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ORIGINAL ARTICLE

Comparison of platelet pellet with or without guided tissue regeneration in the treatment of class II furcation defects in dogs

Gonca Cayir Keles · Burcu Ozkan Cetinkaya · Sancar Baris · Davut Albayrak · Selen Burcak Simsek

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Abstract The purpose of this study was to compare histological effectiveness of platelet pellet (PP), which has higher platelet content than platelet-rich plasma, and the combination of PP/guided tissue regeneration (GTR) for class II furcation defects in dogs. The mandibular second, third, and fourth premolars of both sides in four dogs were used. Class II furcation defects (5 mm in height and 2 mm in depth) were surgically created. Five weeks after the first operation, second premolars were treated with scaling and root planing (group 1); right third and fourth premolars received PP (group 2), and left premolars received the combination of PP/GTR (group 3). Percentage of cementum and alveolar bone formation were evaluated by histometric analysis after a healing period of 12 weeks. There was new cementum along with periodontal ligament and coronal growth of alveolar bone in all groups. Cementum formation was significantly higher in groups 2 and 3 compared to the control group ($P<0.05$) with no significant difference between groups 2 and 3. Limited alveolar bone formation was statistically similar in all groups ($P>0.05$). It is concluded that both PP and the combination of PP/GTR are effective in the treatment of class II furcation defects in dogs. PP thus appears to be a suitable alternative material in the regenerative periodontal therapy.

Keywords Furcation defects · Guided tissue regeneration · Histopathology · Platelet pellet · Dog

G. C. Keles (✉) · B. O. Cetinkaya · S. B. Simsek
Department of Periodontology, Faculty of Dentistry,
Ondokuzmayis University,
55139 Samsun, Turkey
e-mail: goncakeles@hotmail.com

S. Baris
Department of Pathology, Faculty of Medicine,
Ondokuzmayis University,
Samsun, Turkey

D. Albayrak
Department of Hematology, Faculty of Medicine,
Ondokuzmayis University,
Samsun, Turkey

Introduction

Periodontitis, oral infectious disease, is characterized by clinical attachment loss, alveolar bone resorption, periodontal pocketing, and gingival inflammation [13]. The goals of periodontal therapy are the elimination of the infection, arrest the disease progression, and regeneration of the periodontium [17, 37]. Although regeneration of periodontium is the ultimate goal of periodontal therapy, complete regeneration is not a predictable healing outcome following traditional periodontal treatment [38]. In the literature, several regenerative procedures including open flap debridement with bone replacement grafts or in combination with guided tissue regeneration (GTR) have been used [25, 38].

GTR, achieved using barrier membranes, is one of the most widely used treatments which increase regeneration of periodontal tissues [18]. The bioabsorbable barrier membranes are thought to have an advantage over the nonabsorbable membranes in that they do not require a second surgical procedure. In order to enhance outcome of periodontal therapy, the combination of one or more techniques have currently been available for periodontal regeneration [4].

In recent years, platelet-rich plasma (PRP) combined with graft materials, has been used for the purpose of periodontal regeneration [9, 16]. PRP is a volume of autologous plasma that has a higher platelet concentration

than baseline [28]. It is known that the increased number of platelets deliver an increased number of polypeptide growth factors that regulate cell proliferation, chemotaxis, and differentiation to the surgical area within PRP [29]. Platelets contain these growth factors in their alpha granules [2]. Concentration of platelets increases by up to 338% due to the application of PRP in surgical sites [28]. Platelet pellet (PP) which has higher platelet content than PRP was used in the present study. In addition to PP's higher platelet and lower white blood cell content, it is also more adhesive than PRP due to its gel consistency [24].

Findings in the clinical studies have reported that treatments with a combination of PRP/bone graft material/GTR and a combination of PRP/bone graft materials improve clinical parameters in intrabony periodontal defects [4, 5, 16]. Additionally, significant pocket depth reduction, gain in clinical attachment, and alveolar bone level have been shown following both the combination of PP/GTR and synthetic bone grafting material/GTR treatments in our previous clinical study [24]. However, recent controlled clinical trials have suggested that PRP failed to improve the results obtained with the combinations of bone graft materials/bioresorbable and nonresorbable GTR membranes [7, 8, 10, 11]. When interpreting the results obtained in these clinical studies, it has to be pointed out that the findings might have been influenced by the defect characteristics and center and/or operator effect which may depend on differences in the enrolled patients, technical ability, clinical organization, and experience of the clinicians or a combination of these factors [34].

