J Clin Periodontol 2006; 33: 908–921 doi: 10.1111/j.1600-051X.2006.00999.x

Journal of Clinical Periodontology

Influence of autologous platelet concentrate on healing in intra-bony defects following guided tissue regeneration therapy: a randomized prospective clinical split-mouth study

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Christgau M, Moder D, Wagner J, Gläßl M, Hiller K-A, Wenzel A, Schmalz G. Influence of autologous platelet concentrate on healing in intrabony defects following guided tissue regeneration therapy: a randomized prospective clinical split-mouth study. J Clin Periodontol 2006; 33: 908–921. doi: 10.1111/j.1600-051x.2006.00999.x.

Abstract

Objectives: To investigate the influence of autologous platelet concentrate (APC) on early wound healing and regeneration outcomes following guided tissue regeneration (GTR) therapy.

Material and Methods: In 25 patients, two contralateral deep intra-bony defects were treated with β-TCP and a bioresorbable GTR membrane. They were randomly assigned to test and control procedure. In test defects, APC was additionally applied. After 3, 6, and 12 months, healing results were assessed by clinical parameters and quantitative digital subtraction radiography.

Results: Post-operative membrane exposures occurred in 48% of the test sites and 80% of the control sites. Both groups revealed a significant clinical attachment level (CAL) gain of 5 mm after 12 months. Eighty-eight per cent of test and control sites showed a CAL gain of $\geqslant$4 mm. No clinical parameter revealed significant differences between test and control sites. A significant bone density gain was found in both groups after 3, 6, and 12 months. Only after 6 months, the bone density gain was significantly greater in the test defects.

Conclusion: Within the limits of this study, autologous platelet concentrate did not seem to have a noticeable influence on the clinical and most of the radiographic outcomes following GTR. However, APC might reduce the occurrence of post-operative membrane exposures and accelerate bone density gain.

Key words: guided tissue regeneration; intra-bony defects; membranes; periodontal disease/surgery; platelets; subtraction radiography; tricalcium phosphate

Accepted for publication 1 September 2006

Intra-bony defects associated with deep periodontal pockets are ecological niches for periodontal pathogens. They are site-specific risk factors and may be indicators for further disease progression (Armitage 1996, Papapanou & Tonetti 2000). Müller & Ulbrich (2005) found radiographic evidence of intra-bony pockets of 4 mm and deeper in about 20% of the patients older than 50 years. Guided tissue regeneration (GTR) with non-resorbable or bioresorbable cell-occlusive membranes has become a widely accepted method in periodontal therapy of intra-bony defects to restore at least in part the periodontal tissues lost due to the inflammatory process (Caffesse et al. 1997, Christgau et al. 1998a, 2002, Sculean et al. 1999, Cortellini & Tonetti 2000, Eickholz et al. 2000, 2004, Kim et al. 2003). Numerous histological studies in animals (Isidor et al. 1985, Karring et al. 1985, Magnusson et al. 1985b, Caffesse et al. 1988, Claffey et al. 1989, Pontoriero et al. 1992, Elharar et al. 1998) and humans (Nyman et al. 1982, Becker et al. 1987,

Stahl et al. 1990, Zappa 1991, Camelo et al. 1998, Sculean et al. 1999) have proven that the formation of new cementum, new alveolar bone, and new periodontal ligament can be achieved by GTR procedures. Although several clinical studies have shown that the attachment gain achieved by this procedure is stable on a long-term basis (Gottlow et al. 1992, Eickholz et al. 2004, Cortellini & Tonetti 2004), a common problem of GTR therapy is the high variability and low predictability of the healing outcomes (MacNeill & Somerman 1999, Kornman & Robertson 2000). Besides several known impact factors (Kornman & Robertson 2000), a major reason is the fact that GTR membranes act only as mechanical barriers, facilitating a selective cell growth and protecting the blood clot in the defect space. However, the actual amount of regenerated tissue and the course of soft tissue healing are dependent on the individual healing potential (Cortellini & Tonetti 2000, Kornman & Robertson 2000), which is significantly influenced by the presence and amount of the growth factors naturally available in the wound (Cochran & Wozney 1999, MacNeill & Somerman 1999, Wikesjö & Selvig 1999, Kornman & Robertson 2000). Further drawbacks of GTR therapy are early soft tissue healing problems, like membrane exposures, making the post-operative maintenance much more difficult and involving the risk for bacterial contamination of the defect area (Nowzari et al. 1995, Nowzari et al. 1996, Slots et al. 1999). Although controversially discussed in the literature, there are some indications that the prognosis of healing outcomes following GTR therapy might be improved by the additional use of bone graft materials (Needleman et al. 2002, Trombelli et al. 2002, Reynolds et al. 2003, Tonetti et al. 2004, Cortellini & Tonetti 2005, Sculean et al. 2005, Linares et al. 2006).

In contrast to cell-occlusive membranes, therapeutically applied biological mediators, e.g. growth factors and morphogenic proteins, can directly influence cell metabolism and cell activities. In the past, numerous histological animal studies (Giannobile et al. 1994, 1996, Wikesjö et al. 1998, Cochran & Wozney 1999), in vitro experiments (Dennisson et al. 1994, Gamal & Mailhot 2000, Mumford et al. 2001, Marcopoulou et al. 2003, Okuda et al. 2003, Papadopoulos et al. 2003) as well as clinical studies (Howell et al. 1997, Camelo et al. 2003, Nevins et al. 2003, 2005, Sarment et al. 2006) have indicated a potential influence of different growth factors on the regeneration of periodontal tissues. They were used alone (Howell et al. 1997), in combination with bone substitutes (Camelo et al. 2003, Nevins et al. 2003, 2005, Sarment et al. 2006), or in addition to GTR therapy (Cho et al. 1995). Pre-clinical and initial clinical data for growth factors appear promising, although they are insufficient to draw definitive conclusions at this time (Giannobile & Somerman 2003). Unfortunately, so far, recombinant human growth factors have not become readily available for clinical use in periodontal therapy.

Thrombocytes are involved in the wound-healing process and represent a natural source of growth factors, like transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and epithelial growth factor (EGF; Gawaz 2001, Giannobile & Somerman 2003). These factors have mitogenic and chemotactic effects on fibroblasts and can promote angiogenesis by inducing the proliferation of the endothelial cells (Gawaz 2001). Furthermore, PDGF has a mitogenic effect on cells of the osteoblastic lineage (Canalis 1981, 1992, Strayhorn et al. 1999). Competence factors like PDGF have to be distinguished from progression factors, e.g. insulin-like growth factor (IGF), which are only effective after the cells have been made responsive by progression factors (Pledger & Stiles 1977).

Although controversial, some studies have suggested that autologous platelet concentrate (APC) might have a positive influence on hard and soft tissues healing (Marx et al. 1998, Anitua 1999, Kassolis et al. 2000, Robiony et al. 2002, Maiorana et al. 2003, Kassolis & Reynolds 2005). The studies on hard tissue formation were limited to alveolar bone augmentation and sinus floor procedures. Additionally, APC has been reported to support the osseointegration of dental implants (Kim et al. 2002). In some recent studies, APC was applied in addition to other regenerative procedures in intra-bony periodontal defects (Camargo et al. 2002, 2005, Lekovic et al. 2002) and class II furcation defects (Lekovic et al. 2003). However, to the best of our knowledge, no study has reported on the isolated influence of APC in periodontal regeneration. Besides a possibly positive impact on the new formation of bone and periodontal ligament, it would be conceivable that the growth factors of APC might accelerate soft tissue healing and maturation, preventing post-operative membrane exposures.

