Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2009; 36: 581–588 doi: 10.1111/j.1600-051X.2009.01428.x

Journal of Clinical Periodontology

A randomized clinical trial of autologous platelet-rich plasma in the treatment of mandibular degree II furcation defects

Avani Rangaraju Pradeep, Sujatha Pai, Garima Garg, Prapulla Devi and Sharath Kumara Shetty

Department of Periodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India

Pradeep AR, Pai S, Garg G, Devi P, Shetty SK. A randomized clinical trial of autologous platelet rich plasma in treatment of mandibular degree II furcation defects. J Clin Periodontol 2009; 36: 581–588. doi: 10.1111/j.1600-051X.2009.01428.x.

Abstract

Aim: A combined technique using a platelet-rich plasma (PRP)/bovine porous bone mineral/guided tissue regeneration membrane was found to be an effective modality for the treatment of mandibular grade II furcation defects. To elucidate the role played by each component, the present randomized, double-blind study is designed to evaluate the effectiveness of autologous PRP alone in the treatment of mandibular degree II furcation defects compared with open flap debridement (OFD).

Material and Methods: Using a split-mouth design, 40 mandibular degree II furcation defects were treated either with autologous PRP or OFD. Plaque index, sulcus bleeding index, vertical probing depth, relative vertical and horizontal clinical attachment level and gingival marginal level were recorded at baseline and 6 months post-operatively. Vertical and horizontal defect depths were also recorded using spiral computed tomography.

Results: A statistically significant difference was observed in all the clinical and radiographic parameters at the sites treated with PRP as compared with those with OFD. However, all the furcation defects retained their degree II status.

Conclusion: Despite a significant improvement, lack of complete closure of furcation defects implies a limited role of autologous PRP as a regenerative material in the treatment of furcation defects, necessitating further long-term studies.

Key words: furcation defects; open flap debridement; platelet-rich plasma; randomized clinical trial; spiral CT

Accepted for publication 5 April 2009

Furcations are frequently not accessible for adequate professional debridement, because their entrance is very small for the size of periodontal instruments, and they present with ridges, convexities and concavities that frequently render the defect impossible to instrument effectively (DeSanctis & Murphy 2000).

Conflict of interests and source of funding:

The authors have no conflict of interests. The present study was partly funded by Colgate Research Grant, Colgate-Palmolive India Ltd., Mumbai, India.

Regeneration of the periodontium within the furcation defect is considered one of the most challenging aspects of periodontal therapy (Newell 1998). Despite anatomical limitations, reports in the literature have demonstrated a reduction of probing depth and a gain in the clinical attachment level of Class II furcal invasions using coronally positioned flaps (Fuentes et al. 1993), hard tissue grafts (Brunsvold & Mellonig 1993) and guided tissue regeneration (GTR) with and without various grafting materials (Pontoriero et al. 1988, De Leonardis et al. 1999, Lekovic et al. 2003). Recent literature also supports the use of enamel matrix derivatives with or without a combination of bone graft (Aimetti et al. 2007, Chitsazi et al. 2007). However, only GTR therapy has provided histological evidence, in both animals and humans, that clinical resolution of the defect corresponds to regeneration of the supporting apparatus within a previously exposed furcation area (Gottlow et al. 1986, Caffesse et al. 1990a, b).

There has been an increasing interest in polypeptide growth factors (PGF) in the field of periodontal regeneration. PGFs [i.e., platelet-derived growth factor (PDGF), transforming growth

factor-β (TGF-β) and insulin-like growth factor (IGF)] are biologic mediators that have the ability to regulate cell proliferation, chemotaxis and differentiation (Lekovic et al. 2003). PDGF has the primary effect of a mitogen, initiating cell division (Oates et al. 1993). Recombinant PDGF-BB, in combination with recombinant IGF-1 (150 μg/ml of each), has been shown to exert a favourable effect on periodontal regeneration as measured by a gain in clinical attachment and osseous defect fill in humans (Howell et al. 1997). Upon activation, TGF-β facilitates wound healing under inflammatory conditions (Sporn & Roberts 1992). PGFs like PDGF and TGF-β are known to be abundant in the α granules of platelets (Assoian et al. 1984). A convenient technique to obtain a high concentration of PGFs is by preparing autologous platelet-rich plasma (PRP) (Lekovic et al. 2003). It basically involves the sequestration and concentration of platelets in plasma, with subsequent application of this preparation to wound-healing sites. It has been shown that application of PRP to the wound healing site increases the concentration of platelets (and theoretically of PDGF and TGF-β) by up to 338% (Marx et al. 1998).

A human clinical trial using recombinant PDGF and IGF has shown promising results in intra-bony defects and furcations (Howell et al. 1997). The use of purified recombinant PDGF-BB mixed with a bone allograft resulted in robust periodontal regeneration in class II furcation defects (Nevins et al. 2003). It was concluded by Lekovic et al. (2003) that the PRP/bovine porous bone mineral (BPBM)/GTR combined technique was an effective modality of regenerative treatment for mandibular grade II furcation defects and that it is necessary to elucidate the role played by each component of the combined therapy in achieving these results. Hence, the current study is designed to evaluate the effectiveness of autologous PRP alone in the treatment of human mandibular degree II furcation defects compared with open flap debridement (OFD).

Material and Methods

Patient selection

Twenty systemically healthy subjects (10 females and 10 males, mean age of 42.8 years) undergoing periodontal therapy at the Department of Periodontics, Government Dental College and Research Institute, Bangalore, India, from March 2006 to June 2007 were selected for the study (Fig. 1).