To date, there is a clinical study investigating the individual role of autologous platelet concentrate, or GTR membrane in the treatment of intrabony defects has suggested that platelet concentrate and GTR treatments led to similar improvement in clinical and radiographic parameters [31]. In fact, histological analysis of regenerative periodontal therapy is required to determine if regeneration has occurred as positive clinical results are not necessarily representatives of true regeneration [3]. The use of PP for periodontal regeneration offers an interesting and clinically useful modality to the clinician in treating periodontal defects as PP is autologous and cheaper than bone graft materials. The present study was undertaken to test the hypothesis that using PP alone or with GTR membrane is an effective regenerative treatment modality in the treatment of class II furcation defects in dogs. No data are available in comparing PP alone and to the combination of PP/GTR for the periodontal treatment. Therefore, the purpose of this study was to compare the histomorphometrical effectiveness of PP and the combination of PP/GTR with bioabsorbable barrier treatments for class II furcation defects in dogs.

Materials and methods

The research protocol was approved by the Ethical Committee for Animal Research of the University with the assignment protocol CAM 02/58.

Animals

Four young adult mongrel dogs, weighing about 10 kg, with intact maxillary and mandibular teeth showing good systemic and periodontal health were selected for the study. The animals received complete oral prophylaxis, antiparasitic treatment, multivitamins, and complete vaccines. All dogs were fed a water-softened dog food to prevent traumas of mastication to the surgical areas.

Preparation of class II furcation defects

The animals were not fed the night before surgery. All surgical procedures were performed under general anesthesia with xylazine (Rompun, Bayer, Istanbul, Turkey)–ketamine HCl (Pfizer, Istanbul, Turkey; 5 mg/kg, intramuscularly) and local infiltrated anesthesia with 2% lidocaine hydrochloride and 1/80,000 andrenaline (Jetokain ampule, Adeka Pharmaceutical Company, Samsun, Turkey). The mandibular second, third, and fourth premolars (P2, P3, and P4) of both sides in each dog were selected for experimentation. Under aseptic conditions, following sulcular incisions, mucoperiosteal flaps were elevated from the distal of the canine to the mesial of the mandibular first molars. With a round bur, class II furcation lesions, 5 mm in the apicoocclusal direction and 2 mm in the buccolingual direction, were surgically created on the buccal surface of these premolars. The roots were scaled to remove all periodontal ligament fibers. The bilateral defects were filled with a rubber base impression material (Zetaplus putty, Zhermack, Italy) to induce an inflammatory response and prevent spontaneous repair. The flaps were repositioned, and the wounds were closed with bioresorbable sutures.

The impression material remained in place for 3 weeks. After this period, the impression material was removed with curets, and scaling and root planing were performed on the teeth. Plaque control by daily topical application of 0.2% chlorhexidine and mechanical tooth cleaning was maintained during 2 weeks.

PP preparation

The day of periodontal surgery, 100 ml blood was drawn from the *arteria femoralis* on the hind leg and placed in the top and bottom with four bags that contained 15 ml citrate solution. Citrated blood was centrifuged in a standard laboratory centrifuge (ALC PK 130, Cologno Monzese,

Italy) for 15 min at 1,250 revolutions per minute (rpm) to obtain PRP without erythrocyte and leukocyte. A second centrifugation was performed for 10 min at 4,000 rpm. The PP concentrated at the bottom was taken, whereas the platelet-poor plasma was removed [24]. Platelet counts in the PP were done with an automatic hematology analyzer (Mindray BC 3000 Plus, Shenzhen, China).

Treatment of defects

Five weeks after the first operation, mucoperiosteal flaps were raised to expose the inflamed furcations, granulation tissues were removed, and the root surfaces were scaled and planed (Figs. 1a and 2a). A reference notch was made on the mesial and distal root surfaces at the bone crest level with a small 0.5 round bur as a guideline for histomorphometric analysis (Figs. 1b and 2b). The surgical sites were rinsed with sterile saline.

The P2s on both sides of the mandible were used as controls [group 1] and received scaling and root planing. The furcation defects of P3 and P4 on the right side were filled by PP [group 2] (Fig. 1c). At the time of application, PP was coagulated by adding 10% calcium chloride at 1:10 ratio (*v*/*v*) [24]. Atrisorb (Atrix Laboratories, Fort Collins, CO, USA), an absorbable membrane made of polylactic acid, was prepared according to the manufacturer's instructions and placed over the PP-filled furcation defects of P3 and P4 on the left side (group 3; Fig. 2c, d).

