites) could be observed. Although the 7 year results are located at the upper border compared to other studies, they were reduced compared to the 1 year data (median ΔCAL 5.0 mm). While the literature reports a new attachment loss of 0.0–1.6 mm between 1 and 5 years post-operatively (Eickholz et al. 2004, Sculean et al. 2004), the present data showed a median CAL loss between 1 and 7 years of 0.0 mm (control sites) to 2.0 mm (test sites) (Table 2). The new clinical attachment loss in the test sites is greater than in other studies over a period of 10 years: 0.2 –1.62 mm (Pretzl et al. 2008b, 2009, Nickles et al. 2009). The changes in PPD and CAL as well as the vertical relative attachment gain between 1 and 7 years differed significantly between test and control sites.

Tooth survival analysis

The reasons for tooth extraction are multiple (Avila et al. 2009). In the present study the decision for tooth maintenance or extraction was made by the referring dentists. It is only known, that the reasons for tooth extraction were based on prosthodontic and not on periodontal considerations. In this sense, the extracted teeth are not associated with classical periodontal tooth-related

risk factors (Tonetti et al. 1993). Especially the fact that three teeth with three-wall defects had been extracted was surprising because it was shown by Schallhorn et al. (1970) that the number of residual walls was related to the clinical outcomes. In contrast, many studies demonstrated no relation to the defect configuration in terms of one-wall, two-wall or three-wall subcomponents (Tonetti et al. 1993, 1996). Correspondingly, it was surprising that three of six patients who lost teeth within the 7 years participated regularly in SPT until tooth extraction because typically regular supportive periodontal therapy is a key factor for the long-term success in periodontal therapy (Axelsson & Lindhe 1981, Axelsson et al. 2004, Leininger et al. 2010).

Influence of APC

Interestingly, the increase in probing pocket depth and clinical attachment loss as well as the decrease in the vertical relative attachment gain from 1 to 7 years differs significantly between the test and the control sites. The test sites provided worse healing outcomes than the control sites after 7 years. The baseline characteristics of the test sites were not significantly different from the control sites and due to the split-mouth design no systemic influences have to be expected. Nevertheless, the factors influencing the healing outcomes are multiple (Tonetti et al. 1993, Pretzl et al. 2008a). Although one might have expected positive effects of APC, already in the original study (Christgau et al. 2006b) only minor effects of APC on the wound healing could be observed. No noticeable influences of APC had been detected after 1 year. Similarly, Döri et al. (2007a,b) also found no additional effect of APC.

To date, it is not explainable, why the additional use of APC could have a possibly negative effect on the long-term healing results. Further long-term studies are required to elucidate and confirm this problem.

Conclusion

Within its limitations, the present study has shown that in the majority of defects the new attachment gain after GTR therapy could be maintained over a 7 year period, although some new attachment loss had to be observed. The additional use of APC did not reveal any significant advantages; in contrast, the 7 year data indicate a possibly worse long-term stability compared to GTR therapy alone.

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References

Anitua, E. (1999) Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *International Journal of Oral and Maxillofacial Implants* **14**, 529–535.

Avila, G., Galindo-Moreno, P., Soehren, S., Misch, C. E., Morelli, T. & Wang, H. L. (2009) A novel decision-making process for tooth retention or extraction. *Journal of Periodontology* **80**, 476–491.

Axelsson, P. & Lindhe, J. (1981) Effect of controlled oral hygiene procedures on caries and periodontal disease in adults. Results after 6 years. *Journal of Clinical Periodontology* **8**, 239–248.

Axelsson, P., Nystrom, B. & Lindhe, J. (2004) The long-term effect of a plaque control program on tooth mortality, caries and periodontal disease in adults. Results after 30 years of maintenance. *Journal of Clinical Periodontology* **31**, 749–757.

Bosshardt, D. D. & Sculean, A. (2000) Does periodontal tissue regeneration really work? *Periodontology 2000* **51**, 208–219.

Caffesse, R. G., Smith, B. A., Castelli, W. A. & Nasjleti, C. E. (1988) New attachment achieved by guided tissue regeneration in beagle dogs. *Journal of Periodontology* **59**, 589–594.

Camargo, P. M., Lekovic, V., Weinlaender, M., Vasilic, N., Madzarevic, M. & Kenney, E. B. (2002) Platelet-rich plasma and bovine porous bone mineral combined with guided tissue regeneration in the treatment of intrabony defects in humans. *Journal of Periodontal Research* **37**, 300–306.