The inclusion criteria for the study included the presence of paired, contralateral buccal degree II furcation defects (Hamp et al. 1975) in vital, asymptomatic mandibular first molars with a radiolucency in the furcation area on an intra-oral periapical radiograph, vertical probing depth (VPD) $\geqslant$5 mm and horizontal probing depth $\geqslant$3 mm following phase I therapy (scaling and root planning, SRP).

Patients with any systemic illness known to affect the outcomes of periodontal therapy, insufficient platelet count for PRP preparation, compromised immune system, pregnancy and/or lactation, a habit of smoking or use of other tobacco products, those taking drugs known to interfere with wound healing, allergy or sensitivity to any medication to be used in the study and those with unacceptable oral hygiene [plaque index (Silness & Löe 1964) > 1.5] after the re-evaluation of phase-I therapy were excluded from the study. In addition, teeth with inter-proximal intra-bony defects, gingival recession, endodontic involvement or mobility of $\geqslant$ Miller's (1950) Grade II were also excluded.

It was made clear to the potential subjects that participation should be voluntary. Written informed consent was obtained from those who agreed to participate. Ethical clearance was obtained for the study from the Ethical Committee, Rajiv Gandhi University of Health Sciences, Bangalore.

Pre-surgical therapy

Before the surgery, each patient was given careful instructions on proper oral hygiene measures. A full-mouth supra- and subgingival SRP procedure was performed under local anaesthesia.

Six to eight weeks following phase I therapy, a periodontal evaluation was performed to confirm the suitability of the sites for the study.

The selected sites were divided randomly (toss of a coin) into control and test sites according to a split-mouth design, where the control sites were treated with OFD and the test sites with flap surgery, followed by placement of autologous PRP.

The clinical parameters recorded before surgical procedures included VPD from the gingival margin, relative vertical and horizontal clinical attachment level (RVCAL, RHCAL) along with gingival marginal level (GML) from the apical level of customized acrylic stents with grooves to ensure a reproducible placement of the University of North Carolina no. 15 periodontal probe (for vertical measurement) and PQ2N probe (for horizontal measure-

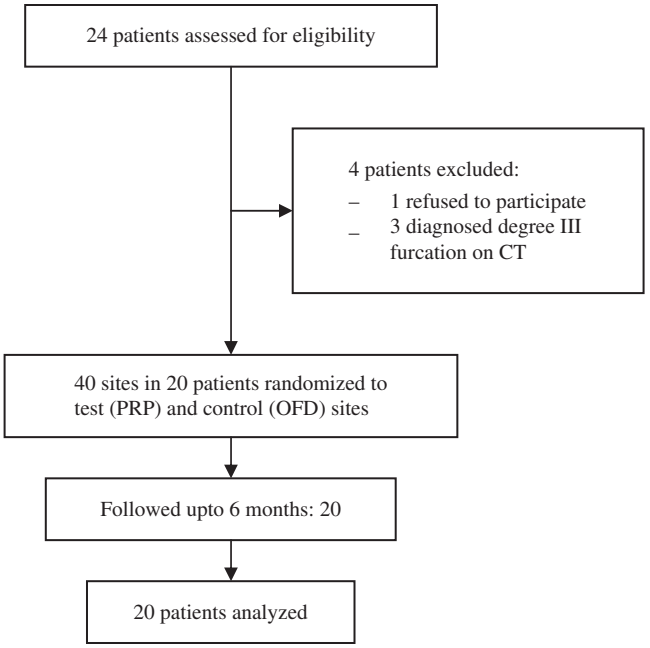

Fig. 1. Flow chart for study patients.

Fig. 2. Pre-operative axial view of the test site for PRP.

Fig. 3. Pre-operative axial view of the control site for OFD.

Fig. 4. Post-operative axial view of the test and control sites.

ments). The site-specific plaque index (PI) of Silness & Löe (1964) and the sulcus bleeding index (SBI) of Muhleman & Son (1971) were also measured.

Bone defect morphology was assessed using spiral computed tomography (CT, Siemens Somotom Emotion, Spiral CT, Erlangen, Germany). A high-resolution CT scanner equipped with a three-dimensional (3D) image reconstruction software package was used with following technical parameters: slice thickness of 1 mm, exposure of 130 kV, 90 mA with a scan time of 1 s/slice in the spiral scan mode, bone algorithm for axial slice orientation and zero degree gantry orientation. 3D images were reconstructed from spiral CT images using a software (Syngo Dental Siemens Software, Germany). The image slice with the maximum defect depth was used to measure the hard tissue parameters. The region of interest was extracted and threshold processing was performed to obtain the images containing bone and teeth. Bone defect morphology was assessed and the measurements were made using the caliper provided with the software with accuracy closest to 0.1 mm. The measurements included the vertical defect depth (VDD: distance from the furcation fornix to the base of the defect in sagittal view) and horizontal defect depth (HDD: distance from an imaginary line joining the maximum convexity of the mesial and distal roots to the base of the defect in axial view).

Examiner calibration

Intra-examiner calibration was achieved by examination of 10 patients twice, 24 h apart before beginning the study. Calibration was accepted if measurements at baseline and 24 h were similar to $\pm$ 1 mm at the 90% level.