For this reason, the aim of this clinical prospective split-mouth study was to investigate the influence of APC on the early wound-healing events and regeneration outcomes following GTR in deep intra-bony periodontal defects. This paper reports the clinical and radiographic data, while another paper will present the laboratory data and their correlation to the healing outcomes (Christgau et al. 2006).

Material and Methods

Study design

The present clinical study was designed as a randomized prospective split-mouth study investigating the additional influence of APC on the early wound-healing events and regeneration outcome in deep intra-bony periodontal defects following GTR. The study design 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 and gave informed consent.

Patient selection

The study included 25 systemically healthy patients (15 females, 10 males), with a median age of 42 years (range: 26–62 years), recruited from the patient pool of the Department of Operative Dentistry and Periodontology at the University of Regensburg. One patient had aggressive periodontitis, while 24 patients had chronic periodontitis. Five patients were active smokers (median: 8 cigarettes/day). Each patient showed one pair of contralateral deep intra-bony, inter-proximal 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 intra-bony defects showed a furcation involvement.

Preparation of the APC

Two weeks before surgery, a medical examination was performed to check the suitability of each patient as a blood

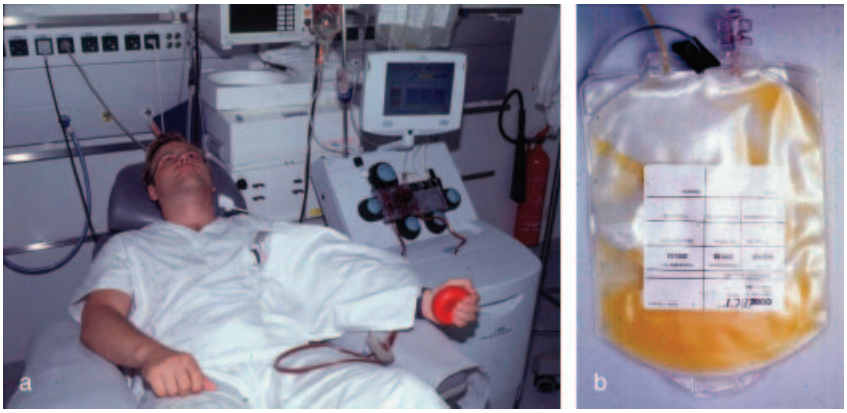

Fig. 1. Apheresis procedure (a) and collection bag for the autologous platelet concentrate (b).

donor. Immediately before periodontal surgery, the thrombocytes for the treatment were collected and APCs were prepared at the Division of Transfusion Medicine at the University Clinic of Regensburg using an apheresis technique (Fig. 1). The procedure has been described in detail elsewhere (Christgau et al. 2006).

2.5 ml of the platelet concentrate was used for clinical application, while the rest of the 50 ml was used for laboratory analyses (Christgau et al. 2006). Complete blood counts were performed in patients' pre-apheresis and post-apheresis samples with an automatic counting system (ADVIA 120, Bayer, Leverkusen, Germany).

For clinical use, the platelet concentrate was reactivated in the following way: 2.5 ml of the platelet concentrate was transferred to a 5 ml syringe. In a 6:1 ratio, 0.5 ml of a sterile 10% calcium chloride ($CaCl_2$) solution was added in order to compensate for the anticoagulating citrate effect (Fig. 2a). A preliminary experiment showed that this 6:1 ratio corresponds to the minimal amount of $CaCl_2$, inducing a clotting of the platelet concentrate.

Periodontal therapeutic procedure

At least 4–6 weeks after completion of full-mouth supra- and subgingival scaling and root-planing as well as splinting of extremely mobile teeth, all surgeries were performed by one surgeon (M. C.) according to the principles of GTR (Christgau et al. 1998a).

For randomized treatment allocation, a randomizing table was created by our mathematician (K.-A. H.) using the SPSS software (Version 13.0, SPSS Inc., Chicago, IL, USA). The randomization table comprised the patient numbers (1–25) and the corresponding defect numbers (1 and 2) per patient. The therapy methods (test or control) were randomly allocated to the defect numbers. By entering the study, the patient numbers were consecutively allocated to the patients and the defect numbers were allocated to the two teeth to be treated. Treatment allocation was concealed to the surgeon until the beginning of the surgery.

Both defects in each patient were treated in the same session. Following sulcular incisions, buccal and oral mucoperiosteal flaps were elevated. As far as possible, vertical releasing incisions were avoided not to compromise flap vascularization. In wide inter-proximal spaces, the papilla preservation technique was applied to avoid loss of gingival height (Takei et al. 1985). After thorough defect debridement (Fig. 3a), the selected *test site* was treated as follows: the root surfaces were conditioned using 24% ethylenediaminetetraacetic acid (EDTA) gel (Prefgel, Straumann, Freiburg, Germany) for 2 min. Following rinsing with sterile 0.9% NaCl solution, reactivated APC was applied to the root surfaces. Then, the defect was filled with β-tricalcium phosphate granules (Ceros, Mathys, Bettlach, Switzerland; Fig. 3b), which were soaked in APC for at least 30 min. before application (Fig. 2b). Afterwards, a synthetic bioresorbable GTR membrane (Resolut XT, Gore, Flagstaff, AZ, USA) was adjusted to the individual defect morphology and secured with bioresorbable polyglycolic sutures (Dexon-II 6-0, Braun-Dexon, Tuttlingen, Germany; Fig. 3c). Apical periosteal releasing incisions were followed by coronal flap repositioning for tension-free primary membrane coverage. The coronal position of the mucoperiosteal flaps was secured by deep, inter-proximal horizontal mattress sutures (Premilene 5-0 DSM19, Braun-Dexon). Tension-free adaptation of the papilla tips was achieved by single sutures (Premilene 6-0 DSM15 or 7-0 DSM11, Braun-Dexon). *Control sites* were treated in the same way without application of APC. In control sites, TCP granules were soaked with patient blood obtained from the defect area.