The flaps were repositioned and secured by 4-0 interrupted silk sutures. The sutures were removed after 1 week. Chemical plaque control was performed once a day by topical application of 0.2% chlorhexidine at the end of the experimental period.

After a healing period of 12 weeks, the animals were euthanized by excess anesthesia.

Histomorphometric analysis

The mandibles were dissected, fixed in 10% formalin, and decalcified in 5% nitric acid. After decalcification, blocks containing the individual teeth were immersed in paraffin. Semiserial sections (6 μm) were cut in the mesial–distal plane throughout the buccal–lingual extension of the teeth and stained with hematoxylin and eosin (H&E). Three sections which are representative of the central area of the furcation of each tooth were observed. The histomorphometric analysis was performed using a light microscope (Zeiss Axiophot Microscope, Carl Zeiss, Thornwood, NY, USA). Images were digitized with a camera (Insight Firewire 2 MegaSample Colour Mosaic Camera, Spot; SciTech, Preston, Victoria, Australia) and analyzed with the IPS 32 Software (Samba Technologies, Grenoble, France) by an examiner with no prior knowledge of the experimental design. Analysis of new cementum and new bone formation were carried out. The percentage of new cementum was evaluated at the dentin surface facing the defect, determined

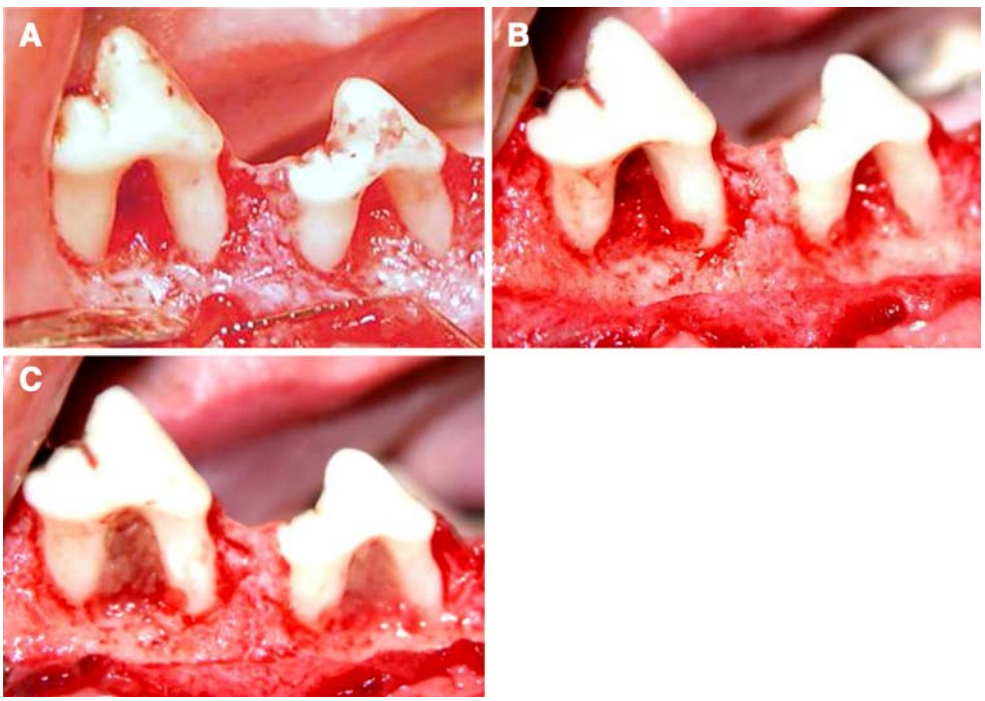

Fig. 1 **a** Clinical aspects of class II furcation defects 5 weeks after creation in group 2. **b** The reference notches made on the root surfaces in group 2. **c** Class II furcation defects treated with PP