PRP preparation

On the day of surgery, 20 ml blood was drawn from each patient by venipuncture of the antecubital vein. Blood was collected in sterile plastic test tubes that contained Citrate Phosphate Dextrose-Adenine (CPD-A, HL Haemopack, Thiruvanathapuram, Kerala, India) as an anticoagulant in the ratio of 2.8 ml CPD-A to 20 ml of blood. The blood-containing test tubes were shaken gently to enhance complete mixing of the blood with the anticoagulant. Then it was kept at room temperature for a minimum of 45 min. to minimize the complement activity. Later, blood-containing test tubes were centrifuged using a refrigerated centrifugal machine at 3000 rpm for 10 min., which resulted in separation of three basic fractions: the bottom red blood cells (RBC), middle PRP and top layer of Platelet-poor plasma (PPP), because of differential densities. Two to three millilitres of the top layer corresponding to the PPP was aspirated with a pipette and collected in a separate sterile plastic tube. The same aspirated PPP was used to obtain the autologous thrombin at the time of application. The PRP was collected in conjunction with the top 1–2 mm of the RBC fraction because the latter is also rich in newly synthesized platelets (Lekovic et al. 2003).

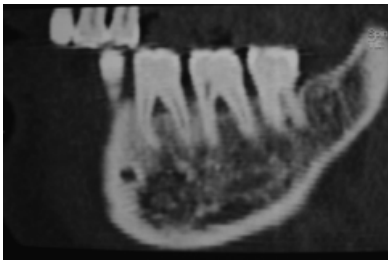

Fig. 5. Pre-operative sagittal view of the test site for PRP.

Surgical procedure

Intra-oral antisepsis was performed with 0.12% chlorhexidine digluconate rinse and extraoral antisepsis was carried out with iodine solution. Following administration of local anaesthesia, sulcular incisions were made and mucoperiosteal flaps were raised. Care was taken to preserve as much inter-proximal soft tissue as possible. Meticulous defect debridement and root planing were carried out using curettes (Gracey, Hu-Friedy, Chicago, IL, USA) and ultrasonic instruments (Cavitron, Dentsply, Tulsa, OK, USA). No osseous recontouring was carried out.

PRP gel was prepared according to the method described by Su et al. (2004). Beads (5 g) and 10% $CaCl_2$ (0.3 ml) were added to PPP (10 ml) to activate coagulation. The mixture was agitated once a minute for 8–10 min. at room temperature. The supernatant containing human thrombin (HT) was recovered after an additional 10 min. HT and PRP, in equal quantities from the same blood donor, were mixed to prepare the gel (Su et al. 2004).

Autologous PRP in the gel form of the required size was packed into the defect in the test group. The mucoperiosteal flaps were repositioned and secured in place using 4-0 non-absorbable black braided silk surgical suture (Ethicon, Johnson and Johnson Ltd., Somerville, NJ, USA). Interrupted or sling sutures were placed and the surgical area was protected and covered with periodontal dressing (Coe-Pak, GC America Inc., Chicago, IL, USA). Later, suitable antibiotics and analgesics (amoxicillin 500 mg four times per day

and ibuprofen 800 mg three times per day as necessary) were prescribed, along with chlorhexidine digluconate rinses (0.12%) twice daily for 3 weeks.

One surgeon performed all the surgeries. An examiner other than the operator performed all the clinical measurements without knowledge of the treatment groups. Patients underwent the two surgical procedures at the same appointment. The test and control sites were not revealed to the patients. A coin toss (done by a person other than the surgeon and the examiner) was used to determine the site to be operated first.

Post-operative care

Periodontal dressing and sutures were removed 2 weeks post-operatively. Surgical wounds were gently cleansed with 0.12% of chlorhexidine digluconate on a cotton swab. Thereafter, gentle brushing with a soft toothbrush was recommended. At 8 weeks post-operatively, each patient was re-instructed for proper oral hygiene measures. Patients were examined weekly up to 1 month after surgery and then at 3 and 6 months. No subgingival instrumentation was attempted at any of these appointments. Post-operative care included reinforcement of oral hygiene and mechanical plaque control whenever necessary.

Post-surgical measurements

Six months after the initial surgery, PI and SBI were recorded. Soft and hard tissue evaluation was performed. Soft tissue measurements were repeated with previously used acrylic stents. A second CT scan of the study sites was carried out (after obtaining the ethical approval from the university) and all the 3D reconstruction and hard tissue measurements were repeated for comparison.

Primary and secondary outcome measures

The primary outcome of the study was defect fill (vertical and horizontal). The secondary outcomes included RVCAL, RHCAL, VPD, GML, PI and SBI.

Statistical analysis

The results were averaged (mean standard deviation) for each clinical and radiographical parameter at both time intervals. The difference between each pair of measurement was then calculated (Baseline – 6 months). Paired *t*-test was applied to assess the statistical significance between time points within each group for clinical and radiographic parameters. One-way analysis of variance was used to test the difference between groups. SBI and PI were expressed as absolute and relative counts. Comparison between indices between the test and control groups was performed using the non-parametric Mann–Whitney test. The data were analysed using statistical software (SPSS® version 10.5, SPSS Inc., Chicago, IL, USA). A sample size of 20 was estimated to achieve 91% power to detect a difference of 0.5 between the null hypothesis and the alternative mean.

Results

All 20 patients completed the study. Healing of all the control and test sites was uneventful, and no cases of post-operative infection were observed.

A statistically significant reduction in the PI and SBI was observed in both test and control sites at 6 months post-operatively. However, the difference between the test and the control sites was statistically insignificant (Table 1).