For peri- and post-operative infection prophylaxis, systemic doxycyclin (doxycylin ratiopharm 100, Ratiopharm GmbH, Ulm, Germany) was given for 19 days (first day: 200 mg, from the second day on 100 mg) beginning 2 h before surgery. Mechanical toothbrushing and flossing were avoided in the operated regions in favour of rinsing with a 0.2% chlorhexidine solution (Corsodyl Lösung, SmithKline Beecham, Bühl, Germany) three times a day for about 6 weeks. During this period, weekly inspections were performed including professional tooth-cleaning, rinsing with 0.2% chlorhexidine solution,

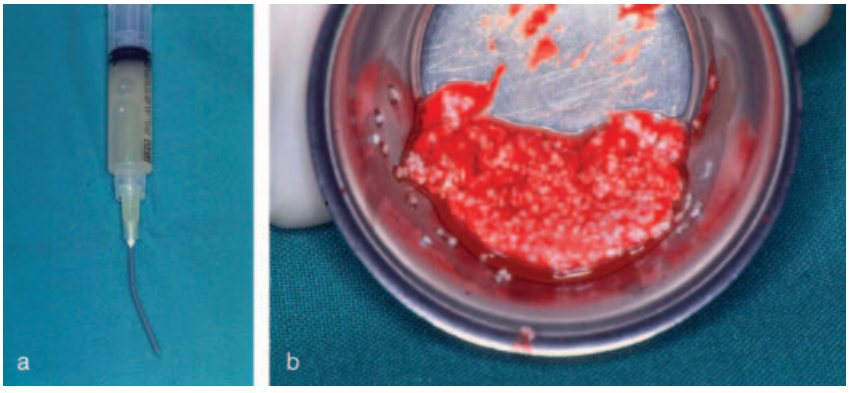

Fig. 2. Activated autologous platelet concentrate in a syringe (a), mixed with β-TCP granules and a few drops of venous blood (b).

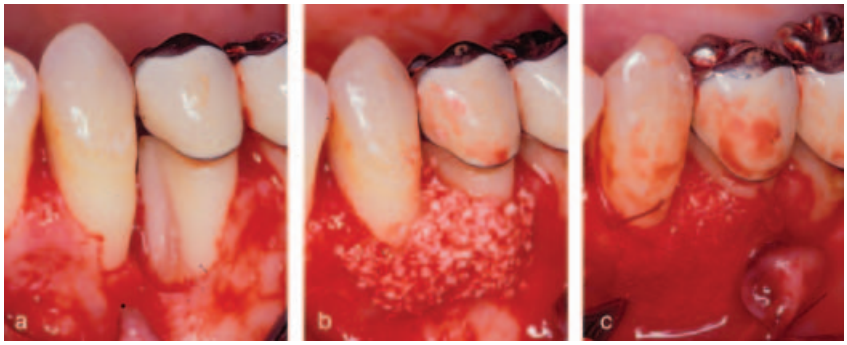

Fig. 3. Therapeutic procedure at a mandibular left premolar (test site): (a) after defect debridement, (b) defect fill with TCP soaked in autologous platelet concentrate, and (c) adaptation and fixation of the bioresorbable guided tissue regeneration membrane.

and application of a 1% chlorhexidine gel (Corsodyl Gel, SmithKline Beecham). Sutures were removed 7 days after surgery. At 6 weeks after completion of GTR therapy, all patients were set on a 2- to 3-month schedule for supportive periodontal therapy.

Clinical examination

Clinical examination was performed by two masked examiners (M. G., J. W.), who were calibrated and who had no knowledge about the treatment modality used in the individual defect.

Early wound-healing problems such as inter-proximal flap dehiscence with membrane exposure or infections were recorded after 3, 7, 14, 21, 28, 35, and 42 days. The buccolingual extension of a membrane exposure, i.e. the distance between the receding inter-proximal gingival margins, was measured to the nearest millimetre.

The healing outcomes were assessed immediately before as well as 3, 6, and 12 months after surgery using the following parameters: gingival inflammation at the surgical site was assessed by the papillary bleeding index (PBI; Saxer & Mühlemann 1975). Furthermore, the full-mouth PBI was calculated as the percentage of the maximum bleeding score after gentle probing of the inter-proximal sites. Oral hygiene was assessed by the full-mouth approximal plaque index (API; Lange et al. 1977), which was calculated as the percentage of inter-proximal sites depicting plaque. The following clinical parameters were recorded for the assessment of the healing results due to the GTR therapy: the clinical attachment level (CAL) as the distance from the cemento-enamel junction (CEJ) or the margin of a restoration to the fundus of the periodontal pocket was used as the primary outcome measure. For further analysis, the gingival recession (REC) as the distance between the CEJ or the margin of a restoration to the free gingival margin and the PPD as the distance from the gingival margin to the fundus of the periodontal pocket were recorded. The baseline depth of the osseous defect (DOD) was measured intra-operatively as the distance from the alveolar crest to the fundus of the osseous defect. A pressure-calibrated probe (Brodontic 25 g, Ash, Dentsply, UK) with a PCP15-UNC tip (HuFriedy, Chicago, IL, USA) and customized acrylic stents with guiding grooves provided standardized probing conditions.

REC, PPD, CAL, and DOD were recorded to the nearest millimetre at 10 molar sites (d/db/b/mb/m; d/dl/l/ml/m) and six pre-molar, canine, and incisor sites (d/b/m; d/l/m). Based on the intra-operative findings, only those probing sites were considered for further evaluation that described the extension of the intra-bony defect under investigation. As previously described (Christgau et al. 1995, 1997, 1998a), for each examination time, the maximum of the clinical parameter values (REC, PPD, CAL, DOD) describing the single defect were used for the statistical evaluation. The healing results of each defect were assessed by the difference of the maximum parameter values (REC, PPD, CAL) found at each examination time (baseline, 3, 6, and 12 months). Thus, as previously discussed in detail (Christgau et al. 1995, 1997, 1998a), the worst healing result of each defect was used for the assessment of the treatment effect. Additionally, for comparison of the attachment changes of sites with different baseline depths, the vertical *relative* attachment gain (V-rAG) was calculated as the percentage of the CAL gain (ΔCAL) after 3, 6, and 12 months related to the DOD value, which determined the space under the membrane available for periodontal regeneration (Christgau et al. 1997).

Radiographic examination

Hard tissue changes following GTR therapy in the defect region were assessed using quantitative digital subtraction radiography (DSR). For this purpose, geometrically standardized intra-oral radiographs (Insight, Eastman Kodak, Rochester, NY, USA) were obtained from each surgical site with the paralleling technique at baseline as well as 3, 6, and 12 months post-operatively using customized acrylic filmholders. The radiographs were developed in a processing automat (XR24-II, Dürr, Germany) and then digitized using a slide scanner (Nikon Film Scanner LS-1000, Nikon GmbH, Düsseldorf, Germany) at a resolution of 400 dpi. Quantitative DSR (WDENS Version 2.42, Torben Jörgensen, Lystrup, Denmark; Wenzel 1989, Christgau et al. 1998b) was used for the assessment of bone changes (density, area) as previously described (Christgau et al. 1998a). Briefly, within the baseline image, a window called ''defect region'' (DR) was defined including the whole radiographically visible osseous defect at baseline. During the subtraction process, this window was automatically transferred to the subtraction image by the computer. Then, in the subtraction image an ''experimental region'' (ER) was defined including all visible radiographic density changes within the DR. A similarly sized ''control region'' (CR) located in an area not affected by the GTR procedure was used as a reference parameter to compare actual GTR-caused bone changes in the ER with the background noise error of the method. Within all examination windows (DR, ER, CR), the computer calculated the pixel number as a parameter for the window area and the mean value of the grey-level histograms as a parameter for bone density. The difference between the grey levels of the ER and CR was called the corrected mean grey level [Δmean (ER–CR)] and was used as a parameter for bone density changes due to GTR. A higher mean value of the ER compared with the CR indicated bone density gain (Christgau et al. 1998b). The area of bone density changes due to GTR was expressed as

percentage of the ER area in pixels related to the DR area in pixels: % relative area of bone density changes = (ER area/DR area) × 100%.