Camargo, P. M., Leković, V., Weinlaender, M., Vasilic, N., Madzarevic, M. & Kenney, E. B. (2005) A reentry study on the use of bovine porous bone mineral, GTR, and platelet-rich plasma in the regenerative treatment of intrabony defects in humans. *Int J Periodontics Restorative Dent* **25**, 49–59.

Christgau, M., Bader, N., Felden, A., Gradl, J., Wenzel, A. & Schmalz, G. (2002) Guided tissue regeneration in intrabony defects using an experimental bioresorbable polydioxanon (PDS) membrane. A 24-month split-mouth study. *Journal of Clinical Periodontology* **29**, 710–723.

Christgau, M., Bader, N., Schmalz, G., Hiller, K. A. & Wenzel, A. (1998) GTR therapy of intrabony defects using 2 different bioresorbable membranes: 12-month results. *Journal of Clinical Periodontology* **25**, 499–509.

Christgau, M., Caffesse, R. G., Schmalz, G. & D'Souza, R. N. (2007) Extracellular matrix expression and periodontal wound-healing dynamics following guided tissue regeneration therapy in canine furcation defects. *Journal of Clinical Periodontology* **34**, 691–708.

Christgau, M., Moder, D., Hiller, K. A., Dada, A., Schmitz, G. & Schmalz, G. (2006a) Growth factors and cytokines in autologous platelet concentrate and their correlation to periodontal regeneration outcomes. *Journal of Clinical Periodontology* **33**, 837–845.

Christgau, M., Moder, D., Wagner, J., Glassl, M., Hiller, K. A., Wenzel, A. & Schmalz, G. (2006b) Influence of autologous platelet concentrate on healing in intra-bony defects following guided tissue regeneration therapy: a randomized prospective clinical split-mouth study. *Journal of Clinical Periodontology* **33**, 908–921.

Christgau, M., Schmalz, G., Wenzel, A. & Hiller, K. A. (1997) Periodontal regeneration of intrabony defects with resorbable and non-resorbable membranes: 30-month results. *Journal of Clinical Periodontology* **24**, 17–27.

Cortellini, P., Pini, P. G. & Tonetti, M. S. (1996a) Periodontal regeneration of human intrabony defects with bioresorbable membranes. A controlled clinical trial. *Journal of Periodontology* **67**, 217–223.

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

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

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

Demir, B., Sengun, D. & Berberoglu, A. (2007) Clinical evaluation of platelet-rich plasma and bioactive glass in the treatment of intra-bony defects. *Journal of Clinical Periodontology* **34**, 709–715.

Dennisson, D. K., Vallone, D. R., Pinero, G. J., Rittmann, B. & Caffesse, R. G. (1994) Differential effect of TGF-ß1 and PDGF on proliferation of periodontal ligament cells and gingival fibroblasts. *Journal of Periodontology* **65**, 641–648.

Dereka, X. E., Markopoulou, C. E. & Vrotsos, I. A. (2006) Role of growth factors on periodontal repair. *Growth Factors* **24**, 260–267.

Döri, F., Huszar, T., Nikolidakis, D., Arweiler, N. B., Gera, I. & Sculean, A. (2007a) Effect of platelet-rich plasma on the healing of intrabony defects treated with a natural bone mineral and a collagen membrane. *Journal of Clinical Periodontology* **34**, 254–261.

Döri, F., Huszar, T., Nikolidakis, D., Arweiler, N. B., Gera, I. & Sculean, A. (2007b) Effect of platelet-rich plasma on the healing of intrabony defects treated with an anorganic bovine bone mineral and expanded polytetrafluoroethylene membranes. *Journal of Periodontology* **78**, 983–990.

Döri, F., Huszar, T., Nikolidakis, D., Tihanyi, D., Horvath, A., Arweiler, N. B., Gera, I. & Sculean, A. (2008a) Effect of platelet-rich plasma on the healing of intrabony defects treated with Beta tricalcium phosphate and expanded polytetrafluoroethylene membranes. *Journal of Periodontology* **79**, 660–669.

Döri, F., Kovacs, V., Arweiler, N. B., Huszar, T., Gera, I., Nikolidakis, D. & Sculean, A. (2009) Effect of platelet-rich plasma on the healing of

intrabony defects treated with an anorganic bovine bone mineral: a pilot study. *Journal of Periodontology* **80**, 1599–1605.

Döri, F., Nikolidakis, D., Huszar, T., Arweiler, N. B., Gera, I. & Sculean, A. (2008b) Effect of platelet-rich plasma on the healing of intrabony defects treated with an enamel matrix protein derivative and a natural bone mineral. *Journal of Clinical Periodontology* **35**, 44–50.