The clinical parameters at baseline and 6 months are reported in Table 2 and their mean change in Table 4. At 6 months post-operatively, test sites presented with a significantly greater reduction in VPD than control sites, with a difference of 1.5 ± 0.10 mm. The gain in attachment level was also significantly greater in the test sites, with a difference between the two groups of 2.4 ± 0.54 mm in RVCAL and 1.7 ± 0.54 mm in RHCAL. Control sites did not reveal any significant reduction in the clinical parameters recorded except for the RHCAL over a period of 6 months unlike the test sites, with significant changes in all the parameters recorded. Gingival recession was found to be similar between the test and the control sites.

Vertical and horizontal defect fill are reported in Tables 3 and 4. Test sites presented with a vertical defect fill of 1.23 ± 0.43 mm ($32.08 \pm 10.37\%$) and a horizontal defect fill of 1.33 ± 0.93 mm ($28.74 \pm 21.09\%$), significantly greater than the control sites with a vertical defect fill of 0.64 ± 0.66 mm ($16.54 \pm 18.80\%$) and a horizontal defect fill of 0.09 ± 0.48 mm ($1.74 \pm 16.95\%$) over a period of 6 months (Figs 2–8).

Discussion

For the present clinical study, with the aim to evaluate the clinical effectiveness of the autologous PRP in treating mandibular buccal degree II furcation defects in humans, 40 sites in 20 patients were treated using the split-mouth design so as to avoid the effect of a natural variation between different individuals.

The limitations of conventional radiographs have been emphasized in the literature. They are 2D representations of the 3D alveolar bone, tooth and soft tissue (Jeffcoat et al. 1995). They mask the osseous changes by other anatomic structures. Radiographs underestimate the true amount of bone loss (Armitage 1996). Foreshortening or elongation of radiographic images caused by cone indication (Hausmann et al. 1989) and variations in the contrast and density caused by poor control of film processing may prevent the accurate detection of osseous changes by the clinician (Reddy 1992). In the dental radiographs, the artificial furcation involvement in 6

Table 1. Bleeding and Plaque index

	Test group		Control group	
	Baseline	6 months	Baseline	6 months
Bleeding index	*N* (%)	*N* (%)	*N* (%)	*N* (%)
Score 0–0.5	0 (0)	10 (50.0)	0 (0)	16 (80.0)
Score 0.6–1	6 (30.0)	10 (50.0)	8 (40.0)	4 (20.0)
Score 1.1–1.5	14 (70.0)	0 (0)	12 (60.0)	0 (0)
p value	0.002*		0.001*	
Plaque index	*N* (%)	*N* (%)	*N* (%)	*N* (%)
Score 0–0.5	0 (0)	12 (60.0)	0 (0)	14 (70.0)
Score 0.6–1	8 (40.0)	8 (40.0)	6 (30.0)	6 (30.0)
Score 1.1–1.5	12 (60.0)	0 (0)	14 (70.0)	0 (0)
p value	0.002*		0.001*	

*$p < 0.05$ significant.

of 28 (21%) molars was identified while all 28 molars with involved furcations (100%) were identified in the axial CT-scans. Therefore, it was concluded that high-resolution-CT offers a 3D assessment of the inter-radicular bone morphology in furcation involvement without overlying structures and the periradicular alveolar bone could be assessed on all sides of the roots, thus permitting a high identification rate and classification of molars with involved furcations (Fuhrmann et al. 1997). The usefulness of CT as an aid in periodontal examination and diagnosis, and in the prediction and evaluation of periodontal regenerative treatment outcomes has been reported in a case report (Ito et al. 2001). However, it is difficult to precisely compare the risk of radiation dosage between CT and conventional radiographs for periodontal examinations (Naito et al. 1998).

Table 2. Clinical parameters at baseline and 6 months

	Test	Control
Vertical probing depth (mm)		
Baseline	6.00 ± 0.94	5.10 ± 1.20
6 months	3.70 ± 0.95	4.30 ± 1.64
p value	<0.05*	>0.05
Vertical attachment level (mm)		
Baseline	8.40 ± 1.71	7.00 ± 1.05
6 months	6.40 ± 1.71	6.90 ± 1.66
p value	<0.05*	>0.05
Horizontal attachment level (mm)		
Baseline	10.60 ± 2.07	8.70 ± 1.64
6 months	8.10 ± 2.13	7.90 ± 1.85
p value	<0.05*	<0.05*
Change in gingival level (mm)		
Baseline	1.60 ± 0.52	1.90 ± 0.74
6 months	1.80 ± 0.79	2.20 ± 0.92
p value	>0.05	>0.05

*$p<0.05$ significant.
NA, not applicable.

Table 3. Radiographic parameters at baseline and 6 months

	Test	Control
Vertical defect depth		
Baseline (mm)	3.86 ± 0.59	3.81 ± 1.39
6 months (mm)	2.63 ± 0.63	3.17 ± 1.35
p value	<0.05*	<0.05*
Horizontal defect depth		
Baseline (mm)	4.53 ± 1.25	3.79 ± 2.09
6 months (mm)	3.20 ± 1.32	3.70 ± 2.13
p value	<0.05*	>0.05

*$p<0.05$ significant.
NA, not applicable.