Data analysis

In this study, the single patient was regarded as the evaluation unit. As previously discussed (Christgau et al. 1997), clinical and radiographic measurements were expressed as median values (with 25th and 75th percentiles) and were statistically evaluated using a non-parametric test. Taking into account the paired nature of the split-mouth design, the Wilcoxon-signed-rank test (SPSS Version 13.0, SPSS Inc.) was applied for pairwise statistical analysis of the clinical and radiographic data of test and control defects (Woolson 1987, Page et al. 1995). The pairwise analysis between the examination times was also performed by using the Wilcoxon-signed-rank test. The significance level was set to $\alpha = 0.05$. For testing the overall influence of the parameters, the error rates method was applied. Hereby, the significance level was adjusted to $\alpha^*(k) = 1-(1-\alpha)^{1/k}$ (k: number of pairwise tests to be considered).

For comparability reasons to other studies, mean values and standard deviations were included in our tables, in spite of using non-parametric tests for statistical evaluation. The evaluation of the early healing problems was performed descriptively.

Results

Thrombocytes in the pristine venous blood samples and platelet concentrates

The pristine venous blood samples revealed 276×10^3 thrombocytes/μl (median), while the APCs obtained by apheresis technique revealed 2163×10^3 thrombocytes/μl (median). This corresponded to a median enrichment factor of 7.9 (Christgau et al. 2006).

Clinical healing results

Baseline situation

Table 1 shows the distribution of the different defect and tooth categories in test and control sites as well as the number of mandibular and maxillary teeth treated. The majority of sites revealed two-wall, three-wall, or combined two-/three-wall defects (84% of test sites and 88% of control sites).

Table 1. Distribution of defect and tooth categories and the distribution of the jaws in test and control sites

	Test sites ($n = 25$)	Control sites ($n = 25$)
Defect categories		
1-wall	0	0
2-wall	3	4
3-wall	11	11
Combined 1-/2-wall	0	0
Combined 1-/3-wall	2	1
Combined 2-/3-wall	7	7
Combined 1-/2-/3-wall	2	2
Tooth categories		
Incisors, canines	9	6
Pre-molars	10	6
Molars	6	13
Jaw distribution		
Maxilla	8	8
Mandible	17	17

n, number of defects.

All patients showed good oral hygiene with a full-mouth PBI of 12% before surgery. The median PBI at the surgical site was 1.0 in both test and control sites. Both defect groups revealed a median baseline PPD of 10.0 mm and a median CAL of 11.0 mm. The pre-operative REC was 1.0 mm in test sites and 2.0 mm in control sites. The median DOD, recorded intra-operatively, was 7.0 mm in test defects and 8.0 mm in control defects. None of these baseline parameters revealed statistically significant differences between test and control sites (Table 3).

Early wound-healing events

None of the test and control defects revealed any clinically visible signs of post-operative infection. Post-operative membrane exposures occurred in 48% of test sites and 80% of control sites at any examination time. Table 2 reports the number of exposures at the healing times as well as the corresponding buccolingual extensions of inter-proximal membrane exposures. There was a clear tendency towards more pronounced exposures in control sites compared with test sites. Four patients (16%) did not show any exposed membrane. The membrane exposures were distributed as follows: in nine patients (36%) only in control sites, and in one patient (4%) only in a test site, in 11 patients (44%) in both test and control sites.

Healing results 3, 6, and 12 months after surgery

The clinical healing results are reported in Tables 3–5. All patients showed good oral hygiene with a full-mouth API of 10%, 15%, and 18% after 3, 6, and 12 months, respectively. The full-mouth PBI was 12%, 15%, and 12% after 3, 6, and 12 months, respectively. Compared with baseline, both test and control sites revealed a statistically significant increase in REC of 1 mm after 3, 6, and 12 months. Furthermore, test and control defects showed statistically significant reductions in PPD and gain in CAL. In both defect groups, the median reduction of PPD (ΔPPD) was 6 mm after 3, 6, and 12 months. This corresponded to a median gain in CAL (ΔCAL) of 6 mm after 3 months and 5 mm after 6 and 12 months in test and control sites (Table 4). In test sites, the median V-rAG was 83.3% of the DOD after 3 months and declined to 71.4% after 6 and 12 months. In control sites, the V-rAG was 75% after 3 months, 70% after 6 months, and 71.4% after 12 months (Table 4). None of the clinical parameters (ΔREC, ΔPPD, ΔCAL, V-rAG) revealed a statistically significant difference between test and control sites at any time after surgery. The error rates method revealed a statistically significant influence of the time ($k = 5$) on all the clinical parameters tested.

Table 5 shows the frequency distribution of CAL changes found in test and control sites in the different observation periods. After 12 months, a CAL gain of at least 2 mm was found in 96% of the test sites and in all of the control sites. Eighty-eight per cent of both test and control sites revealed a CAL gain of at least 4 mm 12 months after GTR surgery. None of the defects showed attachment loss compared with the baseline situation. However, in some of the test and control sites, newly formed attachment was lost between 3 and 6 months as well as between 6 and 12 months.

Radiographic healing results

Figures 4 and 5 show the radiographic healing results and bone density gain in a test and control site. The data of the radiographic evaluation by quantitative DSR are reported in Table 6. Significant bone density gain in test and control defects was indicated by statistically significant differences between the mean grey levels of ER and CR as

well as by positive Δmean(ER–CR) values after 3, 6, and 12 months. Generally, the error rates method revealed a statistically significant influence of the time on these parameters for both therapy methods.

After 3 and 6 months, the test sites showed a greater bone density gain compared with the control sites; after 6 months, this difference reached the level of significance. In contrast, after 12 months, control sites showed a tendency towards a greater bone density gain. The error rates method did not reveal a statistically significant influence of the therapy method independent of the time. The percentage of the radiographically detectable defect fill was 84.5% after 3 months and 77.9% after 12 months in the test sites, while it was 78.3% after 3 months and 80.7% after 12 months in the control sites. These differences between the defect groups were not statistically significant.

Discussion

Discussion of the methods

To the best of our knowledge, the present study is the first investigation facilitating the assessment of the isolated effect of APC on periodontal regeneration by keeping all other possible factors and surgical procedures as constant as possible. For this purpose, a split-mouth-design was applied to compare test and control defects under similar healing conditions. Thus, every patient served as her/his own control (Hujoel & Moulton 1988, Page et al. 1995, Koch & Paquette 1997), although the complexity of factors influencing the healing outcomes still needs to be considered. Several studies (Tonetti et al. 1995, Trombelli & Scabbia 1997, Ehmke et al. 2003, Stavropoulos et al. 2004) have shown that strong cigarette smoking has a negative influence on periodontal regeneration. In the present study, five light smokers (8 cigarettes/day) had to be included. However, due to the split-mouth design, the test and control defects were similarly influenced by this habit.

