Platelet-rich plasma in the treatment of Class II furcation defects: a histometrical study in dogs

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ABSTRACT

Objective: This study was designed to evaluate the potential adjunctive benefits of platelet-rich plasma (PRP) when used with guided-tissue regeneration (GTR) and bioactive glass (BG) in the treatment of Class II furcation lesions. Material and Methods: Bilateral Class II furcation lesions were surgically created and allowed to become chronic in the mandibular third premolars of 9 dogs. The defects were randomly assigned to: A) GTR+BG and B) GTR+BG+PRP. Similar defects were created in the maxillary third premolars and received the same treatments after 45 days. Dogs were sacrificed 90 days after the first treatment. The histometric parameters evaluated were: connective tissue adaptation, new cementum, new bone, mineralized bone area, non-mineralized bone area, and residual BG particle area. Results: Data analysis showed a superior length of new cementum and a greater mineralized bone area for group B in both periods (p<0.05). The non-mineralized bone area was greater in the control group (p<0.05) in both periods. Conclusion: Within the limits of this study, it can be concluded that the use of PRP in the treatment of Class II furcation defects may enhance the amount of new cementum and provide a more mineralized bone in a shorter period of time.

Key words: Periodontal diseases. Platelet-rich plasma (PRP). Regeneration. Furcation defects. Dogs.

INTRODUCTION

It is recognized that teeth with furcation involvement may undergo a more extensive and rapid clinical probing attachment loss and are lost with greater frequency than single-rooted teeth[18]. Although regeneration of periodontal tissue is the ultimate goal of periodontal therapy, regeneration is not a predictable healing outcome after conventional treatment.

With the advent of regenerative techniques, a new concept of periodontal treatment based on the principle of cellular selection and guided tissue regeneration[5], permits the treatment of teeth with furcation lesions by conservative surgical methods. Various studies reported gains in horizontal and vertical clinical attachment, with complete closure of the furcation in up to 67% of the cases using different types of mechanical barriers[20,24]. Despite positive results based on reports of substantial attachment gain and bone fill of furcations, guided-tissue regeneration (GTR) is technique sensitive and could be associated with increased postoperative problems. A major drawback to expanded polytetrafluoroethylene (ePTFE) and other non-resorbable membranes is the need for

their removal after 4 to 6 weeks, often with a surgical procedure that adds to patient morbidity and may disturb the healing tissue[30].

Bone replacement grafts have achieved similar results to GTR, demonstrating a 55% overall improvement, with either complete or partial furcation fill compared to 52% for GTR[2]. The use of bioactive glass (BG) when compared to an ePTFE barrier may produce similar clinical results[30]. When used in a variety of periodontal and oral surgical applications, BG has shown positive clinical and histological outcomes[6,16,27].

In the past decade, several cytokines, or growth factors, have received attention because they can regulate migration, attachment, proliferation and/or differentiation of periodontal ligament cells into hard tissue cells and have been shown to enhance regeneration with *in vivo* experiments[4,23]. A convenient technique to obtain transforming growth factor *β* (TGF*β*) and platelet-derived growth factor (PDGF) is by preparing autologous platelet-rich plasma (PRP)[14]. PDGF has shown to exert a favorable effect on periodontal regeneration as measured by gain in clinical attachment and defect fill in humans[8].

Considering that the most favorable outcome of periodontal regenerative procedures in furcations lesions has been achieved with a combination of graft material and GTR[14,15], the goal of the present study was to investigate, histologically and histometrically, the potential adjunctive benefits of PRP when used with GTR and BG in the treatment of Class II furcation lesions.

MATERIAL AND METHODS

Nine adult female mongrel dogs were included in the experiment (mean weight=15 kg). The study protocol has been approved by the Institutional Committee of Animals Research. The surgical procedures were performed under general anesthesia with intravenous injection of a 3% sodium pentobarbital solution (0.5 mL/kg). The maxillary and mandibular second and fourth premolars had been bilaterally extracted previously and the extraction sites had been allowed to heal for 2 months. Bilateral Class II furcation lesions were surgically created at the buccal aspect of mandibular third premolars. In order to create the defects a buccal mucoperiosteal flap was raised to expose the alveolar buccal bone. Osteotomy was done in the furcation area with high-speed diamond burs with constant irrigation and with Nos. 1 and 2 Oschenbein chisels. The furcations lesions were standardized with a millimeter probe and measured 5.0 mm in the apico-occlusal direction and 2.0 mm in the buccolingual direction (Figure 1), following a technique previously described[22].