It was suggested that PRP contains high concentrations of several growth factors such as PDGF and TGF-β, which may strongly modulate the regeneration process. Data from in vitro studies have shown that PRP stimulates the proliferation of periodontal ligament and osteoblastic cells while, at the same time, epithelial cell proliferation is inhibited (Okuda et al. 2003). It was also speculated that due to its fibrinogen content, PRP reacts with thrombin and induces fibrin clot formation, which in turn is capable of upregulating collagen synthesis in the extracellular matrix and provides a favourable scaffold for cellular migration and adhesion (Camargo et al. 2005). The fibrin component of PRP gel not only works as a haemostatic agent aiding in stabilization of the graft material and the blood clot (Polson & Proye 1983, Wikesjo et al. 1992), but also adheres to the root surface and may impede the apical migration of epithelial cells and connective tissue cells from the flap (Gottlow 1994), and thus PRP may exert a GTR-like effect (may act as a barrier to cover bone and periodontal ligament, temporarily separating them from the gingival epithelium) in the treated defects. While increasing the concentrated delivery of various growth factors, PRP also stimulates monocyte chemotaxis in a dose-dependent fashion and RANTES (regulated on activation normal T cell expressed and secreted), in part, was found to be responsible for PRP-mediated monocyte migration. Lipoxin A_4 was found to be increased in PRP, suggesting that PRP may suppress cytokine release, limit inflammation and thereby promote tissue regeneration. This suggests that PRP facilitates healing by controlling the local inflammatory response (El-Sharkawy et al. 2007).

To date, only a few studies have been conducted to evaluate the influence of PRP alone on bone healing. The treatment of bony defects with PRP alone, in either femurs or calvaria of rabbits or mandibles of dogs, showed no improvement in bone formation (Polson & Proye 1983, Okuda et al. 2003, Camargo et al. 2005). Conversely, favourable results were reported by Anitua (1999), Sammartino et al. (2005) and Simon et al. (2004) in bone healing of extraction sockets treated with PRP only. Therefore, there are still no definitive conclusions about the biologic effect of PRP when it is used alone.

There are differences in the last step of PRP preparation, which includes addition of an agent to start gelation and activation of platelets. Some authors suggest agents such as bovine thrombin (BT) or fibrin adhesive (Whitman et al. 1997, Marx et al. 1998, Anitua 1999, Landesberg et al. 2000, Sonnleitner et al. 2000), whereas others demonstrated the utilization of patient's own blood (Kassolis et al. 2000, Gonshor 2002, Robiony et al. 2002). There are two uncommon, but potentially serious complications that relate to the blood-derived surgical adjuncts. First, when PRP is collected from allogeneic donors, there is a risk of transfusion-transmitted virus infections. Second, when platelet gels are prepared with BT, there is a risk of inducing a serious bleeding disorder from the formation of

Table 4. Comparison of mean change of parameters in 6 months between the groups

	Test	Control	*p* value	*t* value
	Clinical parameters			
Mean vertical probing depth reduction (mm)	2.30 ± 1.41	0.80 ± 1.31	0.025*	2.451
Mean vertical attachment level change (mm)	2.50 ± 1.64	0.10 ± 1.10	0.003*	3.475
Mean horizontal attachment level change (mm)	2.50 ± 1.17	0.80 ± 0.63	0.001*	4.019
Mean gingival recession (mm)	0.20 ± 0.63	0.30 ± 0.67	0.736	0.342
	Radiographic parameters			
Mean vertical defect fill (mm)	1.23 ± 0.43	0.64 ± 0.66	0.033*	2.343
Percentage of vertical defect fill (%)	32.08 ± 10.37	16.54 ± 18.80	0.038*	2.287
Mean horizontal defect fill (mm)	1.33 ± 0.93	0.09 ± 0.48	0.002*	3.711
Percentage of horizontal defect fill (%)	28.74 ± 21.09	1.74 ± 16.95	<0.001*	177.71

*$p<0.05$ significant.

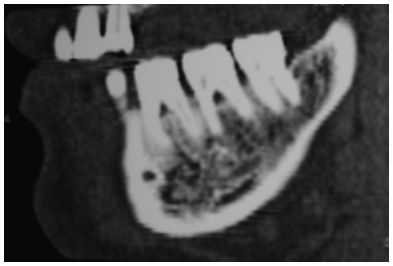

Fig. 6. Post-operative sagittal view of the test site with PRP.

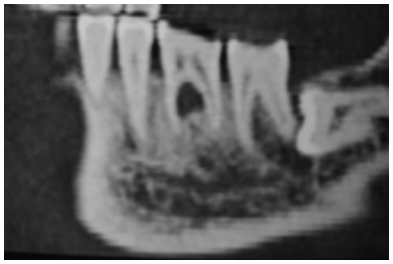

Fig. 7. Pre-operative sagittal view of the control site for OFD.

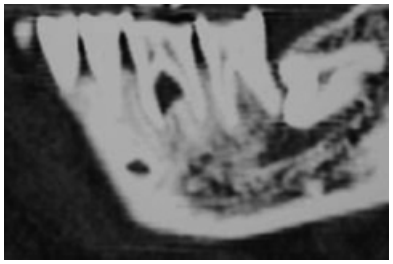

Fig. 8. Post-operative sagittal view of the control site with OFD.

antibodies that cross-react with human coagulation
factor V (Banninger et al. 1993). Also, there is a concern that exposure to bovine blood carries a potential risk of acquiring variant Creutzfeld Jacob disease (Su et al. 2004).