In the present study, the APC was produced by a closed apheresis system applying a continuous cell separation procedure under optimally standardized conditions. In contrast to commercially available chair side systems, this procedure allowed the extraction of the platelets while almost all other blood components were brought back to the blood circulation. Thus, this apheresis method was less invasive for the patient

Table 2. Number and buccolingual extension of membrane exposures during the first 6 weeks

Postoperative (p.o.) observation period	Test sites ($n = 25$)			Control sites ($n = 25$)		
	number of exposures	buccolingual extension (mm)		number of exposures	buccolingual extension (mm)	
		median	range		median	range
3 days p.o.	0	–	–	0	–	–
7 days p.o.	8	3.5	2.0–6.0	9	3.0	2.0–8.0
14 days p.o.	11	4.0	2.0–6.0	17	3.0	1.0–8.0
21 days p.o.	10	3.5	2.0–6.0	15	4.0	3.0–9.0
28 days p.o.	6	3.0	2.0–6.0	14	4.0	1.0–10.0
35 days p.o.	2	2.5	2.0–3.0	4	6.0	3.0–9.0
42 days p.o.	0	–	–	2	6.0	3.0–9.0

n, number of defects.

Table 3. Median values (with 25th/75th percentiles) and mean values (with standard deviations, SD) of the papillary bleeding index (PBI) at the surgical sites, the depth of the osseous defects (DOD), the gingival recession (REC), probing pocket depth (PPD), and the clinical attachment level (CAL) at baseline and after 3, 6, and 12 months ($n = 25$)

	Test sites					Control sites				
	PBI	DOD (mm)	REC (mm)	PPD (mm)	CAL (mm)	PBI	DOD (mm)	REC (mm)	PPD (mm)	CAL (mm)
Baseline										
Median	1.0[1,3]	7.0	1.0[1,2,3]	10.0[1,2,3]	11.0[1,2,3]	1.0[1,3]	8.0	2.0[1,2,3]	10.0[1,2,3]	11.0[1,2,3]
25th/75th percentile	0.0/1.5	6.0/8.0	0.0/3.0	9.0/10.0	10.0/13.0	0.0/2.0	6.0/9.0	0.0/3.0	9.0/10.5	10.0/13.0
Mean (± SD)	0.8 (± 0.8)	7.2 (± 1.9)	1.5 (± 1.8)	9.9 (± 1.4)	11.1 (± 1.9)	0.9 (± 0.9)	7.8 (± 2.1)	1.7 (± 1.5)	9.9 (± 1.3)	11.4 (± 2.1)
3 months										
Median	0.0[1]		2.0[1]	3.0[1]	5.0[1]	0.0[1]		3.0[1]	4.0[1]	6.0[1]
25th/75th percentile	0.0/1.0		0.0/4.5	3.0/4.0	3.5/7.0	0.0/1.0		1.5/3.5	3.0/4.0	5.0/6.5
Mean (± SD)	0.4 (± 0.6)		2.4 (± 2.2)	3.6 (± 1.0)	5.5 (± 2.1)	0.6 (± 0.7)		2.6 (± 1.9)	3.7 (± 1.0)	6.0 (± 2.1)
6 months										
Median	0.0		2.0[2]	3.0[2]	6.0[2]	0.0		3.0[2]	4.0[2]	6.0[2]
25th/75th percentile	0.0/1.0		0.0/4.5	3.0/4.0	4.0/8.0	0.0/1.5		2.0/4.0	3.0/4.0	5.0/7.0
Mean (± SD)	0.4 (± 0.7)		2.4 (± 2.3)	3.5 (± 1.0)	5.8 (± 2.2)	0.6 (± 0.9)		2.6 (± 1.6)	3.8 (± 1.0)	6.1 (± 1.8)
12 months										
Median	0.0[3]		3.0[3]	3.0[3]	6.0[3]	0.0[3]		3.0[3]	4.0[3]	6.0[3]
25th/75th percentile	0.0/0.5		1.0/4.5	3.0/4.0	4.5/7.0	0.0/1.0		2.0/4.0	3.0/5.0	5.0/7.0
Mean (± SD)	0.3 (± 0.6)		2.8 (± 1.9)	3.6 (± 1.0)	6.1 (± 1.7)	0.5 (± 0.6)		2.7 (± 1.5)	3.0 (± 0.9)	6.2 (± 1.5)

n number of split-mouth defects.
*Statistically significant difference between test and control sites ($p \leqslant 0.05$).
[1]Statistically significant difference between baseline and 3 months ($p \leqslant 0.05$).
[2]Statistically significant difference between baseline and 6 months ($p \leqslant 0.05$).
[3]Statistically significant difference between baseline and 12 months ($p \leqslant 0.05$).
[4]Statistically significant difference between 3 and 6 months ($p \leqslant 0.05$).
[5]Statistically significant difference between 6 and 12 months ($p \leqslant 0.05$).

Table 4. Median values (with 25th/75th percentiles) and mean values (with standard deviations, SD) of the changes of the gingival recession (ΔREC), of the probing pocket depth (ΔPPD), of the clinical attachment level (ΔCAL), and the vertical relative attachment gain (V-rAG) after 3, 6, and 12 months ($n = 25$)

	Test sites				Control sites			
	ΔREC (mm)	ΔPPD (mm)	ΔCAL (mm)	V-rAG (%)	ΔREC (mm)	ΔPPD (mm)	ΔCAL (mm)	V-rAG (%)
BL–3 months								
Median	– 1.0[2]	6.0[2]	6.0[2]	83.3[2]	– 1.0[2]	6.0[2]	6.0[2]	75.0[2]
25th/75th percentile	– 2.0/0.0	6.0/7.0	4.5/6.0	64.6/100.0	– 1.5/0.0	5.0/7.0	4.0/7.0	53.5/88.9
Mean (± SD)	– 1.0 (± 1.3)	6.3 (± 1.2)	5.6 (± 1.5)	82.1 (± 25.1)	– 1.0 (± 1.3)	6.2 (± 1.7)	5.4 (± 2.3)	72.4 (± 30.3)
BL–6 months								
Median	– 1.0	6.0[5]	5.0[5]	71.4[5]	– 1.0	6.0[5]	5.0[5]	70.0[5]
25th/75th percentile	– 1.5/0.0	5.0/7.0	5.0/6.0	61.2/88.7	– 2.0/0.0	5.0/7.0	3.5/7.0	50.0/87.5
Mean (± SD)	– 1.0 (± 1.3)	6.4 (± 1.5)	5.3 (± 1.8)	73.3 (± 19.4)	– 0.9 (± 1.3)	6.1 (± 1.3)	5.3 (± 2.0)	70.9 (± 28.4)
BL–12 months								
Median	– 1.0	6.0	5.0	71.4	– 1.0	6.0	5.0	71.4
25th/75th percentile	– 2.0/0.0	5.0/7.0	4.0/6.0	62.5/81.6	– 2.0/0.0	5.5/7.0	4.0/6.5	55.6/85.4
Mean (± SD)	– 1.3 (± 1.3)	6.3 (± 1.2)	5.0 (± 1.5)	70.1 (± 15.8)	– 1.0 (± 1.2)	6.0 (± 1.1)	5.2 (± 1.6)	70.6 (± 21.8)
3–6 months								
Median	0.0[2]	0.0[2]	0.0[2]	0.0[2]	0.0[2]	0.0[2]	0.0[2]	0.0[2]
25th/75th percentile	– 0.5/0.5	– 1.0/1.0	– 1.0/1.0	– 18.3/4.1	– 1.0/0.5	– 1.0/0.0	– 1.0/1.0	– 11.8/11.8
Mean (± SD)	0.0 (± 1.0)	0.0 (± 1.2)	– 0.3 (± 1.4)	– 4.3 (± 21.4)	0.0 (± 0.9)	– 0.1 (± 1.4)	– 0.2 (± 1.6)	– 1.5 (± 21.1)
6–12 months								
Median	0.0	0.0[5]	0.0[5]	0.0[5]	0.0	0.0[5]	0.0[5]	0.0[5]
25th/75th percentile	– 1.0/0.0	– 1.0/1.0	– 1.0/0.0	– 15.5/0.0	– 0.5/1.0	– 1.0/0.0	– 1.0/1.0	– 14.6/12.5
Mean (± SD)	– 0.3 (± 1.2)	0.0 (± 1.0)	0.3 (± 1.1)	– 5.2 (± 18.6)	0.0 (± 1.0)	– 0.2 (± 1.3)	– 0.2 (± 1.5)	– 1.8 (± 20.7)

