Clin Oral Invest (2009) 13:179–187
DOI 10.1007/s00784-008-0223-7

ORIGINAL ARTICLE

Use of platelet-rich plasma in periodontal surgery—a prospective randomised double blind clinical trial

L. Harnack · R. H. Boedeker · I. Kurtulus · S. Boehm · J. Gonzales · J. Meyle

Received: 4 April 2007 / Accepted: 14 August 2008 / Published online: 3 September 2008

Abstract The aim of this prospective controlled randomized clinical trial was to evaluate the additional effect of platelet-rich plasma (PRP) in attachment gain. Twenty-two patients showing contralateral intrabony defects were included. Defects were randomized to β-TCP (Cerasorb®) in combination with PRP (test) or alone (control). Probing pocket depth (PPD), clinical attachment level (CAL), and relative AL (RAL) were assessed at the first, initial, re-evaluation (or basis examinations) and 6 months after surgery. Defect dimensions were recorded at baseline surgery (day 0) and during re-entry surgery (after 6 months), with vertical depth of the defect as primary outcome variable. An early healing index (EHI) was assessed 3 days, 1, 2 and 4 weeks after surgery. Both treatments led to clinical improvements. The median reduction of open vertical depth was 1.9 mm (interquartile intervals, 0.75 and 2.5 mm) at test sites, compared with 2.6 mm (1.8 and 3.5 mm) at control sites (p=0.19, Wilcoxon). The median reductions of PPD and CAL at the four sites in close proximity to the defect in the interproximal area at test sites were 0.8 and 0.28 mm, and at control sites 0.4 and 0.13 mm, respectively. The EHI showed a reduction from grade 3 after 3 days to grade 1 after 4 weeks. PRP did not improve the results achieved with β-TCP in the treatment of intrabony defects.

L. Harnack · I. Kurtulus · S. Boehm · J. Gonzales · J. Meyle (✉)
Department of Periodontology, Justus-Liebig University,
Schlangenzahl 14,
35392 Giessen, Germany
e-mail: joerg.meyle@dentist.med.uni-giessen.de

R. H. Boedeker
Department of Statistics, Justus-Liebig University,
Giessen, Germany

Keywords Platelet-rich plasma · Periodontal surgery · Early healing · β-Tricalcium phosphate · Wound healing

Introduction

Traditional synthetic bone grafts are ceramics of hydroxyapatite (HA; $Ca_{10}(PO_4)_6(OH)_2$), tricalcium phosphate ($Ca_3(PO_4)_2$) or combinations of the two. α- and β-tricalcium phosphate (TCP), although chemically identical, have different physiological characteristics [14, 15, 32]. The β-form is the most stable with a calcium-to-phosphate atomic ratio of 1.5 [18]. Porous β-TCP synthetic cancellous bone resembles human cancellous bone in structure and composition. Generally, β-TCPs contain approximately 39% calcium and 20% phosphorus by weight, similar to natural mineral content in bone [24]. TCP has been evaluated in spinal fusion with results comparable to those with autogenous bone [12].

These materials are considered to be resorbable, since the rate of biodegradation is higher when compared with HA [9, 10]. When used alone for grafting, these synthetic grafting materials function as osteoconductive scaffold for new bone growth and as bridges to the host bone. The scaffold is then removed by a combination of dissolution and phagocytosis (cell-mediated resorption) [16, 17, 25, 35].

β-TCP Cerasorb® (Curasan AG, Kleinostheim, Germany) is a pure phase β-TCP. This material has been used in the treatment of osseous defects in animals and humans [2, 34, 36, 37, 39]. It has been used as synthetic bone substitute in orthopedics and dentistry without adverse events since many years [2, 5, 11, 13, 20, 36, 37]. β-TCP has been used in sinus floor augmentation treatments [44, 49]. The effect on bone regeneration was evaluated in hollow titanium cylinders implanted in the posterior jaws of

five volunteers [41]. The results showed that the pure β-TCP was resorbed simultaneously with new bone formation in a period of 6 months, without interference with the bone matrix formation. However, it also has been reported that the replacement of β-TCP by bone does not occur in an equitable way. This means that there is always less bone volume produced than the volume of β-TCP reabsorbed [19]. For this reason, the clinical use of β-TCP has been recommended as an adjunctive with other less reabsorbable bone graft substitutes or as an expander for autogenous bone graft [31].

Platelet-rich plasma (PRP) is a novel method for gaining autologous growth factors, specifically platelet-derived growth factor (PDGF) and transforming growth factor β (TGF-β) [6, 29, 47]. PDGF and TGF-β are abundant in the alpha granules of platelets [46]. Therefore, the specific sequestration and concentration of platelets in plasma and subsequent application of that preparation to wound healing sites increases the concentration of platelets and theoretically of PDGF and TGF-β. PRP modulates cell proliferation in vitro in a cell-type-specific manner [33]. The growth factors present in PRP might promote wound healing at sites of injury in periodontal tissue by formation of the fibrin clot in combination with up-regulating collagen synthesis in the extracellular matrix [21].

Recently, the combination of autologous platelet-rich plasma (PRP) and different bone substitutes has been used in periodontal and maxillary sinus elevation surgeries [1, 3, 4, 28]. The effects on the rate of bone remodeling of β-TCP in combination with autologous PRP have been studied in animal experiments [22, 23]. Densitometric results obtained after 6 weeks demonstrated that the bone formation was significantly more effective when PRP was used. However, only a few studies have assessed the effects of β-TCP in combination with autologous PRP in oral surgery in humans [7, 43, 48].