The defects were filled with gutta-percha to induce an inflammatory response due to plaque accumulation for a period of one month (Figure 2). After this period, scaling and root planing was performed and a regimen of daily brushing plus topical application of 0.1% chlorhexidine gluconate was instituted for 7 days prior to the surgical procedures.

In the same day of the treatment of the mandibular defects, similar defects were created at the buccal aspect of maxillary third premolars and were exposed to plaque accumulation for 1 month. Forty-five days after this surgical intervention, the maxillary defects were assigned to receive the same treatments.

Figure 1- Clinical aspect of Class II furcation defect (5x 2 mm)

Figure 2- Clinical aspect of Class II furcation defect filled with gutta-percha

PRP Preparation

Blood was obtained several min before the administration of anesthesia. Three tubes (5 mL), containing 0.5 mL of 3.2% sodium citrate solution as an anticoagulant, were drawn from each dog. The tubes were centrifuged at 1,200 rpm for 10 min at room temperature. The blood was thus separated into 3 basic parts: red blood cells (at the bottom of the tube), platelet enriched plasma (a discrete grey line in the middle of the tube) and platelet poor plasma (at the top of the tube). The portion corresponding to the platelet poor plasma was discarded from each tube and the remaining content was centrifuged again at 1,200 rpm for 15 min. Four hundred microliter of the middle portion, corresponding to the platelet enriched plasma, were pipetted from each tube. In order to obtain the gel, 30 μL of 10% calcium chloride was added to PRP and heated in a water bath at 37°C. The gel was achieved after a period of 10 to 15 min[26].

Defect treatment

Mucoperiosteal flaps were carefully reflected on the buccal aspect of the experimental sites, the granulation tissue was removed by curettage, and the exposed root surface was instrumented. On the root surface, the base of the defect was marked with curettes to establish a landmark for the histomorphometric analysis. Each contralateral defect in each animal was randomly assigned to one of the following treatments:

GTR+BG (Perioglas, US Biomaterials, Alachua, FL, USA) – Control group: BG particles were applied filling the defect and absorbable membrane (Resolut XT® Gore-tex, Gore Associates, Flagstaff, AZ, USA) was adapted to the defect;

GTR+BG+PRP - Test group: BG was incorporated to the PRP (1:1) and this assemblage was immediately taken to a water bath at 37ºC what enabled the formation of a mixture of the gel and the BG graft. This mixture was applied filling the defect and an absorbable membrane (Resolut XT® Gore-tex, Gore Associates) was adapted to the defect.

Primary, tension-free wound closure was accomplished following defect treatment, with the gingival flaps positioned and sutured with interrupted sutures (Vicryl, Ethicon INC, São José dos Campos, SP, Brazil) in order to cover the membranes. Immediately after the surgeries, an intramuscular injection of penicillin (1.5 mL – 150,000 IU) was given to the animal.

Forty five days after the mandibular treatment, the maxillary defects (2 defects/ animal) were randomly assigned to receive the same treatments (Group A and Group B).

Postoperative plaque control was performed by irrigation with a solution of 1% chlorhexidine gluconate (every other day). Dogs were sacrificed 90 days after the first treatment, under general anesthesia, with a perfusion of 10% neutral formalin solution. The jaws were dissected and the blocks containing the experimental specimens were obtained. They were decalcified in a solution of equal parts of 50% formic acid and 20% sodium citrate for 5 months. The decalcified specimens were washed in running water, dehydrated and embedded in paraffin. Mesiodistal 7-μm-thick sections were obtained from the tissue blocks longitudinally, in a buccolingual direction, in a way to obtain a panoramic view of the furcation area. The sections were stained with hematoxylin and eosin.

For histometric and descriptive histological analyses, 5 sections *per* site representing the middle portion of the defect (30 μm apart) were used to obtain the mean for each parameter in each dog, measured with an image analysis system (Image Pro Plus Version 3.0, Media Cybernetics, Silver Spring, MD, USA). The sections were blindly presented for measurements by one examiner.

Linear measurements

The following linear measurements (mm) were obtained in the mesial and distal roots under the furcation:

Defect extension (DE): linear extension of the total root surface between the two notches;

New cementum (NC): linear extension of the root surface covered by new cementum;

Connective Tissue Adaptation (CA): extension of the root surface covered by connective tissue *without cementum*.

