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Multi-centre, randomized clinical trial on the efficacy and safety of recombinant human platelet-derived growth factor with β-tricalcium phosphate in human intra-osseous periodontal defects

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Abstract

Aim: The objective of the study was to evaluate the safety and efficacy of a formulation containing recombinant human platelet-derived growth factor (rhPDGF-BB) and β-tricalcium phosphate (β-TCP) in patients with periodontal defects and to compare it with those of β-TCP alone.

Materials and Methods: In this double-blind, prospective, parallel, active-controlled, randomized, multi-centre clinical trial, 54 patients with periodontal osseous defects were randomly assigned to rhPDGF-BB+β-TCP or β-TCP. Following periodontal surgery, respective implantation was performed. The primary and secondary end points of treatment were evaluated at the third and the sixth month.

Results: Among the outcome measures, the extent of linear bone growth ($p < 0.01$) and per cent bone fill ($p < 0.004$) at the sixth month over baseline were significantly higher in the rhPDGF-BB+β-TCP group when compared with the β-TCP group. Similarly, it also resulted in significantly higher area under the curve clinical attachment level gain at 0–6 months ($p < 0.01$), CAL gain and greater reduction in probing depth at the third and the sixth month than that with β-TCP treatment alone. The incidence of adverse events was similar in both the groups and no serious adverse events were reported in any of the patients.

Conclusions: rhPDGF-BB+β-TCP is safe and effective in the treatment of periodontal defects. It increases bone formation and soft tissue healing (clinicaltrials.gov, number NCT00496847; CTRI No.: CTRI/2008/091/000152).

Key words: β-TCP; implantation; periodontal osseous defects; platelet-derived growth factor; randomized clinical trial

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Conflict of interest and source of funding statement

Virchow Biotech supplied investigational drugs – rhPDGF-BB+β-TCP and β-TCP. There was no financial assistance from any other source.

Experimental and clinical studies have shown that purified recombinant human platelet-derived growth factor (rhPDGF-BB) is a potent wound-healing agent and is widely being used in the treatment of diabetic foot ulcers (Smiell et al. 1999). Studies have also shown that rhPDGF has mitogenic and chemotactic effects on periodontal ligament and alveolar bone cells (Lynch et al. 1991, Matsuda et al. 1992, McAllister et al. 1993, Nevins et al. 2005). The beneficial role of rhPDGF in regeneration of bone, periodontal ligament and cementum is documented in

several animal and human studies (Matsuda et al. 1992, Park et al. 1995, Howell et al. 1997, Camelo et al. 2003, Nevins et al. 2003). In vitro studies have shown that the local application of PDGF-BB stimulates periodontal ligament cells to adhere to periodontitis-affected root surfaces (Gamal et al. 1998, Gamal & Mailhot 2000). It is suggested that rhPDGF exerts its action on periodontal tissues by increasing the release of pyridinoline cross-linked carboxyterminal telopeptide of type I collagen, a biomarker of bone turnover (Sarment et al. 2006). Histological evidence of regeneration in human teeth and the formation of new attachment following the use of rhPDGF+β-TCP in four cases of advanced furcation-involved teeth has been reported recently (Mellonig et al. 2009). The local application of PDGF has been shown to stimulate and release vascular endothelial growth factor, resulting in improved angiogenesis and wound healing (Cooke et al. 2006).

In the treatment of periodontal defects, current practice includes the use of alloplastic materials such as β-TCP, which is a synthetic osteoconductive material. Earlier histological studies suggested its osteogenic potential (Saffar et al. 1990). A recent systematic review has also indicated that bone replacement grafts led to demonstrable clinical improvements in periodontal osseous defects (Reynolds et al. 2003). Although β-TCP does not form new connective tissue, it provides a scaffold for new bone formation and also facilitates the stabilization of the blood clot (Szpalski & Gunzburg 2000). As β-TCP is porous, it entraps growth factors such as rhPDGF within its micropores, thereby prolonging their action (Urist et al. 1984). Histological studies have shown that the combination of purified rhPDGF and bone allograft could result in substantial periodontal regeneration (Camelo et al. 2003). In vitro studies show that bone allografts play a synergistic role in the mitogenic effects of PDGF on human periodontal ligament cells. Hence, it is suggested that these might be useful as an adjuvant in the treatment of periodontal defects (Papadopoulos et al. 2003). The combined use of rhPDGF-BB with graft biomaterials has shown beneficial effects in intra-osseous defects (Trombelli & Farina 2008).

The efficacy and safety of rhPDGF+β-TCP in periodontal osseous defects were tested in a large multicentre trial (Nevins et al. 2005). rhPDGF has been developed in India, using recombinant technology through the insertion of the gene for the B chain of human platelet-derived growth factor into the bacteria *Escherichia coli*, and its safety and efficacy have been established in the treatment of diabetic foot ulcers (Hardikar et al. 2005). Periodontal applications of this new material in combination with β-TCP are investigated in this phase III study as genetic and environmental factors are known to influence drug metabolism and drug response (Barbour et al. 1997, Wilkinson 2005).