The purpose of this prospective randomised double-blinded clinical trial was to investigate the effects of PRP in the treatment of periodontal intrabony defects treated with β-TCP.

Materials and methods

Patients and defects

Twenty-two patients with chronic periodontitis were included after informed written consent. The study protocol was approved by the ethic commission of the Justus-Liebig University, Giessen. All patients were older than 30 years. All patients had at least one interproximal site in each contralateral quadrant from the distal area of the canines to the mesial area of the first molar, presenting with probing pocket depth (PPD)>5 mm. The selection criteria of the osseous radiographic defects were contralateral two-wall defects with an intraosseous component as estimated by recent radiographies of good quality and bone sounding. Exclusion criteria were systemic diseases, specially those affecting bone metabolism, smokers (>10 cigarettes per day), poor oral hygiene (PLI≥40%), tooth mobility>grade II, periapical lesions, furcation defects≥grade II, and deficient restorations at the test teeth.

Study design and randomisation

The design of the study is demonstrated in Fig. 1. At the first examination, the following clinical parameters were assessed using the Florida probe® (Gainesville, Florida, USA): PPD, gingival recessions (GR), and bleeding on probing (BOP) at six sites per tooth. The clinical attachment level (CAL) was calculated by adding (in case of gingival recession) or subtracting (in case of gingival enlargement) the value of GR to/from the value of PPD. The relative attachment level (RAL) was measured at the test teeth using individually manufactured acrylic stents with markings at six sites per tooth as reference (from the marking to the bottom of the pocket). A period of repeated professional supragingival oral hygiene (PTC1–PTC4) and motivation sessions followed. Afterwards, an initial examination was performed assessing the clinical parameters previously described. Additionally, subgingival plaque and gingival crevicular fluid (GCF) samples were taken (data are to be analysed and reported in a separate manuscript). Consequently, a period followed where deep scaling and root planing of all sites with PPD≥4 mm was performed. After 2 weeks, a re-evaluation was performed, assessing all clinical parameters, and sampling subgingival plaque and GCF. Radiographs of test and control teeth were taken using a standardised individually manufactured holder, allowing the taking of the radiographs in the same position. If at this examination more than two sites with PPD≥4 mm and BOP remained, the combined antibiotic therapy of amoxicillin and metronidazole was indicated [42]. Only the patients who received antibiotics were re-examined 10 days later (basis examination). After the re-evaluation, the baseline surgical procedure (OP at day 0) and intrasurgical measurements (IM) were performed. Thereafter, oral hygiene was controlled at 1, 3, 4 weeks and 3 months. After 6 months, all clinical parameters were assessed, a re-entry surgery was performed, and the same intrasurgical measurements were recorded. Radiographs were taken.

Additionally, the early healing index (EHI) was assessed 3 days, 1, 2 and 4 weeks after surgery by an independent investigator [45]. This index contains five grades as follows: (1) complete flap closure, no fibrin line in the interproximal area; (2) complete flap closure, fine fibrin line in the

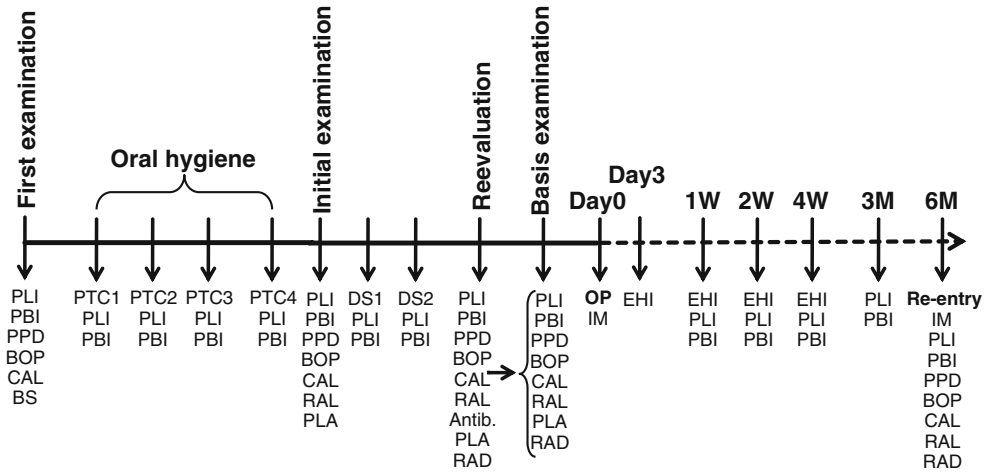

Fig. 1 Schematic illustration of the study design. *PLI* plaque index, *PBI* papillary bleeding index, *PPD* probing pocket depth, *BOP* bleeding on probing, *CAL* clinical attachment level (from the CEJ to the bottom of the pocket), *RAL* relative attachment level (from the stent to the bottom of the pocket),*BS* bone sounding, *PTC* professional teeth cleaning, *PLA* plaque samples, *DS* deep scaling, *Antib* antibiotic therapy, *RAD* radiographs, *OP* baseline surgery, *IM* intraoperative measurements, *EHI* early healing index, *re-entry* re-entry surgery

interproximal area; (3) complete flap closure, fibrin clot in the interproximal area; (4) incomplete flap closure, partial necrosis of the interproximal tissue; and (5) incomplete flap closure, complete necrosis of the interproximal tissue. Photographs were taken during the whole study period.