New bone (NB): measured from a horizontal line connecting both notches (apical limit of the defect) to the most coronal extent of new bone.

Area measurements

A line connecting both notches defined the apical limit of the defect. The following parameters (mm^2) were obtained by the point counting technique:

Mineralized bone area (MBA): portion of the defect area filled with mineralized bone tissue;

Residual BG particle area (RBG): portion of the defect area filled with BG remaining particles;

Non-mineralized bone area (NMBA): portion of the defect area filled with non-mineralized tissue;

The measurements were performed using a microscope (Zeiss Axioskop 2, Carl Zeiss Instruments, Gottingen, Lower Saxony, Germany) with a 1.25/.035 objective associated with a video camera/computer/software (Image Pro Plus Version 3.0, Media Cybernetics).

Statistical analysis

Mean values for each parameter were obtained *per* defect. The mean values for all groups were
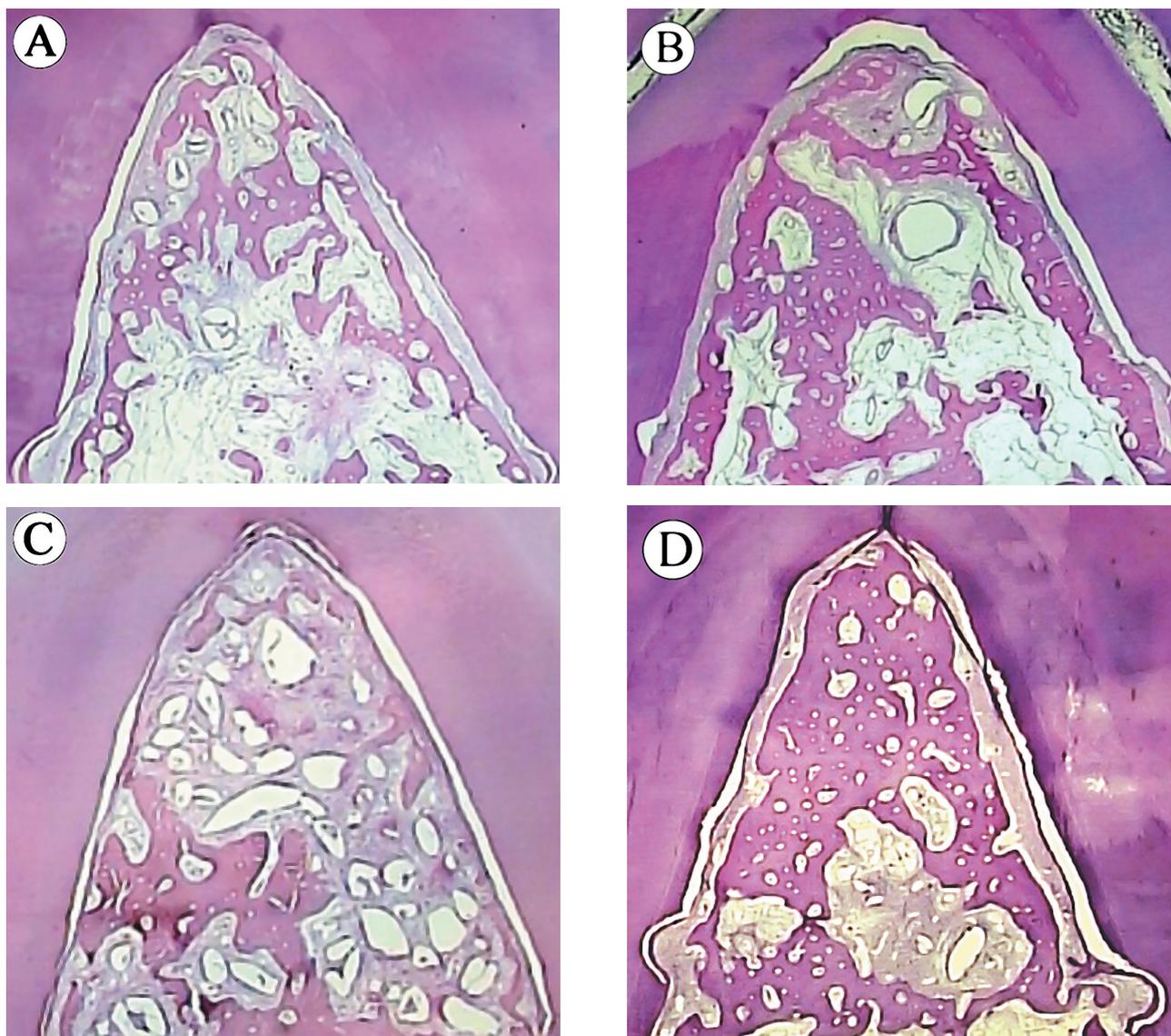
Figure 3- Mesiodistal histologic section of Class II furcation lesion stained with hematoxilyn and eosin (H&E), (original magnification x20); A) Site of the control group guided-tissue regeneration + bioactive glass (GTR+BG) after 45 days; B) Site of the test group guided-tissue regeneration + bioactive glass + platelet-rich plasma (GTR+BG+PRP) after 45 days; C) Site of the control group after 90 days; D) Site of the test group after 90 days. Both treatments promote defect filling in both periods however a greater mineralized bone area was observed for test group in both periods when compare with the control group