Eickholz, P., Krigar, D. M., Kim, T. S., Reitmeir, P. & Rawlinson, A. (2007) Stability of clinical and radiographic results after guided tissue regeneration in infrabony defects. *Journal of Periodontology* **78**, 37–46.

Eickholz, P., Krigar, D. M., Pretzl, B., Steinbrenner, H., Dorfer, C. & Kim, T. S. (2004) Guided tissue regeneration with bioabsorbable barriers. II. Long-term results in infrabony defects. *Journal of Periodontology* **75**, 957–965.

Gamal, A. Y. & Mailhot, J. M. (2000) The effect of local delivery of PDGF-BB on attachment of human periodontal ligament fibroblaststo periodontitis-affected root surfaces - *in vitro*. *Journal of Clinical Periodontology* **27**, 347–353.

Gamal, A. Y., Mailhot, J. M., Garnick, J. J., Newhouse, R. & Sharawy, M. M. (1998) Human periodontal ligament fibroblast response to PDGF-BB and IGF-I application on tetracycline HCl conditioned root surfaces. *Journal of Clinical Periodontology* **15**, 404–412.

Giannobile, W. V., Hernandez, R. A., Finkelman, R. D., Ryan, S., Kiritsy, C. P., D'Andrea, M. & Lynch, S. E. (1996) Comparative effects of platelet-derived growth factor-BB and insulin-like growth factor-I, individually and in combination, on periodontal regeneration in Macaca fascicularis. *Journal of Periodontal Research* **31**, 301–312.

Hanna, R., Trejo, P. M. & Weltman, R. L. (2004) Treatment of intrabony defects with bovine-derived xenograft alone and in combination with platelet-rich plasma: a randomized clinical trial. *Journal of Periodontology* **75**, 1668–1677.

Howell, T. H., Fiorellini, J. P., Paquette, D. W., Offenbacher, S., Giannobile, W. V. & Lynch, S. E. (1997) A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth Factor-I in patients with periodontal disease. *Journal of Periodontology* **68**, 1186–1193.

Isidor, F., Karring, T., Nyman, S. & Lindhe, J. (1985) New attachment-reattachment following reconstructive periodontal surgery. *Journal of Clinical Periodontology* **12**, 728–735.

Kassolis, J. D. & Reynolds, M. A. (2005) Evaluation of the adjunctive benefits of platelet.rich plasma in subantral sinus augmentation. *J Craniofac Surg* **16**, 280–287.

Kassolis, J. D., Rosen, P. S. & Reynolds, M. A. (2000) Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: case series. *Journal of Periodontology* **71**, 1654–1661.

Kornman, K. S. & Robertson, P. B. (2000) Fundamental principles affecting the outcomes of therapy for osseous lesions. *Periodontology 2000* **22**, 22–43.

Lange, D. E., Plagmann, H. C., Eenboom, A. & Promesberger, A. (1977) Clinical methods for the objective evaluationof oral hygiene. *Deutsche Zahnärztliche Zeitschrift* **32**, 44–47.

Leininger, M., Tenenbaum, H. & Davideau, J. L. (2010) Modified periodontal risk assessment score: long-term predictive value of treatment outcomes. A retrospective study. *Journal of Clinical Periodontology* **37**, 427–435.

MacNeil, R. L. & Somerman, M. J. (1999) Development and regeneration of the periodontium: parallels and contrasts. *Periodontology 2000* **19**, 8–20.

Maiorana, C., Sommariva, L., Brivio, P., Sigurta, D. & Santoro, F. (2003) Maxillary sinus augmentation with anorganic bovine bone (BioOss) and autologous platelet-rich plasma: preliminary clinical and histologic evaluation. *International Journal of Periodontics & Restorative Dentistry* **23**, 227–235.

Marcopoulou, C. E., Vavouraki, H. N., Dereka, X. E. & Vrotsos, I. A. (2003) Proliferative effect of growth factors TGF-beta 1, PDGF-BB and rhBMP-2 on human gingival fibroblasts and periodontal ligament cells. *Journal of the International Academy of Periodontology* **5**, 63–70.

Marx, R. E., Carlson, E. R., Eichstaedt, R. M., Schimmele, S. R., Strauss, J. E. & Georgeff, K. R. (1998) Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* **85**, 638–646.