Platelet-rich plasma preparation

The method for PRP preparation was according to the manufacturer's directions (Curasan AG, Kleinostheim, Germany). Ten milliliters of venous blood was collected using vacutainer collection tubes with 10% trisodium citrate solution (Sarstedt, Nümbrecht, Germany). Blood was centrifuged 10 min at 900×*g*. After this step, two fractions in the tube were visible: one containing the plasma and platelets, and one containing erythrocytes, leukocytes, and platelets. Using a second vacutainer tube, the fraction containing the plasma and platelets was carefully aspirated. This PRP was centrifuged at 2000×*g* for 15 min. After this step, again two fractions in the tube were obtained, one upper part containing platelet-free plasma and, in the bottom, one pellet containing the platelets. Using a needle, the platelet-free plasma was aspirated and removed, leaving 0.3 ml plasma in the bottom with the pellet. Then, the tube was mixed for 20 s and the PRP aspirated with a further tube. The PRP was placed in a small stainless steel cup and mixed with the β-TCP. Each patient received its own PRP.

Surgical procedure

Surgical procedures were performed by an experienced surgeon (S.B.). After local anaesthesia, a full thickness access flap using the modified papilla preservation technique was raised [8] at each site simultaneously. Releasing incisions were made if necessary. After the flaps were raised, granulation tissue and any remaining subgingival calculus were removed. The roots were planed with hand instruments. Thereafter, a blinded examiner assessed the morphology of the defect and the tissue boundaries (Fig. 2). The morphology of the defects was recorded as follows:

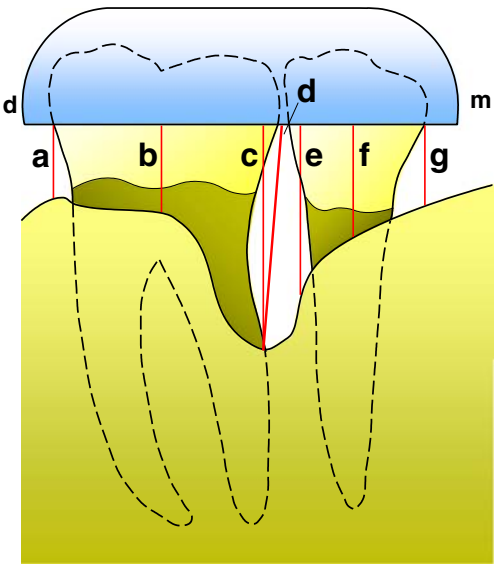

Fig. 2 Measurements of the hard tissue boundaries surrounding the two teeth as assessed during surgical intervention, measured from the stent to the bottom of the defect at the following sites: tooth 1, *a* distobuccal, *b* buccal, and *c* mesiobuccal. Tooth 2, *e* distobuccal, *f* buccal, and *g* mesiobuccal. Primary outcome measurement: *d* deepest part of the defect in the interproximal area, measured from the stent to the bottom of the defect. The same measurements were performed from the lingual/palatal side in order to achieve measurements at six sites per tooth and to measure the primary variable from the buccal and lingual sides

First, the vertical depth of the interdental defect (primary outcome variable, *d*) was measured during surgical intervention with a periodontal probe (PCP-UNC 15) from the stent to the deepest part of the defect. This measurement was performed at the buccal and at the lingual sites. Subsequently, vertical measurements of the hard tissue boundaries surrounding the two operated teeth were performed from the stent to the bottom of the defect at six sites per tooth: mesiobuccal and mesiolingual, distobuccal and distolingual, and buccal and lingual (Fig. 2). The same measurements were performed from the stent to the margin of the crestal bone. Then, the random list was opened, and the defects were consecutively treated with either sequence: sequence 1, quadrant II or III treated only with β-TCP and quadrant I or IV treated with β-TCP and PRP; sequence 2, quadrant II or III treated with β-TCP and PRP, and quadrant I or IV treated only with β-TCP. The granule structure of β-TCP (0.5 g, 150–500 μm, batch number L093B) was applied dry with a periosteal instrument in the defect, without pressing the material. No other vehicle for β-TCP was used. Similarly, the mixture of β-TCP and PRP was applied. The defect was completely filled but not overfilled. The surgical flap was then repositioned after periostal fenestration to ensure that it covered the bone graft. A modified mattress suture was performed using non-irritating sutures (Prolene 6/0, Ethicon, Norderstedt, Germany) to ensure tight adaptation and a stable wound. Sutures were removed after 1 week.

Outcomes of the study

The primary outcome measurement was the vertical depth of the defect recorded intraoperatively at the interdental space from the stent to the deepest point in the defect. The mean difference (from buccal and lingual) in this vertical measurement between the baseline surgery and the re-entry surgery after 6 months reflected the filling of the defect. Secondary parameters were all other clinical measurements, the hard tissue boundaries assessed during the surgeries and the early healing index. The intraosseous defect depth was calculated from the deepest point of the defect to the highest alveolar bone level of the defect. Additionally, standardised radiographs were visualised and evaluated by an independent investigator. Using a magnifying glass (5×), the distance from the cement–enamel junction to the first contact of bone at the root of the respective teeth was measured to the nearest millimeter.