Materials and Methods

Subject population

The study criteria were similar to those followed in a pivotal study (Nevins et al. 2005). Systemically healthy male and female patients, aged between 25 and 75 years, with periodontal defects having a probing depth (PD) of $\geqslant$7 mm at base line; radiographic evidence of an intra-osseous defect depth of $\geqslant$4 mm and base of the defect $\geqslant$3 mm coronal to the apex of the tooth, adequate keratinized tissue at the operative site ($\geqslant$3 mm) and teeth that were vital were included in the study. Patients were excluded on the following grounds: if they failed to maintain adequate oral hygiene [plaque index (PI) > 2]; pregnant and lactating women; previous periodontal surgery on treatment-targeted teeth within the preceding year; tooth mobility with grade III; study tooth exhibiting a class III furcation defect; clinical signs of untreated acute infection at the surgical site; smokers who smoked more than 20 cigarettes per day or current users of tobacco in any smokeless form; known allergy to *E. coli*-derived products; or using rhPDGF or β-TCP in any form within the previous 30 days. A written informed consent was obtained from the patients. The study protocol conformed to ethical guidelines in accordance with the Declaration of Helsinki and ethical guidelines for biomedical research on human participants issued by Indian Council of Medical Research, New Delhi, 2006. The study protocol was approved by the Ethics Committees of the respective investigational centres.

Study design

A double-blind, active-controlled, randomized, parallel, multi-centre, prospective, phase III study was conducted with the approval of the Drug Controller General of India, at three centres in India from July 2007 to August 2009, to assess the efficacy of rhPDGF-BB+β-TCP in comparison with β-TCP alone for the treatment of periodontal defects. A single intra-osseous defect was treated in each patient.

Investigators' meeting and calibration

Before the actual start of the study, a meeting of the investigators was conducted to appraise the study protocol and standardize the clinical, radiological, surgical and post-surgical protocols of the study. The reproducibility and consistency of pre- and post-intervention parameters were ensured during subsequent sessions, where the investigators went through intra- and inter-examiner calibration sessions. The clinical parameters were measured by four investigators (R. S., S. V., B. K., S. D.). These investigators had taken measurements at six probing sites per tooth and the measurements were repeated after 24 h on teeth with $\geqslant$4 mm/depth. From these measurements, the inter-examiner reliability was assessed using the κ coefficient, and the value was found to be 0.8. Investigators (N. A., K. R.), who were totally unaware of the procedure to which the patient was subjected, examined representative sample of pre- and post-surgical radiographs, and from these, the inter-examiner reproducibility was assessed and a variability of 4% was observed.

Randomization and allocation concealment

Sixty-eight patients with chronic periodontitis and radiographic evidence of intra-bony defects were initially screened, and 54 eligible patients were randomly assigned at the time of surgery (visit 3) to the β-TCP group ($n = 27$) or the rhPDGF-BB+β-TCP group ($n = 27$) in a ratio of 1:1. The biostatistician generated the random numbers using block randomization with block sizes of four using SAS program and allocated eligible patients either to group 1 or to group 2. Codes were kept in a sealed cover, with Virchow Biotech (Virchow Biotech, Hyderabad, India), till the completion of the trial. All the investigators enrolled the eligible patients and assigned them at the time of implantation into group 1 and group 2 based on the randomized schedule generated for the purpose. The implantation device was

distributed in a numbered container to all three centres. Neither the patients nor the investigators who implanted the investigational drugs and also those who assessed the outcomes were aware of the group assignment, thereby ensuring double blindness. Unblinding and comparison of both treatment options were performed only after the results of the clinical measurements and radiological parameters were available, and the data were subjected to statistical analysis.

Clinical parameters

To record the clinical parameters, a customized acrylic occlusal stent with a guide groove was fabricated for each patient to fit over the selected tooth and the adjacent teeth. This provided a fixed reference point and fixed angulation for measurements at each site over the entire duration of the study.

The clinical parameters, which included PI, gingival index (GI), PD, clinical attachment level (CAL) and gingival recession (GR) were recorded to the nearest millimetre with the help of a UNC-15 probe at baseline, 3 and 6 months.

Radiographic parameters

Radiographic evaluation of the study was carried out on radiographs taken at baseline and at the end of sixth month (time frames of the study). All the radiographs were taken using the paralleling technique, using the Rinn XCP device. Radiographs were digitized and analysed using AutoCAD 2006 software (Fig. 1).

The anatomical landmarks for the study included cementoenamel junction (CEJ), alveolar crest (AC) and base of the defect (BD) (Schei et al. 1959). Defect depth was measured by drawing an auxiliary line in the direction of the tooth axis and a second auxiliary line (AUX2) perpendicular to the first was drawn from the AC. The defect depth was measured as the distance from the point where the AUX2 crossed the root surface to the BD (Eickholz et al. 2004).

The distance between CEJ and the root apex was also measured and if any difference in this measurement existed between the baseline and the sixth month radiograph, the measurements were corrected for distortion.

The radiographic parameters evaluated were linear bone growth (LBG) and per cent bone fill (%BF). LBG was calculated by subtracting the CEJ to the BD value at the end of 6 months from CEJ to BD at baseline. The %BF was calculated by dividing LBG by radiographic defect depth at baseline.