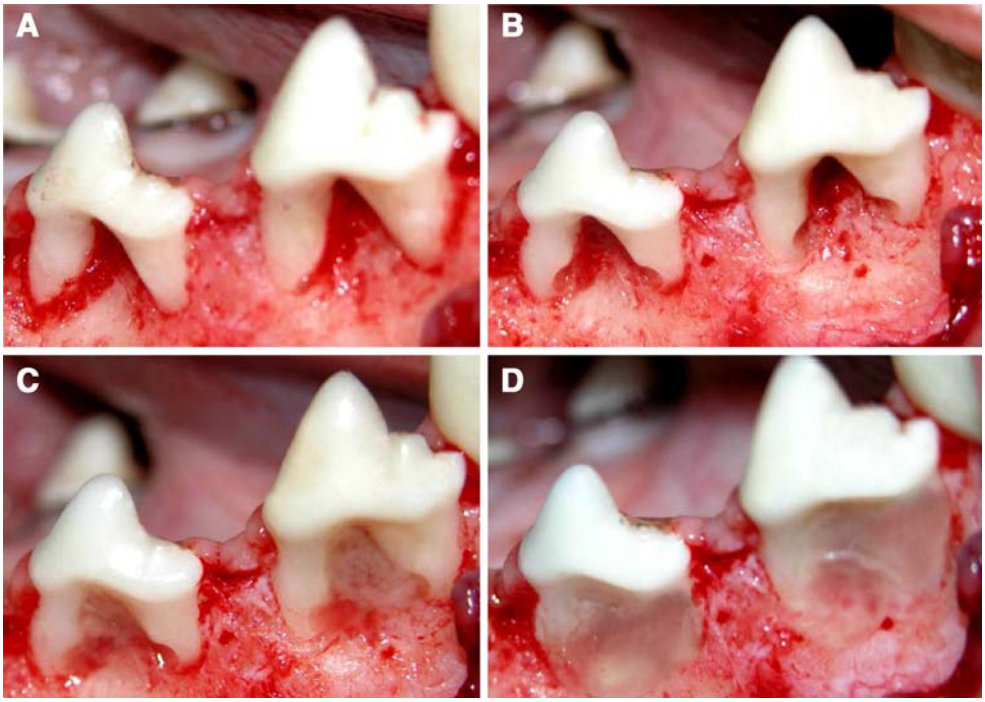

Fig. 2 **a** Clinical aspects of class II furcation defects 5 weeks after creation in group 3. **b** The reference notches made on the root surfaces in group 3. **c**, **d** Class II furcation defects treated with PP and GTR

by the apical limits of the mesial and distal notches. The mineralized matrix deposited on the root dentin was considered to be new cementum [33]. The percentage of new bone from the furcation roof to the bone crest was used to evaluate new bone formation [33].

Statistical analysis

Statistical analysis was performed using a commercially available software program (SPSS 12.0, SPSS., Chicago, IL, USA). The values for each parameter analyzed represented the arithmetic mean of the three measurements obtained from each of the three histologic sections. The Shapiro Wilk test was used to investigate whether or not the data were normally distributed. Statistical comparisons between the treatment modalities were made with Friedman nonparametric test. Comparison among groups was carried out with Wilcoxon test ($P<0.05$, N: 8).

Results

Clinical observations

Eight class II furcation defects were created and surgically treated in each study group. The surgical procedures were well tolerated by all animals, and there were no postoperative complications. No severe inflammation or swelling and dehiscence of the flaps were observed in any of the sites examined throughout the experimental period. There was no significant gingival recession in three groups. The periodontal tissues were clinically healthy on the day of sacrifice.

Histomorphometric assessment

There was new cementum along with periodontal ligament and limited coronal growth of alveolar bone in all groups. These new tissues were observed in the areas above the reference notches. None of the specimens showed root resorption and ankylosis between new bone and the root surfaces.

The newly formed bone was mainly woven bone. The new cementum, deposited over dentin, was of variable thickness, composed of cells with intrinsic and extrinsic collagen fibers distributed randomly on the root surface. The new connective tissue between new bone and the root surface was of cellular and composed of collagen fiber bundles (Fig. 3a–c).

Although the morphologic characteristics of the newly formed tissues were similar in all groups, statistically significant differences were found between test groups (groups 2 and 3) and control group (group 1) in terms of the percentage of the values in cementum analyzed (Table 1). Cementum formation was significantly higher in groups 2 and 3 compared to control group ($P<0.05$) with no significant difference between groups 2 and 3. Limited alveolar bone formation was statistically similar in all groups ($P>0.05$).

The mean ± SEM of platelets in 1 μl PP was $62{,}706\times10^3\pm5{,}028\times10^3$.