Statistical analysis

Descriptive statistics (median, minimum, maximum and interquartiles) were used for the clinical parameters PPD, CAL, RAL and EHI. For the results of the parameters PPD, CAL, RAL and the hard tissue boundaries of the treated teeth, three regions were evaluated: The region surrounding the defect included all four sites in close proximity to the defect in the interdental area (Fig. 2c and e, from buccal and lingual), the region in the middle included all four sites in the middle of the two teeth (Fig. 2b and f, from buccal and lingual) and the third region involved all four sites that were not in the proximity of the defect (Fig. 2a and g, from buccal and lingual).

Due to the split-mouth design of the study, two sequences of treatment were possible: sequence 1, quadrant II or III treated only with β-TCP and quadrant I or IV treated with β-TCP and PRP; sequence 2, quadrant II or III treated with β-TCP and PRP, and quadrant I or quadrant IV treated only with β-TCP. A random list was established by an independent investigator who did not participate in the clinical part of the study.

The description of the results of the two treatment forms (sequences) was performed both separately for each sequence and for the two sequences together. As a normal distribution could not be assumed, the non-parametric Wilcoxon rank sum test was used. For the nominal or ordinal scaled parameters, crossover tables and Fisher's exact test were used. As a non-parametric estimation of the advantage (reduction test versus control) the Hodges–Lehmann estimator and the corresponding 95% confidence interval (CI) were computed.

Results

Patients

Twenty-two patients who met the inclusion criteria were consecutively recruited and randomly assigned to each treatment sequence. Ten patients were treated as follows: β-TCP and PRP on the right side and β-TCP on the left; 12 patients were treated with the converse treatment sequence.

Primary outcome variable and hard tissue boundaries

Both treatment modalities led to improvements regarding the primary outcome, change of open vertical depth (buccal and lingual sites) between the baseline surgery and the re-entry surgeries (Fig. 3). The median change in the test site (β-TCP and PRP) was 1.9 mm (minimum, −3.0 mm; first quartile, 0.8 mm; third quartile, 2.5 mm; maximum, 5.5 mm). The median change in the control site (β-TCP) was 2.6 mm (minimum, −3.8 mm; first quartile, 1.8 mm; third quartile, 3.5 mm; maximum, 6.5 mm). No significant differences between test and control sites were found (p= 0.19, Wilcoxon). In order to get an un-biased estimation and taking in consideration the study design, the Hodges–

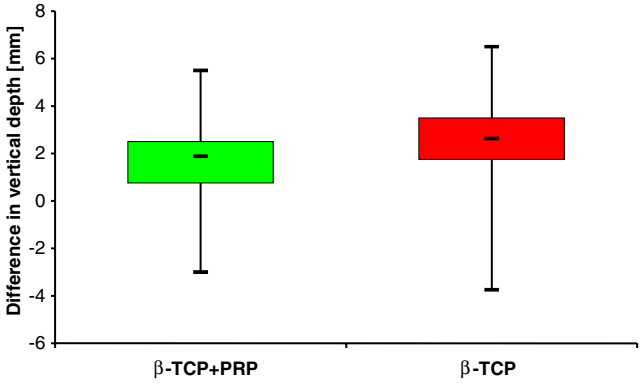

Fig. 3 Change in bony defect vertical depth (measured from the stent to the deepest part of the defect) between the baseline surgery at day 0 and the re-entry surgery after 6 months, separated by treatment (p= 0.19, Wilcoxon; n=22). The *horizontal line* inside the box marks the median. The *lower* and *upper lines* of the box mark the first and third quartile, respectively. The *lower* and *upper horizontal lines at the end of the vertical lines extending from the box* mark the minimum and maximum, respectively

Lehmann estimator of the advantage (reduction test versus control) was 0.81 (95% CI, −1.9; 0.4).

The hard tissue boundaries of the two teeth were measured (as previously described) intrasurgically at the time of baseline and re-entry surgeries. However, there were no differences between both treatment modalities in any of the hard tissue boundaries between the two surgeries (data not shown).

In the control sites (β-TCP), the median intraosseous defect depth was 3.3 mm at the time of baseline surgery and 1.6 mm at the re-entry surgery. In the test sites (β-TCP and PRP), the median intraosseous defect depth was 3.5 mm at the time of baseline surgery and 2.4 mm at the re-entry surgery.

Clinical parameters

Table 1 shows the values of the parameters PPD, CAL and RAL at the re-evaluation (or at the basis examination) and the re-entry surgery after 6 months, assessed at all four sites in close proximity to the defect in the interproximal area. The median change in PPD was 0.8 mm in the sites treated with β-TCP + PRP (minimum, −1.4 mm; first quartile, 0.4 mm; third quartile, 1.2 mm; maximum, 1.9 mm). The median change in the β-TCP sites was 0.4 mm (minimum, −1.8 mm; first quartile, −0.1 mm; third quartile, 1.0 mm; maximum, 3.0 mm; Fig. 4).

The median change in CAL in the β-TCP + PRP sites was 0.3 mm (minimum, −2.0 mm; first quartile, −0.4 mm; third quartile, 0.9 mm; maximum, 3.0 mm). The median change in the sites treated with β-TCP was 0.1 mm (minimum, −2.5 mm; first quartile, −0.4 mm; third quartile, 0.7 mm; maximum, 1.9 mm; Fig. 5).

The median of the difference in RAL was 0.2 mm in the group of patients that were treated with the sequence β-TCP at the left side and β-TCP with PRP at the right side. This value was 0.1 mm in the group of patients that were treated with the sequence β-TCP at the right side and β-TCP with PRP at the left side.