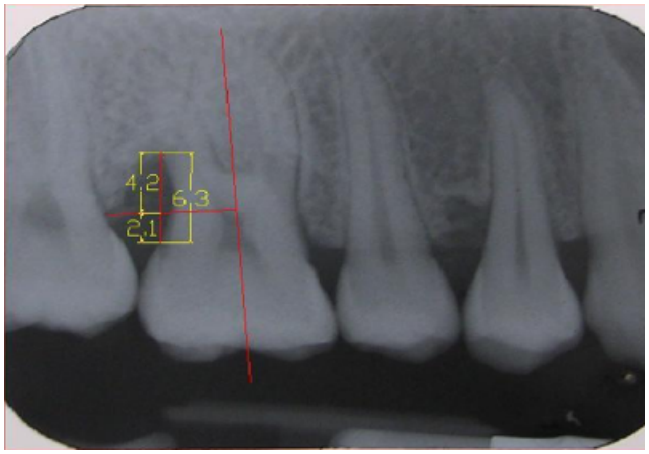

Fig. 1. Measurement of bony defect using AutoCAD software and anatomical reference points (at baseline).

The total study period included three phases – the pre-implantation phase (6 weeks), the implantation phase and the post-implantation phase (6 months) (Fig. S1). During the pre-implantation phase, scaling and root planing were performed. Implantation of rhPDGF-BB (0.3 mg/ml)+β-TCP (0.5 g) or β-TCP (0.5 g) alone was performed during visit 3, which was considered as the baseline visit. Both preparations were formulated in sodium acetate buffer pH 5.5. The study was double-blind and active-controlled but not placebo controlled. Both groups of patients received β-TCP, which was supplied as a powder (0.5 g) in a small sterile bottle (Fig. S2). The powder was mixed with the contents from a prefilled syringe containing either PDGF-BB, the active agent, suspended in sodium acetate buffer pH 5.5 at a concentration of 0.3 mg/ml (the test group), or only sodium acetate buffer. Both the liquids in the prefilled syringes looked similar and the investigators operating on the patient were asked to use the kits as per the block randomization generated for each centre. During the post-implantation phase, all the patients were followed up for 6 months involving five visits at weeks 1, 3, 6 and at 3 and 6 months, respectively (Fig. S3).

Surgical procedure

The surgical procedure included a pre-surgical rinse, administration of local anaesthesia (2% Xylocaine with 1; 80,000 adrenaline) and raising a full-thickness mucoperiosteal flap on both facial and palatal/lingual sides. A thorough surgical debridement of the osseous defect was performed using Gracey curettes. The roots were planed thoroughly and the root surfaces were treated for 3 min. with a tetracycline paste prepared by mixing the contents of one tetracycline hydrochloride 250 mg capsule with a small amount of sterile saline to a paste consistency (Nevins et al. 2003).The paste was carefully applied to the root surface avoiding excessive overflow of the paste onto adjacent bony sites. Following root conditioning, the wound was rinsed thoroughly using sterile saline.

Once the debridement was over, direct measurements of the osseous defect were taken using a UNC-15 periodontal probe. The vertical bone depth (from the bottom of the defect to the AC), the bone defect width and the number of bony walls present were noted. Besides, the distance from the bottom of the defect to the clinically visible CEJ was also measured. If the vertical bone defect depth was $\geqslant$ 4mm, final subject eligibility was confirmed (Fig. 2).

A coded study kit was selected for each patient according to the randomization schedule. β-TCP was mixed with rhPDGF suspended in sodium acetate buffer or only buffer and left for 10 min. to facilitate binding of the rhPDGF-BB protein with β-TCP particles. Pre-suturing was performed before placement of the graft. The required quantity of the graft was delivered in increments into the osseous defect with light pressure. The entire defect was filled avoiding overfilling or underfilling (Fig. 3). The pre-sutured mucoperiosteal flaps

were repositioned and secured with interrupted 3-0 braided silk sutures (Fig. 4). The surgical area was protected and covered with a non-eugenol (Coe Pack, GC American Inc., Alsip, IL, USA) periodontal dressing.

During post-operative care, Amoxicillin 500 mg, three times daily for 5 days, and Ibuprofen (400 mg)+Paracetamol (500 mg), three times daily for 3 days, were prescribed to all patients. One week after surgery, the periodontal dressing was removed and the area was irrigated gently with saline and sutures were removed. Symptoms regarding discomfort, pain, swelling and fever were enquired into and recorded. Periodontal assessment included recording of GR, GI and PI. A radiograph was taken to check the status of the graft. Probing was avoided for 3 months post-surgery. Oral hygiene instructions were reinforced.

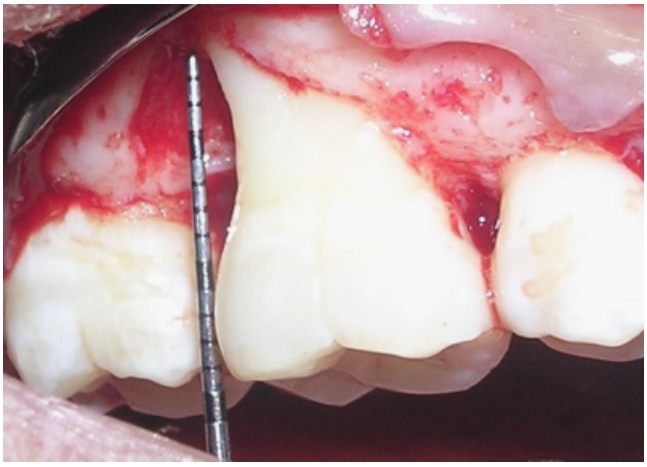

Fig. 2. Surgical site after debridement.