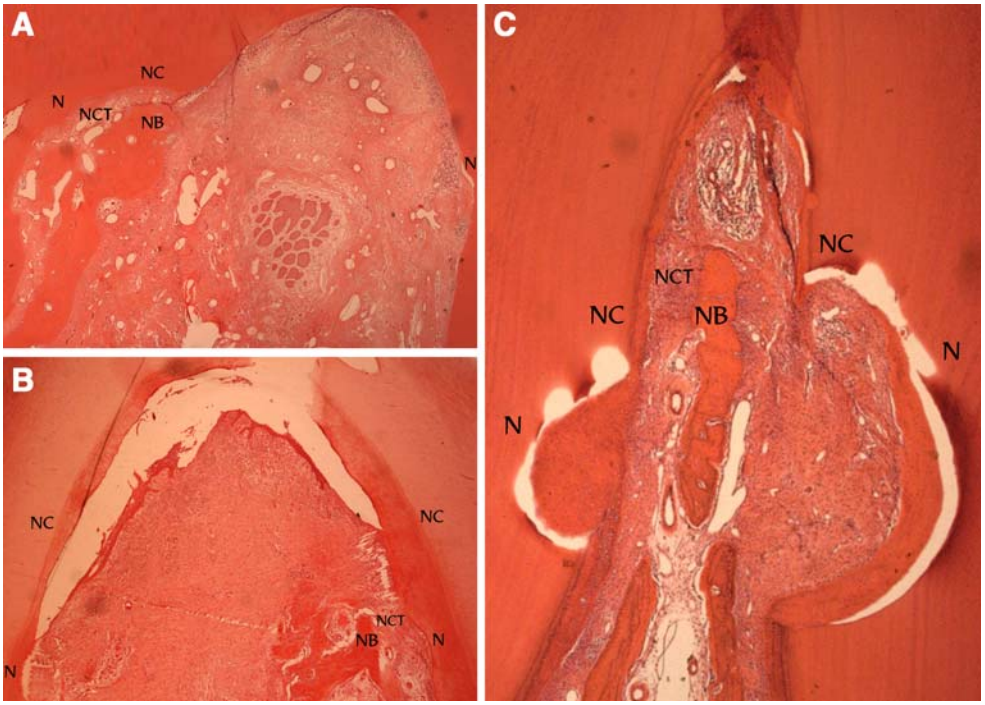

Fig. 3 Histologic overview of class II furcation defects in the mesio–distal plane at 12 weeks after surgical treatments of groups 1, 2, and 3 (H&E). *N* the base of the defects is marked by notch, *NC* new cementum, *NB* new alveolar bone, *NCT* new connective tissue. **a** Group 1, ×25 **b** Group 2, ×25 **c** Group 3, ×25

Discussion

As far as we know, this is the first report comparing the healing activity after surgical therapies with PP, combination of PP/GTR, and flap surgery in class II furcation defects. The results of the present study indicate that the healing success in cementum formation was significantly higher after surgical therapies with both PP and PP/GTR compared to the controls with regard to the histomorphometric values. No significant difference in this parameter was found between PP and PP/GTR treatments. Limited alveolar bone formation was higher in PP and PP/GTR treatments; however, this was not statistically significant compared to that of the control group. The weakness of the present study is not including GTR treatment group as we tested the hypothesis of using PP alone or with GTR membrane that is an effective treatment modality in the treatment of class II furcation defects in dogs. Significant differences might have been found between the groups if GTR treatment group was included in the study as GTR treatment has resulted in very good results in class II furcation defects in dogs [3, 6].

Histologic analysis is necessary to evaluate the effectiveness of the regenerative techniques. The most widely used animal models are dogs and nonhuman primates in the periodontology literature [3]. Class II furcation defect is one of the main indications for the regenerative procedures [19]. A chronic class II furcation defect was chosen due to the possibility of spontaneous regeneration that reduces the sensitivity of the interpretation of regenerative techniques in acute defects [36]. However, acute class II furcation defects have been used in some experimental dog studies [6, 36]. Healing of class II furcation defects in dogs was analyzed histomorphometrically on a mesial–distal plane. Similar to our study, the mesial–distal section plane is the