There were no significant differences in the clinical parameters PPD, CAL and RAL between both treatment modalities in the region surrounding the defect. Similar results were found in the other regions of the test teeth (data not shown).

Early healing index

Figure 6 shows the EHI after 3 days, 1, 2 and 4 weeks after the first surgery separated by treatment [45]. Three days post-surgery, the median of the EHI showed a score of 3 in the two treatment modalities. The same score was observed in both treatments after 1 week. After 2 and 4 weeks, the median score was similarly reduced in both treatment modalities from 3 to 2 and from 2 to 1, respectively.

Table 1 Clinical measurements of the two treatment modalities

Parameter	β-TCP + PRP				β-TCP			
	Baseline surgery	Re-entry surgery (after 6 months)	Re-evaluation	6 months	Baseline surgery	Re-entry surgery (after 6 months)	Re-evaluation	6 months
Vertical defect depth (stent, intrasurgically)	13 (10.5–14)	11 (9.8–11.8)			12.3 (11–13.5)	10.1 (8.3–11.3)		
PPD[a]			3.8 (3.5–4.4)	3.2 (2.4–3.9)			2.9 (2.5–3.6)	2.5 (2.1–3.3)
CAL[a]			4.9 (3.1–5.85)	4.2 (3.9–5.9)			4.3 (2.6–5.7)	3.2 (2.7–5.5)
RAL[a] (stent)			7.2 (6.5–7.6)	7.1 (6.6–7.9)			7.4 (6.9–8.5)	6.9 (6.8–8.5)

Median, first and third quartiles in parentheses
[a] Median, first and third quartiles of the four sites surrounding the defect in the interdental area

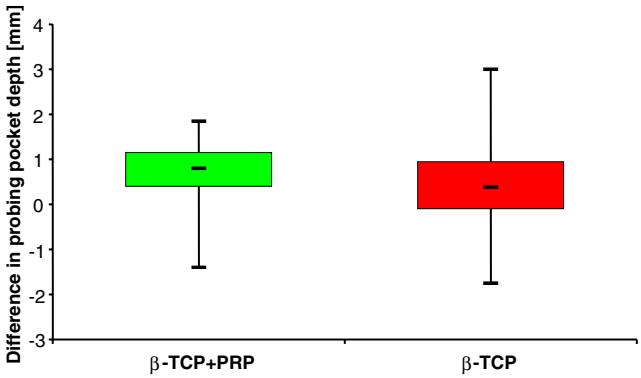

Fig. 4 Change in probing pocket depth (*PPD*) measured with the Florida Probe® at all four sites in close proximity to the defect in the interdental area between the re-evaluation and re-entry surgery after 6 months, separated by treatment (see legend to Fig. 3 for further explanation)

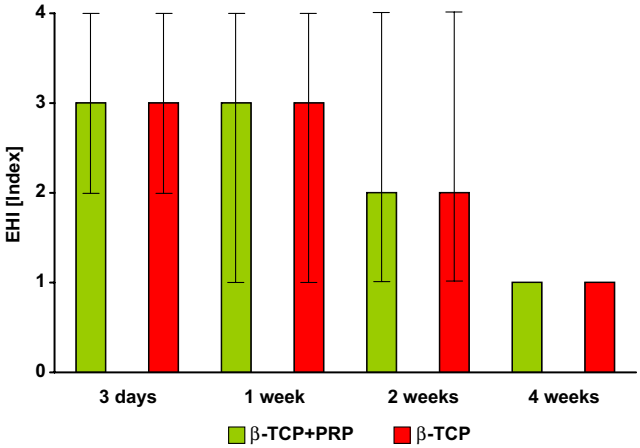

Fig. 6 Early healing index as recorded at different times after baseline surgery, separated by treatment

Radiographic analysis

At the time of surgery, there was a mean defect depth of 5.8 mm in the teeth treated with β-TCP. At the time of re-entry surgery 6 months later, the mean value was 4.5 mm (difference, 1.3 mm). At the sites treated with β-TCP + PRP, there was a mean defect depth of 6.7 mm at the time of surgery and 5.5 mm at the time of re-entry surgery (difference, 1.1 mm).

Discussion

The results of the present prospective randomised double-blind clinical trial demonstrate that for the treatment of periodontal intraosseous defects, both the application of β-TCP alone and β-TCP with PRP result in an improvement of the vertical depth assessed during re-entry after 6 months.

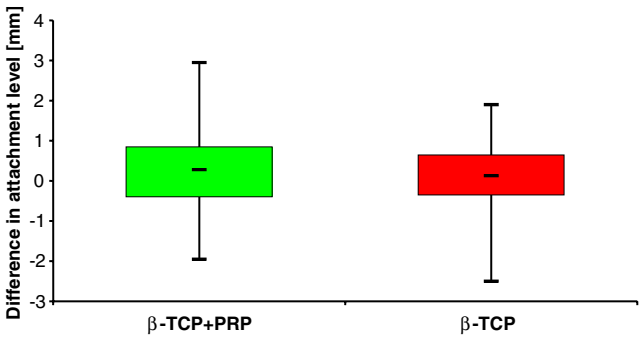

Fig. 5 Change in clinical attachment level (*CAL*) measured with the Florida Probe® at all four sites in close proximity to the defect in the interdental area between the re-evaluation and re-entry surgery after 6 months, separated by treatment (see legend to Fig. 3 for further explanation)

Both treatment modalities resulted in pocket depth reduction and clinical attachment gain as compared to baseline values. However, the difference between the treatment modalities was not statistically significant.