determined using the individual means from the 9 dogs. After testing the assumptions confirming normal distribution, two-way ANOVA was carried out to check any significance among the different treatment groups, any significance among the different time periods and the interaction between them. The p-value ≤0.05 was considered statistically significant.

RESULTS

Clinical observations

Clinically, the healing response was favorable for all treatments. No suppuration or abscess formation was observed during the 90 days and no exposure of membranes was observed in both groups during the healing period. A clinical examination performed immediately before sacrifice, disclosed that the gingival margin of all sites was clinically normal and positioned coronal to the fornix of the buccal furcation.

Histological observations

There were no cases of epithelial downgrowth, ankylosis or extensive root resorption observed in all sites. No inflammation and foreign body reactions indicating toxicity were observed. A similar healing pattern was observed in the two groups, in both periods, regarding the filling of the defect (Figure 3).

A continuous layer of new cementum had formed in the specimens of both groups extending coronally from the apical notches to a varying degree. However, in the group B, a more evident layer of new cementum was observed in the fornix region (coronal portion of the defect). In both groups, some small areas of the defects presented a very dense connective tissue surrounding the remaining BG particles. However, connective tissue adaptation with collagen fibers running parallel to the root surface seemed to be more frequent in group A.

Various defects in all groups presented bone surrounding BG particles and bone in direct contact with these graft particles (Figure 4). However, in
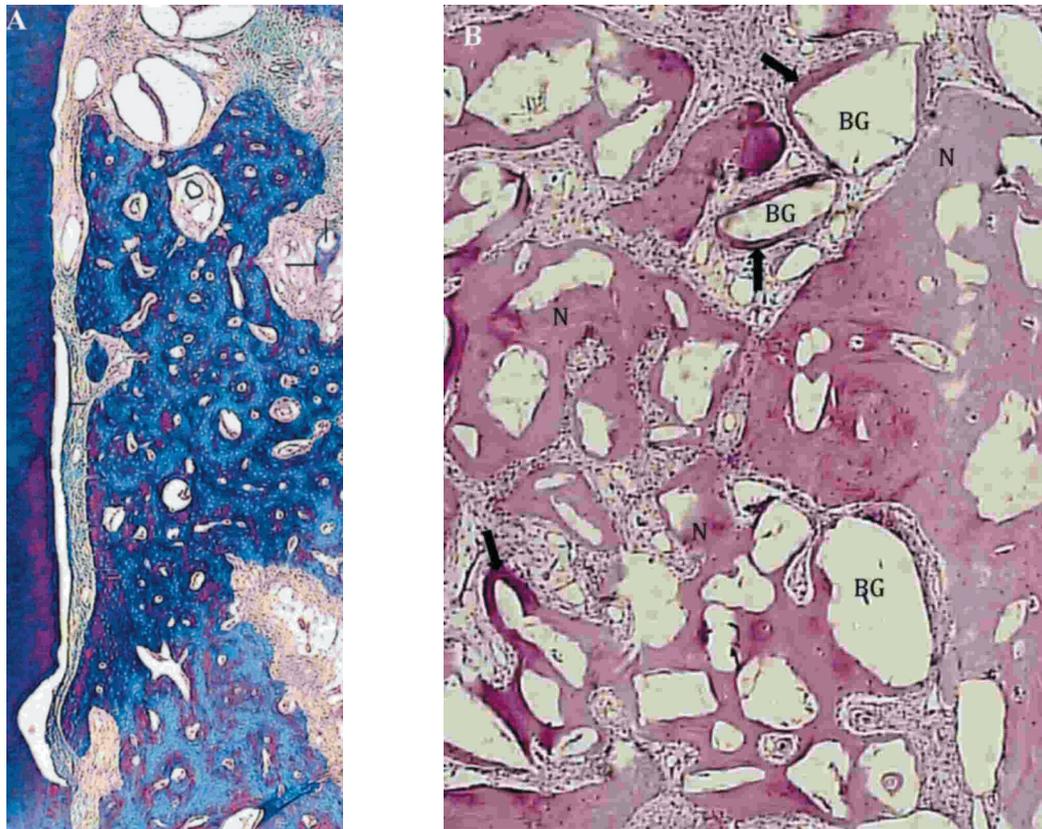
Figure 4- A) Photomicrograph of a mesiodistal histologic section of Class II furcation lesion platelet-rich plasma + bioactive glass + guided-tissue regeneration (PRP+BG+GTR) 90 days after treatment defect (Masson Trichrome staining, original magnification x40). New cementum (NC) was observed above the notch (N). Remaining BG particles were observed surrounded by new bone (NB), connective tissue (CT) and areas suggesting the presence of immature bone (IB) in contact with BG; B) Histologic section of the PRP+BG+GTR defect, 90 days after staining with hematoxilyn and eosin (H&E), (original magnification x100). Remaining BG particles could be observed surrounded by NB and CT. Some areas indicated the new bone formation occurring around BG particles (arrows)