Mayfield, L., Soderholm, G., Hallstrom, H., Kullendorff, B., Edwardsson, S., Bratthall, G., Bragger, U. & Attstrom, R. (1998) Guided tissue regeneration for the treatment of intraosseous defects using a biabsorbable membrane. A controlled clinical study. *Journal of Clinical Periodontology* **25**, 585–595.

Mengel, R., Schreiber, D. & Flores-de-Jacoby, L. (2006) Bioabsorbable membrane and bioactive glass in the treatment of intrabony defects in patients with generalized aggressive periodontitis: results of a 5-year clinical and radiological study. *Journal of Periodontology* **77**, 1781–1787.

Mumford, J. H., Carnes, D. L., Cochran, D. L. & Oates, T. W. (2001) The Effects of Platelet-Derived Growth Factor-BB on Periodontal Cells in an *In Vitro* Wound Model. *Journal of Periodontology* **72**, 331–340.

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

Needleman, I. G., Worthington, H. V., Giedrys-Leeper, E. & Tucker, R. J. (2006) Guided tissue regeneration for periodontal infra-bony defects. *Cochrane Database Systematic Review* **19**, 1724.

Needleman, I., Worthington, H., Moher, D., Schulz, K. & Altman, D. G. (2008) Improving the completeness and transparency of reports of randomized trials in oral health: the CONSORT statement. *American Journal of Dentistry* **21**, 7–12.

Nevins, M., Camelo, M., Nevins, M. L., Schenk, R. K. & Lynch, S. E. (2003) Periodontal regeneration in humans using recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and allogenic bone. *Journal of Periodontology* **74**, 1282–1292.

Nickles, K., Ratka-Kruger, P., Neukranz, E., Raetzke, P. & Eickholz, P. (2009) Open flap debridement and guided tissue regeneration after 10 years in infrabony defects. *Journal of Clinical Periodontology* **36**, 976–983.

Nygaard-Ostby, P., Bakke, V., Nesdal, O., Susin, C. & Wikesjö, U. M. (2010) Periodontal healing following reconstructive surgery: effect of guided tissue regeneration using a bioresorbable barrier device when combined with autogenous bone grafting. A randomized-controlled trial 10-year follow-up. *Journal of Clinical Periodontology* **37**, 366–373.

Nyman, S., Lindhe, J., Karring, T. & Rylander, H. (1982) New attachment following surgical treatment of human periodontal disease. *Journal of Clinical Periodontology* **9**, 290–296.

Okuda, K., Kawase, T., Momose, M., Murata, M., Saito, Y., Suzuki, H., Wolff, L. F. & Yoshie, H. (2003) Platelet-rich plasma contains high levels of platelet-derived growth factor and transforming growth factor-beta and modulates the proliferation of periodontally related cells *in vitro*. *Journal of Periodontology* **74**, 849–857.

Okuda, K., Tai, H., Tanabe, K., Suzuki, H., Sato, T., Kawase, T., Saito, Y., Wolff, L. F. & Yoshiex, H. (2005) Platelet-rich plasma combined with a porous hydroxyapatite graft for the treatment of intrabony periodontal defects in humans: a comparative controlled clinical study. *Journal of Periodontology* **76**, 890–898.

Page, R. C., Armitage, G. C., DeRouen, T. A., Genco, R. J., Hujoel, P., Jeffcoat, M. K., Kornman, K. S. & Williams, R. C. (1995) *Design and conduct of clinical trials of products designed for the prevention, diagnosis, and herapy of periodontitis*, p. 14–23. Chicago: The Acadamy of periodontology.

Papadopoulos, C. E., Dereka, X. E., Vavouraki, E. N. & Vrotsos, I. A. (2003) *In vitro* evaluation of the mitogenic effect of platelet-derived growth factor-BB on human periodontal ligament cells cultured with various bone allografts. *Journal of Periodontology* **74**, 451–457.

Pretzl, B., Kaltschmitt, J., Kim, T. S., Reitmeir, P. & Eickholz, P. (2008a) Tooth loss after active periodontal therapy. 2: tooth-related factors. *Journal of Clinical Periodontology* **35**, 175–182.

Pretzl, B., Kim, T. S., Holle, R. & Eickholz, P. (2008b) Long-term results of guided tissue regeneration therapy with non-resorbable and bioabsorbable barriers. IV. A case series of infrabony defects after 10 years. *Journal of Periodontology* **79**, 1491–1499.

Pretzl, B., Kim, T. S., Steinbrenner, H., Dorfer, C., Himmer, K. & Eickholz, P. (2009) Guided tissue regeneration with bioabsorbable barriers III 10-year results in infrabony defects. *Journal of Clinical Periodontology* **36**, 349–356.