Only few studies have assessed the effects of β-TCP in combination with autologous PRP in oral surgery in humans [43, 48]. A study investigated whether the combination of β-TCP with autologous PRP enhances bony regeneration and resorption of the TCP material in sinus floor augmentation prior to implant insertion [48]. Six months later, the formation of new bone was about 8–10% higher when PRP was applied in the specimens harvested from the augmented region. The resorption of β-TCP was not accelerated and foreign-body giant cells and soft tissue surrounding the β-TCP granules were present.

Studies showing the effects of PRP in combination with β-TCP in periodontal surgery are scarce. There are studies on the effect of PRP with other materials or in combination with guided tissue regeneration (GTR) alone [3, 4, 26, 27]. The combination of PRP with bovine porous bone mineral (BPBM) and GTR was applied for the treatment of periodontal intraosseous defects in humans [3]. The authors reported a significant difference in clinical attachment gain between the defects treated with the combination of PRP/BPBM and GTR than in the control group treated with an open-flap debridement (4.52 versus 1.47 mm, respectively). Defect fill was also superior in the test group. Lekovic et al. [28] studied the effect of a combination of PRP with BPBM and PRP/BPBM with GTR in promoting reduction in probing depth, gain in clinical attachment and defect fill in intrabony periodontal lesions, revealed by re-entry surgeries at 6 months post-treatment. The results showed that both treatment modalities resulted in significant probing depth reduction and clinical attachment gain compared to baseline values. Re-entry surgeries revealed

similar defect fill for both treatment groups. Similar results were observed in the treatment of human mandibular class II furcation lesions [27] and periodontal intrabony defects [4]. Using PRP in combination with an autologous bone graft, Marx et al. [29] showed a bone maturation increase in large human mandibular defects, 1.62–2.16 times that using autologous bone alone at 6 months postoperatively, as measured by histomorphometry. Other studies have shown that the addition of PRP did not induce significantly more new bone formation [30, 40].However, the results of these studies cannot be compared due to different study designs.

In the present study, it was hypothesised that the use of PRP in conjunction with β-TCP might accelerate the bone formation rate. However, histological samples were not obtained in this study in order to demonstrate if the TCP particles were osseointegrated or encapsulated by connective tissue. Thus, clinical parameters were analysed. Although the results of the present study showed that both treatment modalities resulted in reduction of PPD and CAL, and similar defect fill for both treatment groups was present at the re-entry surgeries, the reduction of the clinical parameters was minimal compared to baseline values. This is not in accordance with the previously reported data on the effect of a combination of PRP with other graft materials after 6 months [3, 28]. However, in the present study, clinical data were analysed including all four sites in close proximity to the defects in the interdental area and not only at the deepest lingual or buccal sites. Since the majority of the studies measured PPD and CAL only at the deepest sites, the clinical data cannot be compared.

In this study, the radiographic measurements differed from the measurements during re-entry. The reason for this difference might be the morphology of the bony defect, since two-wall defects with an intraosseous component were part of the inclusion criteria. The intraoperative measurements were performed from the stent to the deepest point of the defect. In the radiographs, the measurements were performed from the cement–enamel junction to the first contact of bone at the root. This might be the buccal or lingual bone wall of the defect.

More recently, the influence of PRP on early wound healing and regeneration outcomes following GTR therapy was investigated [7]. Intra-bony defects were treated with β-TCP and a bioresorbable GTR membrane. In the test defects, additional PRP was added. A significant gain in CAL after 12 months was observed in both groups, although the differences in clinical outcomes between test and control sites were not significant. PRP did not seem to have a noticeable influence on the clinical and radiographic outcomes following GTR. However, PRP reduced the occurrence of post-operative membrane exposures.

Only few studies evaluating clinical outcomes of β-TCP in intraosseous defects have been performed [36, 37]. Snyder et al. [37] evaluated β-TCP on 17 patients with one-wall, two-wall bone and class II furcation defects using standardised preoperative and postoperative radiographs, clinical measurements and clinical photographs. After 18 months, the results showed an average of 2.8 mm of new bone. The capacity of β-TCP to promote bone formation after grafting in intrabony defects in humans was also studied by Saffar et al. [36]. Five biopsies were collected from four patients during re-entry surgery 16 to 40 months after implantation. They were processed without demineralisation for histological examination. The less mature samples showed a highly fibrous, cellular and poorly vascularised connective tissue surrounding the material in the samples. Howship's lacuna-like cavities were visible at the surface of the material. They contained absorbing mononuclear phagocytes. At a more mature stage, the graft was embedded in an acellular fibrous material that underwent mineralisation from the medullary spaces towards the granules, and the bone formed was subsequently remodeled. The implanted material itself was progressively modified and acquired the staining appearance of bone invaded by cells and vessels. However, in a previous study, eight intrabony lesions in four patients that were treated with TCP implants were removed en bloc 3 to 8 months after periodontal flap debridement and histologically analysed [38]. They were walled off by collagen and did not appear to enhance new attachment nor did they induce an inflammatory infiltrate. Thus, they seemed to act as nonirritating fillers. Microscopically, closure of the lesions demonstrated repair with limited evidence of new connective tissue attachment. Histologic expression of the clinical gain in closure was the result of closure by long junctional epithelium. In view of the minimal gains in clinical parameters found in the present study, it is possible that the β-TCP served as a filler material, and the closure was due to a long junctional epithelium.