*Statistically significant difference between test and control sites ($p \leqslant 0.05$).
[1]Statistically significant difference between BL–3 months and BL–6 months ($p \leqslant 0.05$).
[2]Statistically significant difference between BL–3 months and 3–6 months ($p \leqslant 0.05$).
[3]Statistically significant difference between BL–6 months and 3–6 months ($p \leqslant 0.05$).
[4]Statistically significant difference between BL–6 months and BL–12 months ($p \leqslant 0.05$).
[5]Statistically significant difference between BL–6 months and 6–12 months ($p \leqslant 0.05$).
n, number of split-mouth defects; BL, baseline.

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

	Test sites (%)					Control sites (%)				
	BL–3 mo	BL–6 mo	BL–12 mo	3–6 mo	6–12 mo	BL–3 mo	BL–6 mo	BL–12 mo	3–6 mo	6–12 mo
≤ – 2 mm	–	–	–	12	16	–	–	–	12	8
0 ± 1 mm	–	4	4	80	76	4	–	–	84	80
2–3 mm	12	8	8	8	8	12	24	12	–	12
4–5 mm	24	52	60	–	–	32	32	52	4	–
≥ 6 mm	64	36	28	–	–	52	44	36	–	–
≥ 2 mm	100	96	96	8	8	96	100	100	4	12
≥ 4 mm	88	88	88	–	–	84	76	88	4	–

BL, baseline; mo, months.

and enabled a minimal leucocyte and erythrocyte contamination (Christgau et al. 2006). Furthermore, this apheresis method provided thrombocyte numbers in the resulting APC that were higher than in most other studies (Weibrich et al. 2002a, b; 2003a, b).

Similar to previous studies on rh-PDGF (Nevins et al. 2005, Sarment et al. 2006), an alloplastic bioresorbable β-tricalciumphosphate was used as a defect filler and carrier for the APC. So far, in most studies investigating the influence of APC on bone regeneration, APC was used in combination with autologous bone grafts, which were expected to transfer vital bone cells into the defect area (Marx et al. 1998, Anitua 1999, Rosen et al. 2000, Aghaloo et al. 2002, 2004, Fennis et al. 2002, Jakse et al. 2003). Regeneration of tissue is only possible if there are vital cells in the defect area that can be affected by signalling molecules like growth factors (Lynch et al. 1999). In contrast to the very wide bony defects in those previous studies on bone healing (Marx et al. 1998, Anitua 1999, Aghaloo et al. 2002, Fennis et al. 2002, 2004, Jakse et al. 2003), in the present study relatively narrow intra-bony periodontal defects were treated. We assumed that the surrounding vital tissues provided enough periodontal target cells to be affected by the growth factors in the APC. Furthermore, TCP was used to analyse a feasible practical concept, avoiding an additional wound for bone grafting, less invasive for the patient. In line with this, a recent multicentre study (Nevins et al. 2005) reported positive clinical and radiographic healing outcomes using β-TCP, combined with recombinant PDGF in intra-bony periodontal defects. Additionally, a recent

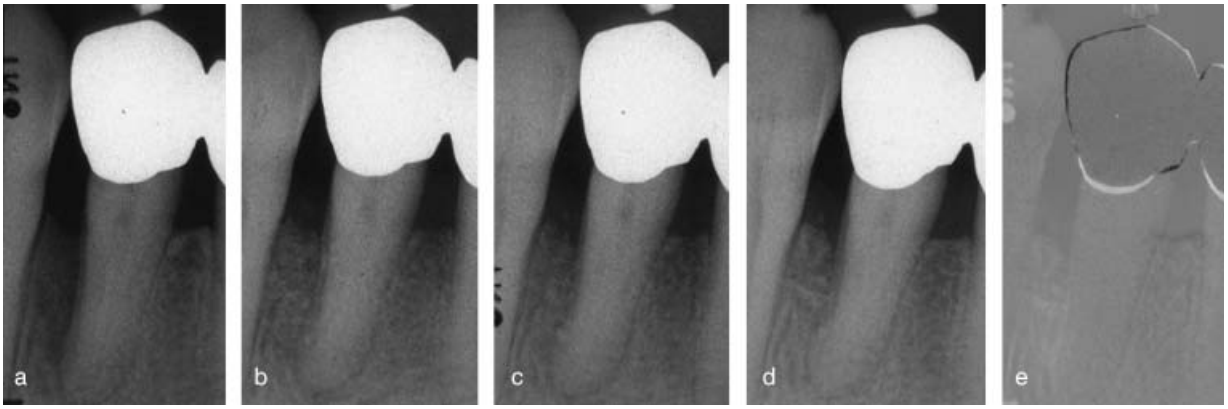

Fig. 4. Radiographically visible healing results at the mandibular left premolar (test site) shown in Fig. 3: (a) at baseline, (b) after 3 months, (c) after 6 months, (d) after 12 months, and (e) digital subtraction image (12 months – baseline): bright grey shades (arrows) indicate bone density gain after 12 months.

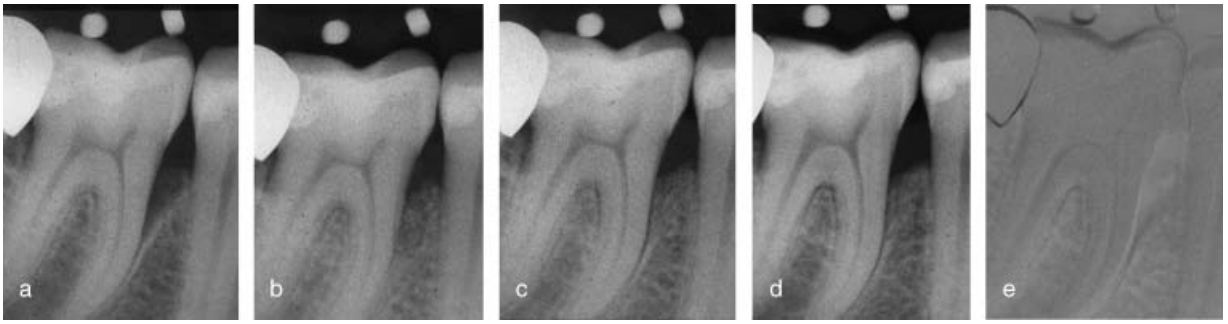

Fig. 5. Radiographically visible healing results at a mandibular right molar (control site): (a) at baseline, (b) after 3 months, (c) after 6 months, (d) after 12 months, and (e) digital subtraction image (12 months – baseline): bright grey shades (arrows) indicate bone density gain after 12 months.

animal study (Kovacs et al. 2005) found strong indications by histomorphometric and densitometric analysis, that platelet concentrates may accelerate the remodelling process of β-TCP and facilitate the formation of hard tissue similar to autologous bone. However, to the best of our knowledge, there is no histological study in the literature proving that TCP alone promotes periodontal regeneration or is a suitable carrier material for the APC and its growth factors.