Su et al. (2004) have shown that the clotting time for BT-induced platelet gel (PG) (9.6 ± 1.2 s) was shorter than that for HT-induced PG (12.0 ± 2.1 s), indicating that the thrombin concentration, determined by the time to clot fibrinogen in the PRP, was slightly less in the HT (approximately 50 US units/ml). PRP volumes prepared using this technique contain an optimal platelet count of $1071 \times 10^3 \pm 227 \times 10^3$ per μL. PGs obtained with HT or BT had similar fibrin consistencies and both were suitable for use as carriers for grafts in clinical practice. The concentrations of PDGF-AB and TGF-β1 levels were significantly higher for PGs using HT compared with BT (Su et al. 2004, Thorn et al. 2004). It has been shown that thrombin obtained from human plasma may be, even at lower concentrations, a more potent trigger for granule secretion and growth factors' release than BT. Replacing BT with HT not only preserves the biologic and physical properties of the PGs but also enhances the safety profile (Su et al. 2004).

To our knowledge, there have been no studies reporting the use of PRP alone in the treatment of furcation defects. However, when interpreting the findings of this study, it has to be kept in mind that at present no other data evaluating the treatment of furcation defects with the use of PRP and spiral CT are available, and therefore, a direct comparison with other studies is not possible.

A mean VPD reduction of 2.30 ± 1.41 mm in the test sites compared with 0.80 ± 1.31 mm in the control sites was observed 6 months post-operatively in the present study. Thus, the test sites presented with a significantly greater VPD reduction than the control sites, with a difference of 1.5 ± 0.10 mm. Gingival recession was found to be similar between both the test and the control sites. Even though the mean VPD reduction in our study is similar to that observed by Lekovic et al. (2003) (1.58 ± 0.22 mm in favour of the test sites), VPD reduction with PRP alone was much less than that with the use of PRP/BPBM/GTR over a period of 6 months in the previous study by Lekovic et al. (2003) (4.07 ± 0.33 mm). VPD reduction using PRP in our study was found to be equivalent to that obtained by OFD (2.49 ± 0.38 mm) in the study of 6 months' duration by Lekovic et al. (2003). Apart from the different regenerative materials used, the difference in VPD reduction in the two studies could also be attributed to the deeper baseline pocket depth in both the groups in the study by Lekovic et al. (2003) as the potential for regeneration is more in initially deeper defects (Machtei et al. 1994). The mean VPD reduction in our study is more than the weighted mean difference of 1.16 mm with GTR as mentioned in the systematic review by Jepsen et al. (2002) and the median VPD reduction of 0.5 mm by EMD (Meyle et al. 2004).

An important clinical outcome of a periodontal regenerative procedure is gain in clinical attachment. A gain of 2.50 ± 1.64 mm in RVCAL achieved using PRP in the present study is lesser than 3.29 ± 0.42 mm achieved by a combination of PRP/BPBM/GTR in mandibular grade II furcations. The reason for the difference could not be given due to lack of data regarding the baseline RVCAL in the other study (Lekovic et al. 2003). The mean RVCAL change in our study is more than the weighted mean difference of 1.77 mm with GTR as mentioned in the systematic review by Jepsen et al. (2002).

Although all the furcation defects retained the degree II status (about 6/20, i.e., 30% of the defects approaching degree I with a 3 mm horizontal probing depth, the deepest horizontal probing within the furcation to gingival margin), there was a significant gain in RHCAL of 2.50 ± 1.17 mm over a period of 6 months in the test group. The RHCAL gain in our study is more than the weighted mean difference of 1.31 mm with GTR reported in the systematic review by Jepsen et al. (2002).

A vertical defect fill of 1.23 ± 0.43 mm and a horizontal defect fill of 1.33 ± 0.93 mm was obtained with PRP in the present study. The defect resolution by GTR alone was more (1.60 ± 1.50 mm vertical defect fill and 2.47 ± 0.99 mm horizontal defect fill) while that by a combination of GTR and bovine-derived anorganic bone was much better (1.80 ± 2.11 mm vertical defect fill and 3.27 ± 1.39 mm horizontal defect fill) in a study by Simonpietri et al. (2000). The defect fill obtained by PRP/BPBM/GTR was almost double in the study by Lekovic et al. (2003) (2.56 ± 0.36 mm vertical defect fill and 2.28 ± 0.33 mm horizontal defect fill). The mean reduction of open horizontal furcation depth by EMD (2.6 ± 1.8 mm) and GTR (1.9 ± 1.4 mm) over a period of 14 months was more than that achieved by PRP in 6 months in the present study (Jepsen et al. 2004). The defect fill by PRP may improve over a longer duration (>6 months), although this remains to be proven.

From a clinician's point of view, the practical aspects related to PRP preparation, which involves an additional step in the surgical procedure, increasing the time for the procedure by 20–30 min., should also be considered. Despite a 29% horizontal defect fill, lack of complete closure of mandibular degree II furcation defects using PRP implies that it has a limited role as a regenerative

material in the treatment of furcation defects.

Conclusion

Within the limits of the present study, despite the statistically significant results obtained by autologous PRP, it did not appear to be clinically beneficial for the treatment of mandibular degree II buccal furcation defects. Future controlled studies should include a histomorphometric analysis to demonstrate periodontal regeneration and also the assessment of bone quality using Hounsfield units on suitable CT images. Further studies need to be carried out on a larger sample size and for a longer duration to further explore the role of PRP in the management of furcation defects.

Acknowledgement

The authors would like to acknowledge Dr. Venkatappa, Managing director and Dr. Girish, Chief radiologist, Kanva Diagnostics, Bangalore, for assistance in CT analysis.

References

Aimetti, M., Romano, F., Pigella, E. & Piemontese, M. (2007) Clinical evaluation of the effectiveness of enamel matrix proteins and autologous bone graft in the treatment of mandibular Class II furcation defects: a series of 11 patients. *International Journal of Periodontics and Restorative Dentistry* **27**, 441–447.