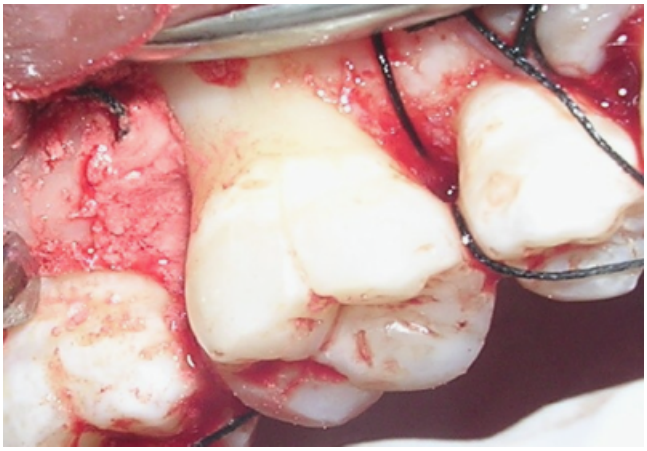

Fig. 3. Intra-osseous defect implanted with a graft.

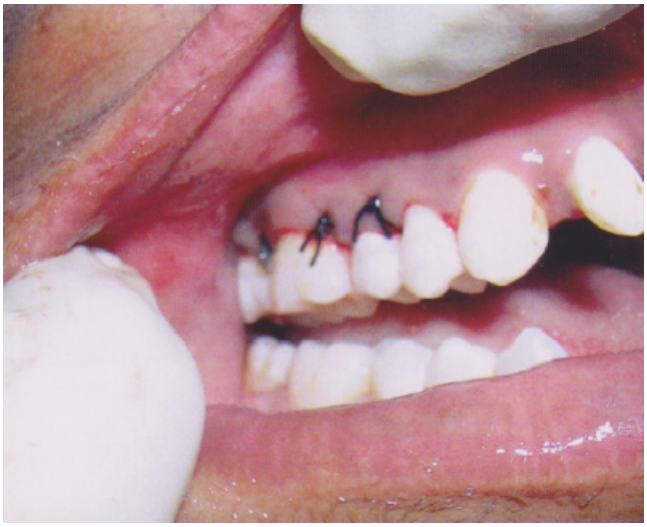

Fig. 4. Mucoperiosteal flap closure.

The post-implantation visits were scheduled to monitor safety and efficacy. Safety was assessed based on periodontal parameters including pain, swelling, mobility and delayed soft tissue healing. In addition, vital signs (temperature, heart rate, respiratory rate, blood pressure) and the incidence and severity of adverse events were also monitored. Haematological parameters such as haemoglobin, total blood picture and prothrombin time were measured at baseline and at the end of the study. Besides, biochemical tests were conducted to monitor blood glucose, renal and liver functions at the first (screening), third (implantation) and eight (termination) visits. Efficacy was assessed by taking clinical and radiographic parameters into account.

Study outcomes

The primary end points considered were the extent of LBG and %BF at the sixth month and area under the curve (AUC) of CAL gain (0–6 months). The secondary end points were CAL gain, changes in PD and GR at the third and the sixth month over baseline and incidence of adverse events.

Sample size and statistical analysis

Based on the reported extent of LBG of 0.8 ± 1.71 mm in the control group (β-TCP) and 2.52 ± 1.96 mm in the drug group (rhPDGF-BB+β-TCP 0.3 mg/ml) of the pivotal study by Nevins et al. 2005, the number of subjects needed per treatment group was calculated, using the following assumptions: 5% level of significance, 80% power (two-sided) and 20% attrition. It was observed that 25 patients in each of the two groups, when randomly allocated, would meet the statistical power. Using this computation in the present trial, 54 patients

were enrolled, with 27 patients in each group.

Data on baseline characteristics were analysed for differences between β-TCP- and rhPDGF-BB+β-TCP-treated groups using a *t*-test for independent samples and a proportion *Z*-test for radiographic parameters. Differences between the β-TCP and rhPDGF-BB+β-TCP groups for the extent of LBG at 6 months, AUC CAL gain (0–6 months), CAL gain, PD, GR and safety parameters, such as haematological, biochemical and vital parameters, were tested by an independent-samples Student's two-tailed *t*-test; differences in %BF at the sixth month and incidence of adverse events between β-TCP and rhPDGF-BB+β-TCP groups were analysed using a proportion *Z*-test; and changes in PD and GR between β-TCP and rhPDGF-BB+β-TCP groups were tested using the Mann–Whitney non-parametric test. All tests were performed using SPSS statistical software, version 16.0.

Table 1. Demographic characteristics of ITT patients

	β-TCP group	rhPDGF-BB+β-TCP group	*p* value
No. of Patients	27	27	
No. of Males	12 (44.4%)	13 (48.1%)	1.0
Age (years)*	30.9 ± 5.1	32.6 ± 7.3	0.327
Weight (kg)*	58.63 ± 10.4	60.48 ± 10.3	0.515
Height (cm)*	162.37 ± 7.2	163.04 ± 7.6	0.742
BMI*	22.12 ± 2.8	22.60 ± 2.7	0.526
Smokers	3 (11.1%)	3 (11.1%)	1.0

Values are number (percentage).
*Values are mean ± SD.
BMI, body mass index; β-TCP, β-tricalcium phosphate; rhPDGF-BB, recombinant human platelet-derived growth factor.