Recently, the effect of β-TCP on bone regeneration was evaluated in hollow titanium cylinders implanted in the posterior jaws of five volunteers [41]. β-TCP particles were inserted inside the cylinders and harvested 6 months after placement. Control cylinders were inserted without β-TCP particles. The density of the newly formed bone inside the bone-growing chambers measured 27.84%±24.67% in test and 17.90%±4.28% in control subjects, without a statistically significant difference. Analysis of the histologic specimens revealed that the density of the regenerated bone was related to the density of the surrounding bone. The study showed that the pure β-TCP was resorbed simultaneously with new bone formation in a period of 6 months, without interference with the bone matrix formation.

In the present study, PRP did not improve the results achieved with β-TCP alone in the treatment of intrabony defects.

Acknowledgement The authors thank Mr. Helge Hudel for his competent contribution in the statistical analysis. This study was supported by Curasan AG, Kleinostheim, Germany

Conflict of interest The authors declare that they have no conflict of interests.

References

1. Anitua E (2001) The use of plasma-rich growth factors (PRGF) in oral surgery. Pract Proced Aesthet Dent 13:487–493
2. Bowers GM, Vargo JW, Levy B, Emerson JR, Bergquist JJ (1986) Histologic observations following the placement of tricalcium phosphate implants in human intrabony defects. J Periodontol 57:286–287
3. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB (2002) Platelet-rich plasma and bovine porous bone mineral combined with guided tissue regeneration in the treatment of intrabony defects in humans. J Periodontal Res 37:300–306
4. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB (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 Restor Dent 25:49–59
5. Cameron HU (1992) Tricalcium phosphate as a bone graft substitute. Contemp Orthop 25:506–508
6. Christgau M, Moder D, Hiller KA, Dada A, Schmitz G, Schmalz G (2006) Growth factors and cytokines in autologous platelet concentrate and their correlation to periodontal regeneration outcomes. J Clin Periodontol 33:837–845
7. Christgau M, Moder D, Wagner J, Glassl M, Hiller KA, Wenzel A, Schmalz G (2006) Influence of autologous platelet concentrate on healing in intra-bony defects following guided tissue regeneration therapy: a randomized prospective clinical split-mouth study. J Clin Periodontol 33:908–921
8. Cortellini P, Pini PG, Tonetti MS (1996) The modified papilla preservation technique with bioresorbable barrier membranes in the treatment of intrabony defects. Case reports. Int J Periodontics Restor Dent 16:546–559
9. Daculsi G, Passuti N (1990) Effect of the macroporosity for osseous substitution of calcium phosphate ceramics. Biomaterials 11:86–87
10. Daculsi G, Passuti N, Martin S, Deudon C, Legeros RZ, Raher S (1990) Macroporous calcium phosphate ceramic for long bone surgery in humans and dogs. Clinical and histological study. J Biomed Mater Res 24:379–396
11. Damien CJ, Parsons JR (1991) Bone graft and bone graft substitutes: a review of current technology and applications. J Appl Biomater 2:187–208
12. Delecrin J, Takahashi S, Gouin F, Passuti N (2000) A synthetic porous ceramic as a bone graft substitute in the surgical management of scoliosis: a prospective, randomized study. Spine 25:563–569
13. Ducheyne P (1988) Titanium and calcium phosphate ceramic dental implants, surfaces, coatings and interfaces. J Oral Implantol 14:325–340
14. Ducheyne P, Radin S, Heughebaert M, Heughebaert JC (1990) Calcium phosphate ceramic coatings on porous titanium: effect of structure and composition on electrophoretic deposition, vacuum sintering and in vitro dissolution. Biomaterials 11:244–254
15. Ducheyne P, Radin S, King L (1993) The effect of calcium phosphate ceramic composition and structure on in vitro behavior. I. Dissolution. J Biomed Mater Res 27:25–34
16. Dupraz A, Delecrin J, Moreau A, Pilet P, Passuti N (1998) Long-term bone response to particulate injectable ceramic. J Biomed Mater Res 42:368–375
17. Erbe EM, Marx JG, Clineff TD, Bellincampi LD (2001) Potential of an ultraporous beta-tricalcium phosphate synthetic cancellous bone void filler and bone marrow aspirate composite graft. Eur Spine J 10(Suppl 2):S141–S146
18. Hamadouche M, Sedel L (2000) Ceramics in orthopaedics. J Bone Joint Surg Br 82:1095–1099
19. Hollinger JO, Brekke J, Gruskin E, Lee D (1996) Role of bone substitutes. Clin Orthop Relat Res 324:55–65
20. Hossain MZ, Yamada T, Yamauchi K (1989) [Biodegradable ceramic as a bone graft substitute followed by orthodontic tooth movement]. Nippon Kyosei Shika Gakkai Zasshi 48:483–495
21. Kawase T, Okuda K, Wolff LF, Yoshie H (2003) Platelet-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro. J Periodontol 74:858–864
22. Kovacs K, Velich N, Huszar T, Fenyves B, Suba Z, Szabo G (2005) Histomorphometric and densitometric evaluation of the effects of platelet-rich plasma on the remodeling of beta-tricalcium phosphate in beagle dogs. J Craniofac Surg 16:150–154
23. Kovacs K, Velich N, Huszar T, Szabo G, Semjen G, Reiczigel J, Suba Z (2003) Comparative study of beta-tricalcium phosphate mixed with platelet-rich plasma versus beta-tricalcium phosphate, a bone substitute material in dentistry. Acta Vet Hung 51:475–484
24. Legeros RZ (1991) Calcium phosphates in oral biology and medicine. Monogr Oral Sci 15:1–201
25. Legeros RZ (1993) Biodegradation and bioresorption of calcium phosphate ceramics. Clin Mater 14:65–88
26. Lekovic V, Camargo PM, Weinlaender M, Kenney EB, Vasilic N (2001) Combination use of bovine porous bone mineral, enamel matrix proteins, and a bioabsorbable membrane in intrabony periodontal defects in humans. J Periodontol 72:583–589
27. Lekovic V, Camargo PM, Weinlaender M, Vasilic N, Aleksic Z, Kenney EB (2003) Effectiveness of a combination of platelet-rich plasma, bovine porous bone mineral and guided tissue regeneration in the treatment of mandibular grade II molar furcations in humans. J Clin Periodontol 30:746–751
28. Lekovic V, Camargo PM, Weinlaender M, Vasilic N, Kenney EB (2002) Comparison of platelet-rich plasma, bovine porous bone mineral, and guided tissue regeneration versus platelet-rich plasma and bovine porous bone mineral in the treatment of intrabony defects: a reentry study. J Periodontol 73:198–205
29. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR (1998) Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 85:638–646
30. Miranda SR, Nary FH, Padovan LE, Ribeiro DA, Nicolielo D, Matsumoto MA (2006) Use of platelet-rich plasma under autogenous onlay bone grafts. Clin Oral Implants Res 17:694–699
31. Moore WR, Graves SE, Bain GI (2001) Synthetic bone graft substitutes. ANZ J Surg 71:354–361
32. Nagase M, Chen RB, Araya Y, Nakajima T (1991) Evaluation of a bone substitute prepared from alpha-tricalcium phosphate and an acid polysaccharide solution. J Oral Maxillofac Surg 49:1305–1309
33. Okuda K, Kawase T, Momose M, Murata M, Saito Y, Suzuki H, Wolff LF, 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. J Periodontol 74:849–857