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

Armitage, G. C. (1996) Periodontal diseases: diagnosis. *Annals of Periodontology* **3**, 37–215.

Assoian, R. K., Grofendorst, G. R., Miller, D. M. & Sporn, M. B. (1984) Cellular transformation by coordinated action of three peptide growth factors from human platelets. *Nature* **309**, 804–806.

Banninger, H., Hardegger, T., Tobler, A., Barth, A., Schüpbach, P., Reinhart, W., Lämmle, B. & Furlan, M. (1993) Fibrin glue in surgery: frequent development of inhibitors of bovine thrombin and human factor V. *British Journal of Haematology* **85**, 528–532.

Brunsvold, M. A. & Mellonig, J. T. (1993) Bone grafts and periodontal regeneration. *Periodontology 2000* **1**, 80–91.

Caffesse, R. G., Dominguez, L., Nasjleti, C. E., Castelli, W. A., Morrison, E. C. & Smith, B. A. (1990a) Furcation defects in dogs treated by guided tissue regeneration. *Journal of Periodontology* **61**, 45–50.

Caffesse, R. G., Smith, B. A., Duff, B., Morrison, E. C., Merril, D. & Becker, W. (1990b) Class II furcation treated by guided tissue regeneration in humans: case reports. *Journal of Periodontology* **61**, 510–514.

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

Chitsazi, M. T., Mostofi-Zadeh-Farahani, R., Pourabbas, M. & Bahaeddin, N. (2007) Efficacy of open flap debridement with and without enamel matrix derivatives in the treatment of mandibular degree II furcation involvement. *Clinical Oral Investigation* **11**, 385–389.

De Leonardis, D., Garg, A. K., Pedrazzoli, V. & Pecora, G. E. (1999) Clinical evaluation of the treatment of class II furcation involvements with bioabsorbable barriers alone or associated with decalcified freeze-dried bone allograft. *Journal of Periodontology* **70**, 8–12.

DeSanctis, M. & Murphy, K. G. (2000) The role of resective periodontal surgery in the treatment of furcation defects. *Periodontology 2000* **22**, 154–168.

El-Sharkawy, H., Kantarci, A., Deady, J., Hasturk, H., Lieu, H., Alshahat, M. & Vandyke, T. E. (2007) Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. *Journal of Periodontology* **78**, 661–669.

Fuentes, P., Garrett, S., Nilvéus, R. & Egelberg, J. (1993) Treatment of periodontal furcation defects. Coronally positioned flap with or without citric acid root conditioning in class II defects. *Journal of Clinical Periodontology* **20**, 425–430.

Fuhrmann, R. A., Bucker, A. & Diedrich, P. R. (1997) Furcation involvement: comparison of dental radiographs and HR-CT-slices in human specimens. *Journal of Periodontal Research* **32**, 409–418.

Gonshor, A. (2002) Technique for producing platelet-rich plasma and platelet concentrate: background and process. *The International Journal of Periodontics and Restorative Dentistry* **22**, 547–557.

Gottlow, J. (1994) Periodontal regeneration. In: Lang, NP & Karring, T (eds). *Proceedings of the 1st European Workshop in Periodontology*, pp. 72–192. London: Quintessence publishing company.

Gottlow, J., Nyman, S., Lindhe, J., Karring, T. & Wennstrom, J. (1986) New attachment formation in the human periodontium by guided tissue regeneration. Case reports. *Journal of Clinical Periodontology* **13**, 604–616.

Hamp, S. E., Nyman, S. & Lindhe, J. (1975) Periodontal treatment of multirooted teeth. Results after 5 years. *Journal of Clinical Periodontology* **2**, 126–135.

Hausmann, E., Allen, K., Christersson, L. & Genco R, . (1989) Effect of X-ray beam vertical angulation on radiographic alveolar crest level measurements. *Journal of Periodontal Research* **24**, 8–19.

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

Ito, K., Yoshinuma, N., Goke, E., Arai, Y. & Shinoda, K. (2001) Clinical application of a new compact computed tomography system for evaluating the outcome of a regenerative therapy: a case report. *Journal of Periodontology* **72**, 696–702.

Jeffcoat, M. K., Wang, I. C. & Reddy, M. (1995) Radiographic diagnosis in periodontics. *Periodontology 2000* **7**, 54–68.

Jepsen, S., Eberhard, J., Herrera, D. & Needleman, I. (2002) A systematic review of guided tissue regeneration for periodontal furcation defects. What is the effect of guided tissue regeneration compared with surgical debridement in the treatment of furcation defects? *Journal of Clinical Periodontology* **29**, 103–116.

Jepsen, S., Heinz, B., Jepsen, K., Arjomand, M., Hoffmann, T., Richter, S., Reich, E., Schulean, A., Gonzales, J.R., Bodekar, R.H. & Meyle, J. (2004) A randomized clinical trial comparing Enamel matrix derivative and membrane treatment of buccal class II furcation involvement in mandibular molars. Part I: study design and results for primary outcomes. *Journal of Periodontology* **75**, 1150–1160.

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

Landesberg, R., Roy, M. & Glickman, R. S. (2000) Quantification of growth factor levels using a simplified method of platelet-rich plasma gel preparation. *Journal of Oral and Maxillofacial Surgery* **58**, 297–300.

Lekovic, V., Camargo, P. M., Weinlander, M., Vasilic, N., Aleksic, Z. & Kenney, E. B. (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. *Journal of Clinical Periodontology* **30**, 746–751.