Table 1- Mean and Standard Deviation (SD) for the linear parameters evaluated after all the treatments (bioactive glass+guided-tissue regeneration and bioactive glass+platelet-rich plasma+guided-tissue regeneration) in both periods.

Mean±SD Parameters	Group A (control group)		Group B (test group)	
	45 days	90 days	45 days	90 days
New cementum (mm)	9.46±1.23[Ba]	9.20±3.21[Ba]	12.10±1.88[Aa]	12.45±1.73[Aa]
Connective Tissue Adaptation (mm)	2.00±0.71[Ba]	1.84±1.87[Ba]	0.00±0.00 [Aa]	0.33±0.62[Aa]
New bone (mm)	3.68±1.11[Aa]	4.33±0.64[Aa]	4.60±0.39[Aa]	5.01±0.63[Aa]
Defect Extension (mm)	11.88±1.17[Aa]	11.14±2.80[Aa]	12.10±1.88[Aa]	12.78±1.41[Aa]

Capital letters compare treatments within each time and non-capital letter compare times within each treatment. Means followed by different letters are statistically different

Table 2- Mean and Standard Deviation (SD) for the area parameters evaluated after all the treatments (bioactive glass+guided-tissue regeneration and bioactive glass+platelet-rich plasma+guided-tissue regeneration) in both periods

Mean±SD Parameters	Group A (control group)		Group B (test group)	
	45 days	90 days	45 days	90 days
Mineralized Bone Area (mm²)	4.66±1.52[Bb]	7.63±1.53[Ba]	9.20±2.31[Ab]	10.31±4,51[Aa]
Non-mineralized bone Area (mm²)	7.16±2.49[Aa]	4.10±0.63[Ab]	4.34±0.48[Ba]	2.58±0.69[Bb]
Residual BG particles Area (mm²)	1.77±0.51[Aa]	1.74±0.44[Aa]	1.72±0.80[Aa]	1.40±0.68[Aa]

Capital letters compare treatments within each time and non-capital letter compare times within each treatment. Means followed by different letters are statistically different

group B the defects were filled by a higher density bone with a more evident mineralized bone area with islands of highly cellular bone marrow. In group A, the bone presented larger marrow spaces what could indicate a more immature bone when compared to group B.

Histometric measurements

The histometric results are shown in Tables 1 and 2. Data analysis showed a superior length of new cementum for group B in both periods (45 days and 90 days) (p<0.05). No statistically significant differences were observed between periods for the linear parameters for the two groups. The addition of PRP provided a greater mineralized bone area in both periods (p<0.05). The non-mineralized bone area was greater in the control group (p<0.05) in both periods while no statistically significant difference was observed in the area occupied by the remaining BG particles between groups. Significant differences between periods were observed in the mineralized bone area and non-mineralized bone area (p<0.05).