Table 2. Clinical and radiographic parameters of patients at baseline

Parameter	β-TCP group (*n* = 27)	rhPDGF-BB+β-TCP group (*n* = 27)	*p* value
CAL (mm)	7.8 ± 1.2	8.4 ± 2.5	0.304
Probing depth (mm)	7.7 ± 1.9	8.7 ± 1.9	0.087
Gingival recession (mm)	0.1 ± 0.4	0.4 ± 1.0	0.166
Gingival index	0.3 ± 0.3	0.3 ± 0.2	0.754
Plaque index	0.5 ± 0.2	0.6 ± 0.2	0.666
Defect location – Arch wise*			
Maxillary	10 (37.0%)	11 (40.7%)	1.0
Mandibular	17 (63.0%)	16 (59.3%)	1.0
Defect tooth*			
Multi-rooted	23 (85.2%)	21 (77.8%)	0.716
Single-rooted	4 (14.8%)	6 (22.2%)	0.726
Osseous defect depth (mm)	6.7 ± 1.9	6.3 ± 1.9	0.447
Osseous defect width (mm)	3.2 ± 0.6	3.2 ± 0.7	0.784
Osseous defect morphology*			
1-wall	4 (14.8%)	5 (18.5%)	1.0
2-wall	6 (22.2%)	7 (25.3%)	1.0
3-wall	10 (37.0%)	9 (33.3%)	1.0
Combined	7 (25.9%)	6 (22.2%)	1.0

Values are mean ± SD.
*Values are number (percentage).
β-TCP, β-tricalcium phosphate; CAL, clinical attachment level; rhPDGF-BB, recombinant human platelet-derived growth factor.

Results

Sixty-eight patients with chronic periodontitis and radiographic evidence of intra-bony defects were initially screened; among them, 54 patients were enrolled, and 50 completed the total study period. One in each group was lost to follow-up during the 6-month study period due to reasons not related to the treatment. Radiological films of two other patients were not of diagnostic quality. Despite this, the clinical parameters of these two patients were also included for efficacy assessment. Each patient underwent one surgical procedure involving one defect only. Thus, a total number of 50 defects in 25 patients in rhPDGF-BB+β-TCP and 25 patients in the β-TCP group were available for statistical evaluation. This represented 92.6% of the patients recruited. All data were subjected to statistical analysis using an intent-to-treat approach last observation carried forward.

The demographic characteristics, clinical and radiographic parameters of all randomized patients assigned to two groups at baseline are presented in Tables 1 and 2. There were no statistically significant differences in the demographic characteristics, clinical and radiographic parameters between the two groups at baseline. Majority of teeth presenting the defects were multi-rooted in both groups (Table 2). The distribution of teeth in each group, according to the presence of furcation lesions, was similar, i.e. 17 (63%) patients had either grades I or II furcation in each group.

The LBG was significantly ($p<0.01$) higher in the rhPDGF-BB+β-TCP group at the end of 6 months post-surgery, compared with that in the β-TCP group (Fig. 5). Similarly, the %BF was also significantly ($p<0.004$) higher in the rhPDGF-BB+β-TCP group compared with that in the β-TCP group (Fig. 6). The changes, reflecting bone formation in radiographs of a representative patient treated with rhPDGF-BB+β-TCP, are depicted in Figs 7 and 8.

CAL gain, which reflects soft tissue healing, was significantly higher in the rhPDGF-BB+β-TCP group compared with that in the β-TCP group both at 3 months ($p<0.05$) and at 6 months ($p<0.005$) (Fig. 9). As a result, AUC CAL gain at 0–6 months was significantly higher ($p<0.01$) in the rhPDGF-BB+β-TCP group (14.9 ± 3.0 mm-months) than that in the β-TCP group (12.7 ± 3.2 mm-months). The representative photographs and radiographs of β-TCP alone are included in the electronic version (Figs S4–S8).

The mean reduction in the PD from baseline to the third month ($p<0.04$) and the sixth month ($p<0.005$) was significantly higher in the rhPDGF-BB+β-TCP group compared with that in the β-TCP group (Fig. 10). However, there were no significant differences in the mean change in GR from baseline to 3 months (0.44 ± 0.77 *versus* 0.52 ± 0.71 mm) and 6 months (0.44 ± 0.77 *versus* 0.54 ± 0.73 mm) between the rhPDGF-BB+β-TCP group and the β-TCP group, respectively.

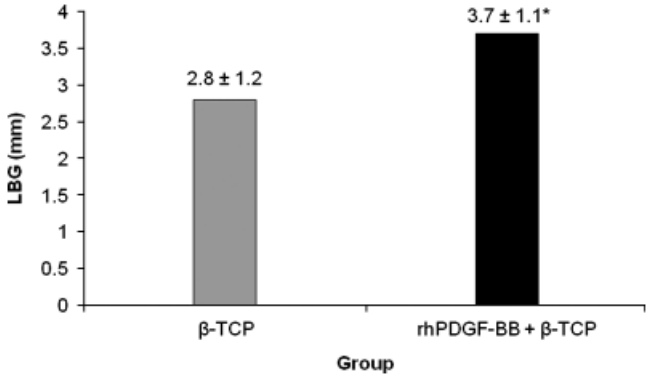

Fig. 5. Comparison of linear bone growth at the sixth month in β-tricalcium phosphate (β-TCP) alone ($n = 25$) and recombinant human platelet-derived growth factor (rhPDGF-BB)+β-TCP ($n = 25$) groups ($^*p < 0.01$).