Robiony, M., Polini, F., Costa, F. & Politi, M. (2002) Osteogenesis distraction and platelet-rich plasma for bone restoration of the severely atrophic mandible: preliminary results. *Journal of Oral and Maxillofacial Surgery* **60**, 630–635.

Sant'Ana, A. C., Marques, M. M., Barroso, T. E., Passanezi, E. & de Rezende, M. L. (2007) Effects of TGF-beta1, PDGF-BB, and IGF-1 on the rate of proliferation and adhesion of a periodontal ligament cell lineage *in vitro*. *Journal of Periodontology* **78**, 2007–2017.

Sarment, D. P., Cooke, J. W., Miller, S. E., Jin, Q., McGuire, M. K., Kao, R. T., McClain, P. K., McAllister, B. S., Lynch, S. E. & Giannobile, W. V. (2006) Effect of rhPDGF-BB on bone turnover during periodontal repair. *Journal of Clinical Periodontology* **33**, 135–140.

Saxer, U. P. & Mühlemann, H. R. (1975) Motivation und Aufklärung. *Schweizer Monatsschrift fur Zahnmedizin* **85**, 905.

Schallhorn, R. G., Hiatt, W. H. & Boyce, W. (1970) Iliac transplants in periodontal therapy. *Journal of Periodontology* **41**, 566–580.

Sculean, A., Chiantella, G. C., Windisch, P., Arweiler, N. B., Brecx, M. & Gera, I. (2005) Healing of intra-bony defects following treatment with a composite bovine-derived xenograft (Bio-Oss Collagen) in combination with a

collagen membrane (Bio-Gide PERIO). *Journal of Clinical Periodontology* **32**, 720–724.

Sculean, A., Donos, N., Chiantella, G. C., Windisch, P., Reich, E. & Brecx, M. (1999) GTR therapy with bioresorbable membranes in the treatment of intrabony defects: a clinical and histologic study. *International Journal of Periodontics & Restorative Dentistry* **19**, 501–509.

Sculean, A., Donos, N., Schwarz, F., Becker, J., Brecx, M. & Arweiler, N. B. (2004) Five-year results following treatment of intrabony defects with enamel matrix proteins and guided tissue regeneration. *Journal of Clinical Periodontology* **31**, 545–549.

Sculean, A., Kiss, A., Miliauskaite, A., Schwarz, F., Arweiler, N. B. & Hannig, M. (2008a) Ten-year results following treatment of intra-bony defects with enamel matrix proteins and guided tissue regeneration. *Journal of Clinical Periodontology* **35**, 817–824.

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.

Shirakata, Y., Taniyama, K., Yoshimoto, T., Miyamoto, M., Takeuchi, N., Matsuyama, T. & Noguchi, K. (2010) Regenerative effect of basic fibroblast growth factor on periodontal healing in two-wall intrabony defects in dogs. *Journal of Clinical Periodontology* **37**, 374–381.

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. & 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.

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

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

Wikesjö, U. M., Razi, S. S., Sigurdsson, T. J., Tatakis, D. N., Lee, M. B., Ongpipattanakul, B., Nguyen, T. & Hardwick, R. (1998) Periodontal repair in dogs: effect of recombinant human transforming growth factor beta 1 on guided tissue engineering. *Journal of Clinical Periodontology* **25**, 475–481.

Wilson, T. G. Jr, Glover, M. E., Schoen, J., Baus, C. & Jacobs, T. (1984) Compliance with maintenance therapy in a private periodontal practice. *Journal of Periodontology* **55**, 468–473.

Woolson, R. F. (1987) *Statistical methods for the analysis of biomedical data*. p. 352. New York: John Wiley & Sons.

Yassibag-Berkman, Z., Tuncer, O., Subasioglu, T. & Kantarci, A. (2007) Combined use of platelet-rich plasma and bone grafting with or without guided tissue regeneration in the treatment of anterior interproximal defects. *Journal of Periodontology* **78**, 801–809.

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Clinical Relevance

Scientific rationale for the study: Long-term data on the potential advantage of the additional application of autogenous platelet concentrate during GTR treatment are lacking.

Principle findings: At 1 and 7 years both therapeutic approaches have achieved significant attachment gains compared to baseline. Nevertheless, in the present study attachment loss had occurred between the 1 and 7 year observation. Beside others, this was possibly due to the fact that not all patients received an adequately structured supportive periodontal therapy.

Practical implications: Within the limitations of the study, APC does not seem to have a positive influence on the long-term outcome of GTR therapy.