34. Palti A, Hoch T (2002) A concept for the treatment of various dental bone defects. Implant Dent 11:73–78
35. Radin S, Ducheyne P, Berthold P, Decker S (1998) Effect of serum proteins and osteoblasts on the surface transformation of a calcium phosphate coating: a physicochemical and ultrastructural study. J Biomed Mater Res 39:234–243
36. Saffar JL, Colombier ML, Detienville R (1990) Bone formation in tricalcium phosphate-filled periodontal intrabony lesions. Histological observations in humans. J Periodontol 61:209–216
37. Snyder AJ, Levin MP, Cutright DE (1984) Alloplastic implants of tricalcium phosphate ceramic in human periodontal osseous defects. J Periodontol 55:273–277
38. Stahl SS, Froum S (1986) Histological evaluation of human intraosseous healing responses to the placement of tricalcium phosphate ceramic implants. I. Three to eight months. J Periodontol 57:211–217
39. Sugaya A, Minabe M, Hori T, Tatsumi J, Watanabe Y, Ikeda K, Numabe Y, Hayashi H, Kamoi K (1990) Effects on wound healing of tricalcium phosphate-collagen complex implants in periodontal osseous defects in the dog. J Periodontal Res 25:60–63
40. Tamura K, Sato S, Kishida M, Asano S, Murai M, Ito K (2007) The use of porous beta-tricalcium phosphate blocks with platelet-rich plasma as an onlay bone graft biomaterial. J Periodontol 78:315–321
41. Trisi P, Rao W, Rebaudi A, Fiore P (2003) Histologic effect of pure-phase beta-tricalcium phosphate on bone regeneration in human artificial jawbone defects. Int J Periodontics Restor Dent 23:69–77
42. Van Winkelhoff AJ, Rodenburg JP, Goene RJ, Abbas F, Winkel EG, de Graaff J (1989) Metronidazole plus amoxycillin in the treatment of *Actinobacillus actinomycetemcomitans* associated periodontitis. J Clin Periodontol 16:128–131
43. Velich N, Nemeth Z, Hrabak K, Suba Z, Szabo G (2004) Repair of bony defect with combination biomaterials. J Craniofac Surg 15:11–15
44. Velich N, Nemeth Z, Toth C, Szabo G (2004) Long-term results with different bone substitutes used for sinus floor elevation. J Craniofac Surg 15:38–41
45. Wachtel H, Schenk G, Bohm S, Weng D, Zuhr O, Hurzeler MB (2003) Microsurgical access flap and enamel matrix derivative for the treatment of periodontal intrabony defects: a controlled clinical study. J Clin Periodontol 30:496–504
46. Weibrich G, Kleis WK, Hafner G (2002) Growth factor levels in the platelet-rich plasma produced by 2 different methods: curasan-type PRP kit versus PCCS PRP system. Int J Oral Maxillofac Surg 17:184–190
47. Whitman DH, Berry RL, Green DM (1997) Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg 55:1294–1299
48. Wiltfang J, Schlegel KA, Schultze-Mosgau S, Nkenke E, Zimmermann R, Kessler P (2003) Sinus floor augmentation with beta-tricalciumphosphate (beta-TCP): does platelet-rich plasma promote its osseous integration and degradation? Clin Oral Impl Res 14:213–218
49. Zerbo IR, Zijderveld SA, de Boer A, Bronckers AL, de Lange G, ten Bruggenkate CM, Burger EH (2004) Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clin Oral Implants Res 15:724–732