Table 1 The percentage of new cementum and alveolar bone

	Group 1 (Control)	Group 2 (PP)	Group 3 (PP/GTR)
New cementum (%)	34.60 (13.57–100)	97.27 (46.95–100)[a,b]	94.14 (43.55–100)[a,b]
	45.60±11.92	83.99±7.70	81.63±8.17
New bone (%)	47.84 (14.24–65.44)[c]	51.70 (44.36–98.76)[c]	49.07 (45.60–97.75)[c]
	42.44±6.07	62.64±7.89	61.06±7.90

n=8 Class II furcation defects in each groups. Friedman and Wilcoxon tests. Data are expressed as the median (minimum-maximum) and as the mean ± SEM

PP platelet pellet, *GTR* Guided tissue regeneration

[a] Significantly different from the values of group 1 ($P<0.05$)

[b] No significant difference was found between the groups ($P>0.05$)

[c] No significant difference was found between the groups ($P>0.05$)

one most commonly reported for histologic analysis in the literature [27, 33].

In GTR, the placement of a barrier membrane preserves a space for coronal migration of periodontal ligament cells and endosteal cells from the defect base which is essential for coronal bone growth [30, 33]. Experimental studies on regenerative therapy appear to favor the notion that regenerative potential of GTR promotes periodontal regeneration [3, 27]. Polylactic acid barrier was selected as the GTR membrane because the data have demonstrated that this type of barrier is successful in regenerative periodontal therapy [3, 26] and shows effective clinical results like nonabsorbable barriers [15].

In most reported studies related to the field of dental surgery, PRP has been used in combination with autogeneous bone graft, allograft, bovine-derived xenograft, and porous hydroxyapatite graft [4, 5, 14, 16, 28]. To date, there is only one case series study using autologous platelet concentrate alone in the treatment of intrabony defects that has suggested that autologous platelet concentrate achieves a similar probing depth reduction, clinical attachment level gain, and radiographic bone fill to GTR using bioabsorbable barrier membrane over a 52-week period [31]. This is the report on the usage of PP alone in class II furcation defects for regeneration. Platelets have been considered to be important in tissue regeneration, so PP with higher platelet content [24] was used in the present study. Platelet counts in this study was approximately similar that our previous clinical study reports clinical and radiological success of PP + bioresorbable GTR membrane combination [24]. To the authors' knowledge, variability of platelet counts, $2{,}163\times10^3$ platelets/μl in autologous platelet concentrate and $3{,}990\times10^3$ platelets/μl in concentrated PRP, has been reported in the literature [7, 12]. Platelet counts in the PP were found to be approximately 15- to 30-fold increased compared to the autologous platelet concentrate and concentrated PRP values.

The animals were sacrificed 3 months after periodontal surgery in this study. The observation period of experimental studies in animals has varied from a couple of weeks up to 3 or 6 months [35]. It has been reported that regenerated tissues are in the process of formation and/or remodeling after 3 months of wound healing [27]. This is consistent with the report that at 4 weeks, dense connective tissue; at 8 weeks, woven bone; and at 20 weeks, lamellar bone has filled the class III furcation defects in dogs after GTR [1]. Newly formed bone with limited amounts in this study showed characteristics of immature bone. Histomorphometric analysis of new cementum formation demonstrated a high percentage of new cementum in group 2 (83.99%) and group 3 (81.63%), and there was no significant difference among groups. Group 1 had the lowest values (45.60%). It is important to consider that the major goals of regenerative periodontal therapy are the new cementum formation and restoration of soft tissue attachment to the cementum [20, 21], since epithelial cells may not be able to migrate on the root surface which is covered by new cementum with extrinsic fibers [21].

From a clinician's perspective, it is notable that PP has a sticky consistency and adheres to the root surface, and so may impede the apical migration of epithelial and connective tissue cells from the flap as a membrane barrier [11]. This might probably be related to the higher new cementum formation in PP and PP/GTR treatments compared to the control group and also related to the lack of benefit provided by GTR to PRP. It may be hypothesized that limited and similar alveolar bone formation in the treatment groups is the result of the other physical and chemical properties of PP.

To the best of our knowledge, the clinical studies in a general sense have been reported in relation to the blood products in periodontal regenerative therapy. Findings from these controlled clinical trials using combination of PRP with bone graft materials have conflicting results [8–11, 16]. Results of some clinical studies have shown that PRP has failed to add clinical benefit to bone graft materials alone [9, 16] or in combination of bone graft materials with GTR [8, 10, 11]. PRP preparation has been suggested to have a limited potential to promote local bone formation histologically in the rat calvaria defects in an experimental study [32]. As has been reported [14], PRP may also be effective in small (periodontal) and larger bone defects if the defects are treated with both autologous graft and PRP. The authors have also considered that PRP needs vital bone cells for stimulation [14]. However, an in vitro study [22] has suggested that PRP has the ability to increase periodontal ligament cell numbers and simultaneously upregulate extracellular matrix production. PRP has also been suggested to stimulate cell proliferation and increase alkaline phosphatase activity in periodontal ligament cells in a recent study [23]. In light of this information, it is relevant to assume that PRP may promote periodontal wound healing with these functions.