Machtei, E., Cho, M., Dunford, R., Norderyd, J., Zambon, J. & Genco, R. (1994) Clinical, microbiological and histological factors which influence the success of regenerative periodontal therapy. *Journal of Periodontology* **65**, 154–161.

Marx, R. E., Carlson, E. R., Eichstaedt, R. M., Schimmele, S. R., Strauss, J. E. & Georgeff, K. R. (1998) Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral*

Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics **85**, 638–646.

Meyle, J., Gonzales, J. R., Bodekar, R. H., Hoffmann, T., Richter, S., Heinz, B., Arjomand, M., Reich, E., Schulean, A., Jepsen, K. & Jepsen, S. (2004) A randomized clinical trial comparing Enamel matrix derivative and membrane treatment of buccal class II furcation involvement in mandibular molars. Part II: secondary outcomes. *Journal of Periodontology* **75**, 1188–1195.

Miller, S. C. (1950) *Textbook of Periodontia*, 3rd edition, **125**. Philadelphia: Blackstone.

Muhleman, H. R. & Son, S. (1971) Gingival sulcus bleeding- a leading symptom in initial gingivitis. *Helvetica Odontologica Acta* **33**, 107–113.

Naito, T., Hosokawa, R. & Yokota, M. (1998) Three-dimensional alveolar bone morphology analysis using computed tomography. *Journal of Periodontology* **69**, 584–589.

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

Newell, D. H. (1998) The diagnosis and treatment of molar furcation invasions. *Dental Clinics of North America* **42**, 301–307.

Oates, T. W., Rouse, C. A. & Cochran, D. L. (1993) Mitogenic effects of growth factors on human periodontal ligament cells in vitro. *Journal of Periodontology* **64**, 142–148.

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

Polson, A. M. & Proye, M. P. (1983) Fibrin linkage: a precursor for new attachment. *Journal of Periodontology* **54**, 141–147.

Pontoriero, R., Lindhe, J., Nyman, S., Karring, T., Rosenberg, E. & Sanavi, F. (1988) Guided tissue regeneration in degree II furcation-involved mandibular molars. *Journal of Clinical Periodontology* **15**, 247–254.

Reddy, M. (1992) Radiographic evaluation of periodontal therapy. *Journal of Periodontology* **63**, 1078–1084.

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

Sammartino, G., Tia, M., Marenzi, G., di Lauro, A. E., D'Agostino, E. & Cláudio, P. P. (2005) Use of autologous platelet-rich plasma (PRP) in periodontal defect treatment after extraction of impacted mandibular third molars. *Journal of Oral and Maxillofacial Surgery* **63**, 766–770.

Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontolgia Scandinavia* **22**, 121–135.

Simon, D., Manuel, S., Geetha, V. & Naik, B. R. (2004) Potential for osseous regeneration of platelet-rich plasma – a comparative study in mandibular third molar sockets. *Indian Journal of Dental Research* **15**, 133–136.

Simonpietri, C. J. J., Novaes, A. B. Jr., Batista, E. L. Jr. & Filho, E. J. (2000) Guided tissue regeneration associated with bovine-derived anorganic bone in mandibular class II furcation defects. 6-month results at re-entry. *Journal of Periodontology* **71**, 904–911.

Sonnleitner, D., Huemer, P. & Sullivan, D. Y. (2000) A simplified technique for producing platelet-rich plasma and platelet concentrate for intraoral bone grafting techniques: a technical note. *The International Journal of Oral and Maxillofacial Implants* **15**, 879–882.

Sporn, M. B. & Roberts, A. B. (1992) Transforming growth factor-β: recent progress and new challenges. *Journal of Cell Biology* **119**, 1017–1021.

Su, C. Y., Chiang, C. C., Lai, W. F., Lin, K. W. & Burnouf, T. (2004) Platelet-derived growth factor-AB and transforming growth factor- β1 in platelet gels activated by single-donor human thrombin. *Transfusion* **44**, 945.

Thorn, J. J., Sorensen, H., Weis-Fogh, U. & Andersen, M. (2004) Autologous fibrin glue with growth factors in reconstructive maxillofacial surgery. *International Journal of Oral Maxillofacial Surgery* **33**, 95–100.

Whitman, D. H., Berry, R. L. & Green, D. M. (1997) Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *International Journal of Oral and Maxillofacial Surgery* **55**, 1294–1299.

Wikesjo, U. M. E., Nilveus, R. E. & Selvig, K. E. (1992) Significance of early healing events on periodontal repair. A review. *Journal of Periodontology* **63**, 158–165.

Address:
Pradeep A.R.
Department of Periodontics
Government Dental College and Research Institute
Bangalore 560002
Karnataka
India
E-mail: periodontics_gdc@yahoo.co.in

Clinical Relevance

Scientific rationale for the study: The combination of PRP/BPBM/GTR was found to be effective for regeneration of mandibular grade II furcation defects. Hence, this study was designed to elucidate the role played by PRP alone.

Principal findings: The use of PRP led to a statistically significant reduction in the VPD, gain in RVCAL and RHCAL and a significantly greater vertical and horizontal defect fill as revealed by Spiral CT images, compared with OFD. Despite the gain in RHCAL, all the furcation defects retained the degree II status.

Practical implications: Despite the lack of complete closure of degree II furcation defects, a statistically significant clinical and radiographic improvement has been observed over 6 months. Hence, long-term follow-up is required to confirm the effectiveness of autologous PRP in the treatment of furcation defects.