In the past, root conditioning alone has not shown any clinically relevant benefit on attachment formation (Magnusson et al. 1985a, Erdinc et al. 1995, Mayfield et al. 1998). For example, the application of an EDTA gel neither accelerated the formation of new cementum nor improved the attachment formation (Mayfield et al. 1998). Nevertheless, Blomlöf et al. (1997) have shown by scanning electron microscopy that a 24% EDTA gel could effectively remove the smear layer and expose superficial collagen fibres of a root surface. In the present study, it seemed suitable that the adhesion of the APC and the growth factors to the root surface might be improved by exposure of the superficial collagen structures. However, similar to the root conditioning before application of enamel matrix derivatives (EMD; Hammarström 1997), the necessity of this additional procedure still needs to be proven.

Before application to the test defect, the platelet concentrate was re-activated by adding a 10% calcium chloride solution in a 6:1 ratio. This ratio was determined as the minimal amount of $CaCl_2$ neutralizing the citrate effect and causing the clotting of the platelet concentrate. This corresponds to the data of Marx et al. (1998). Other authors used ratios of 5:1–3:1 for reactivation (Kassolis et al. 2000, Appel et al. 2002). In contrast to Marx et al. (1998), in the present study no bovine thrombin was added in order to avoid the risk of contamination and infection by a xenogenous material. Appel et al. (2002) recently suggested that bovine thrombin was not necessary for reactivation. Instead, with regard to Appel et al. (2002), only some drops of the patients' own blood were added to accelerate the coagulation process. It has to be mentioned that in the present study, neither before nor after reactivation, the functionality or the activity of the platelets themselves were tested.

Discussion of results

The APC of the present study contained far more platelets ($2163 \times 10^3/\mu$l) than platelet concentrates produced by other laboratory procedures: 1408×10^3 platelets/μl (Weibrich et al. 2002b), 270×10^3 platelets/μl (Appel et al. 2002). However, so far little is known about the optimal thrombocyte number in APC to have a maximal biological effect (Jensen et al. 2005). Recently, one indication was provided by Weibrich et al. (2004), who found in an animal study the best effect with a thrombocyte number of approximately 1000×10^3 platelets/μl.

Platelets contain the growth factors TGF-β, PDGF, and EGF (Gawaz 2001). Marx et al. (1998) could detect PDGF and TGF-β by monoclonal antibodies in

Table 6. Digital subtraction radiography (DSR): median values (with 25th/75th percentiles) and mean values (with standard deviations, SD) of the mean grey levels of ER and CR, the corrected mean grey level Δ(ER–CR), and the relative area (rel. area) of radiographic density changes after 3, 6, and12 months ($n = 25$)

	Test sites			Control sites		
	ER	CR	Δ(ER–CR)	ER	CR	Δ(ER–CR)
3 months − BL						
Mean grey level						
Median	137.2*	126.9*	11.0	137.7*	128.4*	8.0
25th/75th percentile	132.0/146.4	124.9/129.5	4.5/21.5	133.0/141.3	127.2/131.6	4.5/14.0
Mean (± SD)	139.3 (± 10.4)	126.4 (± 5.6)	12.8 (± 12.3)	137.6 (± 7.3)	128.9 (± 4.6)	8.4 (± 7.5)
rel. area (%)						
Median	84.5	–	–	78.3	–	–
25th/75th percentile	57.8/96.2	–	–	63.7/89.3	–	–
Mean (± SD)	80.6 (± 37.6)	–	–	81.6 (± 27.3)	–	–
6 months − BL						
Mean grey level						
Median	138.4*	127.6*	12.0[1]	135.4*	128.3*	9.0[1]
25th/75th percentile	129.8/146.6	121.8/131.0	5.3/18.8	132.3/141.6	126.6/131.2	5.5/13.0
Mean (± SD)	139.4 (± 10.9)	126.3 (± 6.7)	13.2 (± 12.1)	137.0 (± 6.7)	127.9 (± 5.9)	9.2 (± 6.5)
Rel. area (%)						
Median	80.8	–	–	73.8	–	–
25th/75th percentile	55.8/102.3	–	–	61.7/87.1	–	–
Mean (± SD)	81.4 (± 32.9)	–	–	72.3 (± 26.2)	–	–
12 months − BL						
Mean grey level						
Median	136.*	127.8*	11.5	136.3*	126.2*	14.0
25th/75th percentile	131.3/145.2	125.2/129.8	4.3/19.8	132.9/144.6	121.8/128.2	7.0/17.0
Mean (± SD)	13.8 (± 11.7)	125.5 (± 8.1)	12.5 (± 11.0)	138.2 (± 7.1)	125.0 (± 5.3)	13.3 (± 8.6)
Rel. area (%)						
Median	77.9	–	–	80.7	–	–
25th/75th percentile	67.9/100.0	–	–	67.9/100.0	–	–
Mean (± SD)	80.6 (± 19.9)	–	–	79.5 (± 23.8)	–	–

*Statistically significant difference between ER and CR ($p \leqslant 0.05$).
[1]Statistically significant difference between test and control sites ($p \leqslant 0.05$).
BL, baseline; ER, experimental region; CR, control region.

platelets as well as in platelet concentrates. Furthermore, these authors could show an accelerated bone density and maturity after reconstruction of mandibular continuity defects with autologous bone in combination with APC compared with the control group without the additional use of APC. Other clinical studies examining the influence of recombinant PDGF (Park et al. 1995) or TGF-β (Wikesjö et al. 1998) in addition to GTR therapy in animal models could show slight additional positive effects of these growth factors on the periodontal regeneration outcome.

We found post-operative membrane exposures in 48% of test sites and 80% of control sites. Thus, there was a clear tendency towards more pronounced exposures in control sites compared with test sites, which may be explained by the possibly positive influence of the APC growth factors on soft tissue healing in the test sites. For example, PDGF, which is expected to be released from the α-granules of the platelets, initiates the wound-healing process by mitogenic and chemotactic effects on fibroblasts and by proliferative effects on endothelial cells (Ross 1989, Gawaz 2001). TGF-β stimulates tissue growth, supports the production of extracellular matrix (ECM) proteins (Massague 1987), and participates in the stabilization process of the ECM (Ignotz & Massague 1986). However, the fact that still almost half of the test defects showed membrane exposures inspite of APC application and the fact that the difference in membrane exposures had apparently no effect on the healing outcomes question the value and the effort of the additional APC preparation for periodontal wound healing.