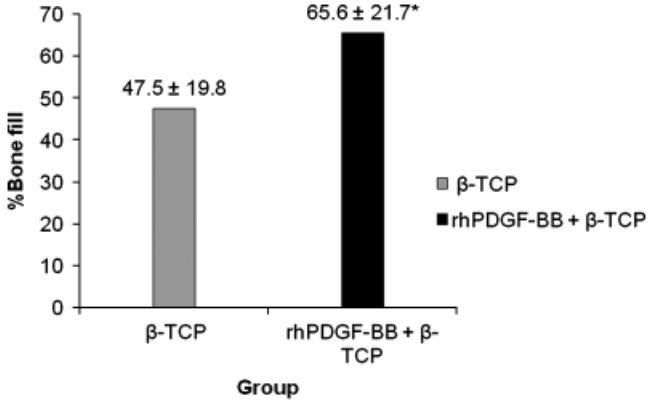

Fig. 6. Comparison of per cent bone fill at the sixth month in β-tricalcium phosphate (β-TCP) alone ($n = 25$) and recombinant human platelet-derived growth factor (rhPDGF-BB)+β-TCP ($n = 25$) groups ($^*p < 0.004$).

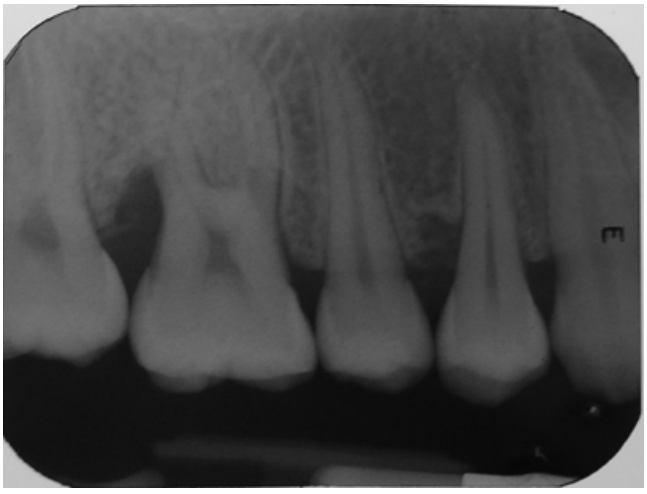

Fig. 7. Radiograph at the baseline period.

Subgroup analysis of %BF was also performed to assess the effects of defect morphology. The %BF was significantly ($p < 0.02$) higher in the rhPDGF-BB+β-TCP group compared with that in the β-TCP group in all defect types. The analysis demonstrated that rhPDGF-BB+β-TCP treatment improved bone fill in all defect types (Fig. S9).

As there was a 1 mm difference in the mean PD between the two treatment groups, covariate analysis was performed for all outcomes (LBG, %BF, AUC CAL gain, CAL gain, change in PD and GR) (Table S1). The significant levels, which were observed earlier, were not altered, thereby indicating that mean differences in PD of 1 mm between two groups had no detrimental effects on the outcome measures.

Implantation of rhPDGF-BB+β-TCP or β-TCP alone was safe and well tolerated. There were no serious adverse events in any of the patients. Only minor side effects such as fever, pain, swelling of gums and tooth mobility were reported immediately following implantation. In the present study, during visits 3–8, 13 patients – 4 (30.8%) patients in the rhPDGF-BB+β-TCP group and 9 (69.2%) patients in the β-TCP group – reported 19 adverse events, which indicated that some of the patients had more than one adverse event (Table 3). Proportion *Z*-test showed that the percentage of patients with adverse events was not significantly different between the rhPDGF-BB+β-TCP group and the β-TCP group. In addition, results on safety showed that both treatments had no effect on periodontal parameters including pain, swelling, mobility and delayed soft tissue healing. Vital signs, and haematological and biochemical parameters studied during the different visits, following implantation, were not significantly different from baseline.

Discussion

Fifty-four subjects were enrolled and randomly divided into the rhPDGF-BB+β-TCP group and the β-TCP group, each group containing 27 patients. Among the 54 patients who were randomized, there was an excellent compliance and 50 (92.6%) patients (25 patients in each rhPDGF-BB+β-TCP group and β-TCP group) completed the study. One patient in the rhPDGF-BB group could not spare time and excused herself (telephonically) at visit 7. Another patient in the β-TCP group moved away to a distant place and could not continue his participation in the study after visit 6. Two patients, one in each group, were not considered at the final recall because of the poor quality of their radiographs. However, the clinical parameters of these patients were considered for statistical analysis. Data obtained in the presented study were subjected to both ITT and per-protocol analysis to see whether it would influence the statistical interferences of the

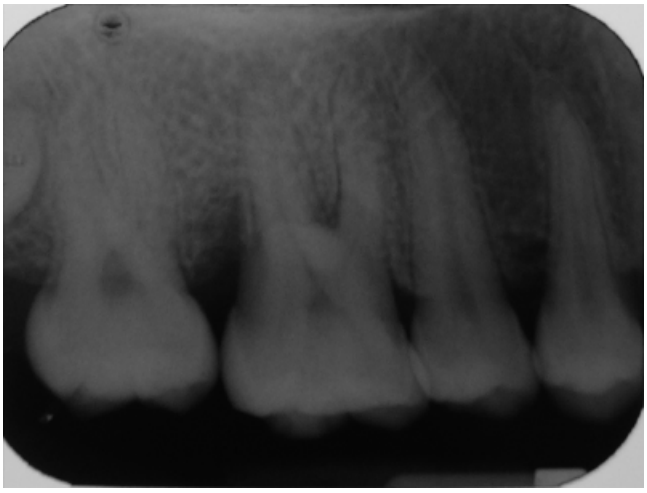

Fig. 8. Same patient after 6 months treated with recombinant human platelet-derived growth factor and β-tricalcium phosphate.