It is important to note that blood products may affect the wound healing due to release of polypeptide growth factors from platelets and also their other properties [5]. PP works as a hemostatic and stabilizing agent, and may aid blood clot formation because of its high fibrin content [9]. The blood clot formation and immobilization has been reported to be the essential events for successful regenerative procedures [5, 33] as replacement of the blood clot eventually leads to the new periodontal tissue formation [27]. In the present study, histologic aspects of the healing process were closely similar in the both PP and PP/GTR treatment modalities. This finding might probably be related to the reported properties of blood products.

Based on the results of the present study, it has been concluded that both PP and the combination of PP/GTR are effective in the treatment of class II furcation defects in dogs. Within the limitations of the period and number of experimental animals, the data have also suggested that GTR adds no histomorphometric benefit to PP. PP is developed from autologous blood, completely safe, and it eliminates concerns about disease transmission and immunogeneic reactions associated with allogeneic or xenogeneic preparations [29]. It has been considered an economical source of growth factors by most clinical dentists. PP thus appears to be a suitable alternative material in the regenerative periodontal therapy.

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Conflict of Interest The authors declare that they have no financial relationships related to any products involved in this study. The study was self-funded by the authors.

References

1. Araujo MG, Berglundh T, Lindhe J (1997) On the dynamics of periodontal tissue formation in degree III furcation defects. An experimental study in dogs. J Clin Periodontol 24:738–746
2. Assoian RK, Grotendorst GR, Miller DM, Sporn MB (1984) Cellular transformation by coordinated action of three peptide growth factors from human platelets. Nature 309:804–806
3. Bogle G, Garrett S, Stoller NH, Swanbom DD, Fulfs JC, Rodgers PW, Whitman S, Dunn RL, Southard GL, Polson AM (1997) Periodontal regeneration in naturally occurring Class II furcation defects in beagle dogs after guided tissue regeneration with bioabsorbable barriers. J Periodontol 68:536–544
4. 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
5. 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 Restorative Dent 25:49–59
6. Cetiner D, Unsal B, Parlar A, Gültekin E, Kurtis B (2004) Evaluation of periodontal healing in class II furcation defects following guided tissue regeneration with two different types of polylactic acid membranes. Chin Med J (Engl) 117:270–274
7. 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
8. Christgau M, Moder D, Wagner J, Glässl 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
9. Demir B, Sengun D, Berberoglu A (2007) Clinical evaluation of platelet-rich plasma and bioactive glass in the treatment of intrabony defects. J Clin Periodontol 34:709–715
10. Döri F, Huszár T, Nikolidaki D, Arweiler NB, Gera I, Sculean A (2007) Effect of platelet-rich plasma on the healing of intra-bony defects treated with a natural bone mineral and a collagen membrane. J Clin Periodontol 34:254–261
11. Döri F, Huszár T, Nikolidakis D, Arweiler NB, Gera I, Sculean A (2007) Effect of platelet-rich plasma on the healing of intrabony defects treated with an anorganic bovine bone mineral and expanded polytetrafluoroethylene membranes. J Periodontol 78:983–990
12. Dugrillon A, Eichler H, Kern S, Klüter H (2002) Autologous concentrated platelet-rich plasma (cPRP) for local application in bone regeneration. Int J Oral Maxillofac Surg 31:615–619
13. Flemmig TF (1999) Periodontitis. Ann Periodontol 4:32–38
14. Froum SJ, Wallace SS, Tarnow DP, Cho SC (2002) Effect of platelet-rich plasma on bone growth and osseointegration in human maxillary sinus grafts: three bilateral case reports. Int J Periodontics Restorative Dent 22:45–53
15. Garrett S, Polson AM, Stoller NH, Drisko CL, Caton JG, Harrold CQ, Bogle G, Greenwell H, Lowenguth RA, Duke SP, DeRouen TA (1997) Comparison of a bioabsorbable GTR barrier to a non-absorbable barrier in treating human class II furcation defects. A multi-center parallel design randomized single-blind trial. J Periodontol 68:667–675
16. Hanna R, Trejo PM, Weltman RL (2004) Treatment of intrabony defects with bovine-derived xenograft alone and in combination with platelet-rich plasma: a randomized clinical trial. J Periodontol 75:1668–1677
17. Heitz-Mayfield LJ (2005) How effective is surgical therapy compared with nonsurgical debridement? Periodontol 2000 37:72–87
18. Ivanovski S, Li H, Daley T, Bartold PM (2000) An immunohistochemical study of matrix molecules associated with barrier membrane-mediated periodontal wound healing. J Periodontal Res 35:115–126
19. 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? J Clin Periodontol 29:103–116, discussion 160–162
20. Karring T, Nyman S, Gottlow J, Laurell L (1993) Development of the biological concept of guided tissue regeneration—animal and human studies. Periodontol 2000 1:26–35
21. Kawaguchi H, Hirachi A, Hasegawa N, Iwata T, Hamaguchi H, Shiba H, Takata T, Kato Y, Kurihara H (2004) Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. J Periodontol 75:1281–1287
22. 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
23. Kawase T, Okuda K, Saito Y, Yoshie H (2005) In vitro evidence that the biological effects of platelet-rich plasma on periodontal ligament cells is not mediated solely by constituent transforming-growth factor-beta or platelet-derived growth factor. J Periodontol 76:760–767
24. Keles GC, Cetinkaya BO, Albayrak D, Koprulu H, Acikgoz G (2006) Comparison of platelet pellet and bioactive glass in periodontal regenerative therapy. Acta Odontol Scand 64:327–333
25. Keles GC, Cetinkaya BO, Ayas B, Isildak I, Diraman E, Koprulu H, Acikgoz G (2007) Levels of gingival tissue platelet activating factor after conventional and regenerative periodontal surgery. Clin Oral Investig 11:369–376
26. Laurell L, Falk H, Fornell J, Johard G, Gottlow J (1994) Clinical use of a bioresorbable matrix barrier in guided tissue regeneration therapy. Case series. J Periodontol 65:967–975