In the present study, both test and control procedures provided statistically significant reductions in PPDs and gain in clinical attachment after 3, 6, and 12 months compared with the baseline situation. In test and control defects, the CAL gain was 5 mm and the PPD reduction was 6 mm after 12 months of healing. These clinical healing results were better than in previous studies without bone substitute materials (Christgau et al. 1995, 1998a, 2002, Cortellini et al. 1996, Tonetti et al. 1998, Sculean et al. 1999, Eickholz et al. 2000, 2004, Murphy & Gunsolley 2003). Furthermore, the radiographically recorded area of bone density gain was around 80%, while it was only between 30% and 40% in a previous study using GTR membranes alone (Christgau et al. 2002). However, neither the clinical measurements nor the radiographic analysis can distinguish between the true periodontal regeneration and the defect fill by the radiopaque TCP granules. The true healing mode can only be verified by histological means.

A further reason for the relatively good attachment gains may be the relatively high baseline DOD in the present study. Previous studies have shown a positive correlation between an increased baseline depth of the intra-

osseous component and the final regeneration outcome (Tonetti et al. 1993, 1996, Kornman & Robertson 2000).

Ninety-six per cent of the test and all of the control sites revealed a clinical attachment gain of at least 2 mm after 12 months, while 88% of test and control sites showed an attachment gain of at least 4 mm after 12 months. In former studies using membranes alone, a CAL gain of at least 4 mm was found in 51.6% (Christgau et al. 2002), 38.7% (Tonetti et al. 2004) or 69% (Sculean et al. 2005). However, in the present study none of the clinical parameters (REC, PPD, CAL, V-rAG) revealed statistically significant differences between the test and the control sites. Apparently, the additional application of the APC had no noticeable effect on the periodontal healing outcomes after 3, 6 and 12 months. In contrast, Nevins et al. (2005) found clinically an accelerated CAL gain and reduced increase in REC 3 months after combined therapy with recombinant PDGF and β-TCP compared with periodontal defect fill with β-TCP alone.

In this study, the CAL gain was accompanied by a significant bone density gain in the defect region of test as well as control sites. 73.8%–84.5% of the baseline defect area revealed a significant bone density gain. The radiographically detectable hard tissue changes are far greater than were described in former studies (Brägger et al. 1992, Christgau et al. 1997, 1998a, 2002). However, the data of the present study have to be interpreted with caution, because the β-TCP granules themselves are radiopaque. As already mentioned, it cannot be distinguished by radiographic means, if β-TCP is really replaced by vital bone. The area of the bone density changes after 3, 6 and 12 months showed no statistically significant differences between test and control sites. This means that APC did not induce more bone formation in test sites.

Interestingly, after 3 and 6 months the test sites showed a higher bone density gain than the control sites, while there was a tendency towards a higher bone density in control defects after 12 months. However, these differences reached a level of significance only after 6 months. Corresponding to previous findings (Marx et al. 1998), these differences in the speed of bone density gain between test and control sites may be explained by an accelerated bone maturation induced by the APC. Apparently, it seems to play a role only during the first months of healing, while it had no influence on the final healing outcome after 12 months. Canalis et al. (1992) could show that PDGF stimulates the activity of the osteoblast-collagenase. Its degradative effect is regarded as a prerequisite for bone remodelling. A clinical study reported an increased bone turnover 6 weeks after application of rh-PDGF (Sarment et al. 2006). Furthermore, Giannobile et al. (1997) and Canalis et al. (1993) showed a positive effect of certain growth factor combinations (IGF-I and PDGF-BB or TGF-β1 and bFGF) on the proliferation and activity of bone cells in vitro. The present study revealed some indications for an accelerated bone maturation, but not for more bone formation by APC in the test defects. A preliminary study (Howell et al. 1997) reported significantly more osseous defect fill and vertical bone gain in periodontal defects after application of a high-dose (150 μg/ml) growth factor combination (PDGF-BB and IGF-I) compared with the low-dose combination (50 μg/ml) and a placebo. Nevins et al. (2005) found more bone gain in intra-bony defects 6 months after combined therapy with recombinant PDGF and β-TCP compared with β-TCP alone.

In the literature, there have already been several studies analysing the influence of APC on bone regeneration in maxillofacial surgery (Marx et al. 1998, Anitua 1999, Dugrillon et al. 2002, Wiltfang et al. 2004, Jensen et al. 2005). Furthermore, there are some studies applying APC in periodontal regeneration procedures (Camargo et al. 2002, 2005 Lekovic et al. 2002, 2003). However, to the best of our knowledge, the present study is the first one examining the isolated influence of an APC on periodontal healing and regeneration. Therefore, a direct comparison with the previous studies (Marx et al. 1998, Anitua 1999, Camargo et al. 2002, 2005 Dugrillon et al. 2002, Lekovic et al. 2002, 2003, Wiltfang et al. 2004) is not possible. But our results in periodontal healing seem to confirm some tendencies found with APC in bone regeneration.

Within the limits of this study, APC seems to have only a few positive effects on the early soft tissue healing, reducing the prevalence of membrane exposures, and on the speed of hard tissue maturation within the first months. The weak effects of the additional application of the APC on the healing outcomes in intra-bony periodontal defects may be explained by the following: the effect of the therapeutically applied thrombocyte growth factors might be neglectable compared with that of the already naturally occurring growth factors in the periodontal wound. Furthermore, the half-life period of the APC growth factors may be too short to have any significant influence on the long-lasting healing procedures and thus on the clinical periodontal healing parameters (CAL, PPD).

At this moment, based on the present findings the additional application of an APC cannot be recommended for periodontal regeneration due to an unfavourable cost/risk to benefit ratio (neclectable healing effects, additional patient discomfort).

Conclusions

In the present study, a significant clinical attachment gain and bone gain was obtained in test and control sites after 3, 6, and 12 months. Apart from fewer post-operative membrane exposures and a significantly higher bone density gain in the test defects after 6 months, the additional application of APC had no clinically or radiographically relevant effect on the periodontal regeneration outcome. Within the limits of this study, the results question the value of the APC for periodontal regeneration and do not justify the enormous additional effort and patients' discomfort for the APC preparation.

Acknowledgements

This study was supported in part by the Robert Mathys Foundation, Bettlach, Schweiz. The authors wish to express their gratitude to Prof. Dr. Raul G. Caffesse (Houston, TX, USA) for his help in preparing this manuscript.

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Clinical relevance

Scientific rationale: While recombinant biological mediators are not yet available for clinical application, APC is a natural source of growth factors. Although some studies have indicated beneficial effects of APC on bone healing, nothing is known about its possible influence on the periodontal regeneration process.

Principle findings: The application of APC in addition to GTR therapy in intra-bony defects seemed to reduce the occurrence of post-operative membrane exposures and accelerate early bone density gain. However, no noticeable effect on the clinical periodontal healing outcomes and the amount of bone gain after 3, 6, and 12 months could be observed.

Practical implications: At this moment, the present findings do not justify the additional efforts and costs for APC application in GTR therapy of intrabony periodontal defects.