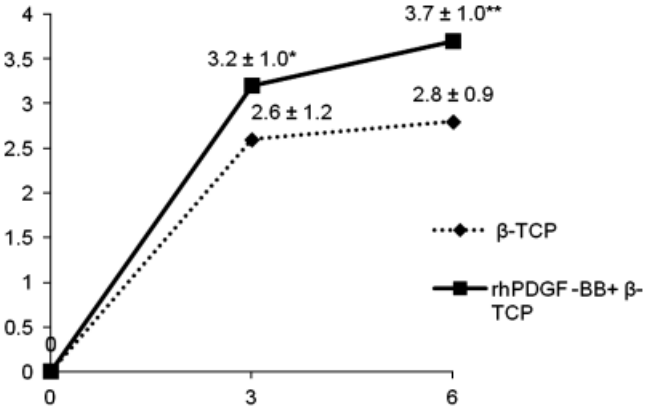

Fig. 9. Mean CAL gain at 3 and 6 months over baseline in β-tricalcium phosphate (β-TCP) alone ($n = 27$) and recombinant human platelet-derived growth factor (rhPDGF-BB)+β-TCP ($n = 27$) groups ($^{*}p < 0.05$; $^{**}p < 0.005$).

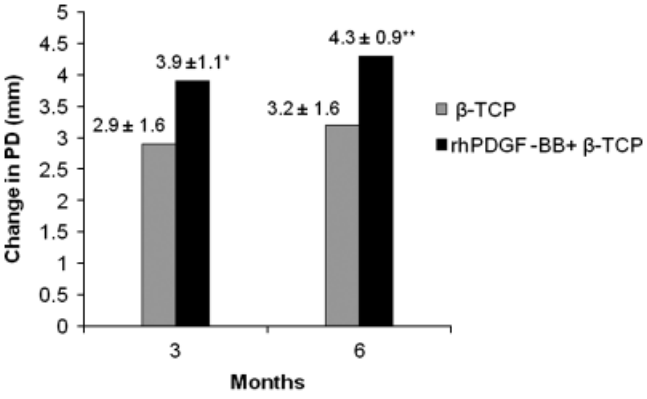

Fig. 10. Changes in probing depth (PD) at the third and sixth month over baseline in β-tricalcium phosphate (β-TCP) alone ($n = 27$) and recombinant human platelet-derived growth factor (rhPDGF-BB)+β-TCP ($n = 27$) groups ($^{*}p < 0.04$; $^{**}p < 0.005$).

outcome measures. However, the results of both statistical methods were essentially similar. No patient was lost to follow-up due to incidence of any adverse events.

The patients in the rhPDGF-BB+β-TCP group received implantation of a combination of β-TCP and 0.3 mg/ml of rhPDGF-BB with buffer. It was shown earlier that rhPDGF-BB 0.3 mg/ml concentration was more effective than 1.0 mg/ml concentration on both clinical and radiographic parameters such as CAL gain, LBG and %BF (Nevins et al. 2005). The efficacy of rhPDGF-BB+β-TCP, as assessed by clinical and radiological parameters, was significantly higher over β-TCP in all centres. Considering the effect of centre as a covariate, data analysis indicated that there were no significant differences in the outcome measures between the centres.

In the pivotal study by Nevins et al. 2005, the average age of the patients was 49.4–52.8 years, whereas in the present study, subjects were comparatively younger (mean age 31.8 years). Although patients between 25 and 75 years were eligible, 80% of patients were in the age range of 25 and 36 years. As a result, the mean age of the patients was 32.6 years in the rhPDGF-BB+β-TCP group and 30.9 years in the β-TCP group. This implies that the majority of patients who participated in the study fall under the category of aggressive periodontitis. In the United States or in most developed and affluent countries, a large number of the elderly also seek periodontal treatment and opt for surgery, while in India, except for the young and middle aged, elderly people are neither aware of nor willing to carry out comprehensive periodontal care. The pivotal study referred to also has an assortment of ethnic groups while the present study consisted of a cohesive group of Indians. These demographic differences constitute a basic departure from the pivotal study.

Results of using 0.3 mg/ml rhPDGF-BB+β-TCP when compared with β-TCP alone, the improvements in LBG, %BF, CAL gain at 6 months, AUC CAL gain 0–6 months, reduction in PD and GR were in accordance with those reported in the pivotal study (Nevins et al. 2005).

Parameters of bone formation such as LBG and %BF were measured only at 6 months, as complete bone maturation and mineralization do not occur by the third month. The rhPDGF-BB+β-TCP combination significantly improved parameters of bone formation at 6 months (Figs 7 and 8) and soft tissue outcomes at the third and the sixth month compared with those of the β-TCP group (Figs 9 and 10). In the present study, great majority of the considered defects were located on multi-rooted teeth with a buccal extension.