27. Macedo GO, Souza SL, Novaes AB Jr, Grisi MF, Taba M Jr, Palioto DB (2006) Effect of early membrane removal on regeneration of Class II furcation defects in dogs. J Periodontol 77:46–53
28. 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
29. Marx RE (2001) Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent 10:225–228
30. Melcher AH (1976) On the repair potential of periodontal tissues. J Periodontol 47:256–260
31. Papli R, Chen S (2007) Surgical treatment of infrabony defects with autologous platelet concentrate or bioabsorbable barrier membrane: a prospective case series. J Periodontol 78:185–193
32. Pryor ME, Polimeni G, Koo KT, Hartman MJ, Gross H, April M, Safadi FF, Wikesjo UM (2005) Analysis of rat calvaria defects implanted with a platelet-rich plasma preparation: histologic and histometric observations. J Clin Periodontol 32:966–972
33. Regazzini PF, Novaes AB, de Oliveira PT, Palioto DB, Taba M, de Souza SL, Grisi MF (2004) Comparative study of enamel matrix derivative with or without GTR in the treatment of class II furcation lesions in dogs. Int J Periodontics Restorative Dent 24:476–487
34. Sanz M, Tonetti MS, Zabalegui I, Sicilia A, Blanco J, Rebelo H, Rasperini G, Merli M, Cortellini P, Suvan JE (2004) Treatment of intrabony defects with enamel matrix proteins or barrier membranes: results from a multicenter practice-based clinical trial. J Periodontol 75:726–733
35. Selvig KA (1994) Discussion: animal models in reconstructive therapy. J Periodontol 65:1169–1172
36. Soares FP, Hayashi F, Yorioka CW, Pannuti CM, Gioso MA, de Lima LA, Romito GA, Pustiglioni FE (2005) Repair of Class II furcation defects after a reparative tissue graft obtained from extraction sockets treated with growth factors: a histologic and histometric study in dogs. J Periodontol 76:1681–1689
37. Suvan JE (2005) Effectiveness of mechanical nonsurgical pocket therapy. Periodontol 2000 37:48–71
38. Trombelli L (2005) Which reconstructive procedures are effective for treating the periodontal intraosseous defect? Periodontol 2000 37:88–105