Table 3. Number (%) of patients with common adverse events in β-TCP and rhPDGF-BB+β-TCP groups

Adverse Event	β-TCP No. of patients (%)	rhPDGF-BB+β-TCP No. of patients (%)	*p* value
Fever	1 (6.7%)	0	–
Pain	3 (20%)	3 (75%)	0.14
Swelling of gums	2 (13.3%)	0	–
Tooth mobility	9 (60%)	1 (25%)	0.5
Total number of adverse events	15	4	–
Total number of patients with adverse events	9 (36%)	4 (16%)	0.2

β-TCP, β-tricalcium phosphate; rhPDGF-BB, recombinant human platelet-derived growth factor.

Irrespective of bone defect morphology, the combination of rhPDGF-BB+β-TCP increased bone formation.

While it is well recognized that morphological defects play an important role in healing and successful outcomes of regenerative technologies and they are also approach-specific (Tonetti et al. 1993, Cortellini & Tonneti 2000, Tonetti et al. 2004), it is also known that furcation-involved teeth are expected to heal less well than those without. However, randomization in the present study has ensured the distribution of equal number of patients with furcation or without furcation in the two treatment groups. Similar number of teeth i.e., 17(63%) in each group had furcation of either grade I or grade II. If furcation had detrimental effects, it would have influenced both groups to a similar extent.

In the evaluation of the efficacy of periodontal therapy, improvement in soft tissue healing as well as enhanced bone formation are critical. Implantation of a combination of rhPDGF-BB+β-TCP has resulted in a significant improvement in both these aspects. However, there was no significant correlation between the parameters reflecting soft tissue healing (CAL gain) and bone formation (%BF).

β-TCP has been shown to have osteogenic potential (Saffar et al. 1990). Therefore, in this study, it was used as an active control as well as in the new formulation of rhPDGF-BB. In this study, β-TCP was used as a positive control group rather than open-flap debridement. Improvements observed in the present study with reference to clinical and radiographic parameters cannot be attributed to open-flap debridement that was performed before implantation of β-TCP as it supports only modest gains in clinical attachment compared with bone replacement grafts (Reynolds et al. 2003, Trombelli & Farina 2008). In this study, tetracycline root conditioning was carried out to render the root surface more conducive for effective growth factor utilization (Madison & Hokett 1997, Shetty et al. 2008). The conditioned root surface may have also served as an additional slow-release delivery system of PDGF-BB, thereby enhancing periodontal regeneration (Lioubavina-Hack et al. 2005). With reference to the surgical technique, braided silk 3-0 was used although its wicking effect is known. However, any such effect was preempted by a properly adopted periodontal dressing, which was placed in all the patients.

The next question that arises is whether the initial beneficial effects in clinical and radiographic parameters, observed with rhPDGF-BB+β-TCP treatment either at the third or at the sixth month, are transient or sustained. A recently published study, although limited to results of four cases, has shown that CAL gain, LBG and %BF observed at the sixth month were maintained even at 18 and 24 months post-surgery (McGuire et al. 2006).

Apart from efficacy, this study also examined the safety aspect of the test product containing rhPDGF-BB+β-TCP. No serious adverse events were observed in both treatment groups, except for minor side effects. There were no statistically significant differences in the vitals, and haematological and biochemical parameters at different visits from baseline. There were no serious adverse events in any of the patients. Although the incidence of adverse events in the β-TCP group was twice that in the test group, the two-tailed *Z*-test for proportions with a confidence interval of 95% showed that these differences were not significantly different between the two groups (Table 3). Although PDGF has been shown to be safe and efficacious, the search for further refinements and novel factors for periodontal regeneration continues. Recently, recombinant human growth/differentiation factor-5 in β-TCP was reported to have significantly enhanced cementum formation and regenerated bone height in Beagle dogs as compared with PDGF. However, its safety and efficacy needs to be evaluated in human clinical trials (Kwon et al. 2010).

In conclusion, it can be assumed that implantation of rhPDGF-BB+β-TCP in intra-osseous periodontal defects was safe, well tolerated and resulted in clinically and statistically significant improvements in bone formation parameters as well as soft tissue outcomes. The product can be incorporated as part of a surgical regimen to treat intra-ossoeous defects.

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Supporting information

Additional supporting information regarding the following may be found in the online version of this article:

Study time-line (Fig. S1), β-TCP bottle and prefilled test or placebo gel syringes (Fig. S2), consort diagram (Fig. S3), photographs, radiographs and surgical procedure of a β-TCP alone case (Fig. S4–S8), per cent bone fill according to defect type (Fig. S9), baseline values of clinical parameters (Table S1).

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Clinical Relevance

Scientific rationale: rhPDGF is known to promote the regeneration of bone, periodontal ligament and cementum in humans. β-TCP provides a scaffold for new bone formation. Therefore, this study examined the efficacy of implantation of this combination in 54 patients with periodontal osseous defects.

Principal findings: rhPDGF-BB+β-TCP significantly increased bone formation as measured by LBG, %BF and soft tissue healing as measured by CAL and AUC CAL gain at the end of the third and the sixth month.

Practical implications: Periodontal disease is highly prevalent in India and this treatment approach could be of some assistance in the management of periodontal disease with intra-osseous defects.