DISCUSSION

The present study evaluated if the application of PRP on Class II furcation lesions would enhance periodontal regeneration when associated with GTR and bioactive glass (BG). Clinical reports have demonstrated that the association of GTR, bone substitutes and PRP could provide good results in the treatment of intrabony defects and class II furcation lesions, as revealed by gain in clinical attachment, defect fill at reentry, reducing probing depth, vertical defect fill and horizontal defect fill[13,14]. However, to the best of our knowledge, this is the first study that evaluated histologically the effect of the association of PRP with GTR and BG on periodontal regeneration of class II furcation lesions in dogs.

It has been demonstrated that when a bone graft or substitute is combined with the GTR procedure in furcation lesions, a greater amount of defect fill is observed[3,14,15]. This advantage can be explained by the osteoconductive and/or osteoinductive properties of the graft materials. One of the bone substitutes that have been investigated is the BG, a material that has been suggested as an osteoconductive graft[6,16,27]. The use of BG in the present study was not associated with any foreign body reaction. Healing events such as ankylosis and root resorption were not observed. This observation is in agreement with previous studies using BG in animals[7,29]. It was observed that some remaining BG granules showed dissolution of their core with the formation of new bone, as described by Vogel, et al.[28] (2001).

The sites treated with GRT+BG (Group A) showed new cementum formation and new bone regeneration in both periods. These results are in accordance with previous reports, which demonstrated a beneficial effect of the association of GTR and bone substitutes[14,15]. However, clinical studies do not provide information about the nature of the healing process after these procedures.

In contrast with previous studies[13,18,22], in the present investigation, there were no instances of epithelial downgrowth in all sites. In similar defects, Andrade, et al.[1] (2007) observed a correlation between the exposure of the membrane and the epithelial migration. The presence of epithelial tissue was observed in all histological sections of the sites that shown a membrane exposure and was ascribed to the degradation of the exposed portions of this material.

The use of PRP for periodontal regeneration has been based on the idea that this preparation contains polypeptide growth factors (PGFs). Some specific PGFs, like PDGF and TGFβ, could promote the growth and differentiation of the periodontal ligament and alveolar bone cells and could be responsible for the clinical improvement observed in experimental sites[4,23]. Other interesting feature

of PRP is its sticky consistency due to its high fibrin content. The fibrin component of PRP may work as a hemostatic agent aiding in stabilizing the graft material and the blood clot[19]. Blood clot immobilization has been suggested as an important event in the early phases of wound healing of periodontal regenerative procedures[20,21].

It has been previously shown that PRP may stimulate PDL cells and fibroblastic cell proliferation[10,11] but may suppress epithelial cell division *in vitro*[17]. Consequently, by ordering these cellular responses into a series of related events, PRP may facilitate wound-healing and tissue regeneration. In the present study, the sites in the Group B showed a superior cementum formation, in both observation periods, when compared with the sites of Group A (p<0.05). To the best of our knowledge, no study provided direct evidence that the PRP could stimulate the differentiation of cells from the periodontal ligament to cementoblasts, inducing cementogenesis. However, previous investigations have suggested that the treatment with recombinant growth factors, such as PDGF and IGF may enhance the formation of cementum on denuded dentin surfaces[4]. In addition to it, a similar result was observed when the PRP was associated with a subepithelial connective tissue graft for the treatment of gingival recessions created in dogs[26].

The positive effect of PRP in the bone response was demonstrated by previous studies[9,21] and could be evidenced in the present study by the largest area of mineralized bone observed in group B. Sites treated with PRP showed a superior mineralized bone area with a concomitant inferior non-mineralized bone area, in both periods, when compared with group A (p<0.05). However, the difference between treatments is more evident in the first period (45 days) when the area of mineralized bone in group B was almost twice the one observed in group A. This observation is in agreement with an *in vitro* study[25] that suggested that PDGF acts upon osteoblastic cell proliferation, exerting most of its effects during the early phase of wound healing. Although the mineralized bone formation was superior in group B of the present study, it should be noticed that recent animals studies could not demonstrate that PRP increases bone regeneration when used in peri-implant bone defects[9].

To date, there is insufficient information about growth factor interactions and how they influence the activations of gene expression and protein production. Also, the ideal platelet and growth factor concentrations to promote periodontal regeneration has not been established. Lacoste, et al.[12] (2003) reported that growth factors may act at specific times and at appropriate concentrations and this may be other explanation for the different results in studies with PRP. In the present study, the achieved percentage mean of platelet concentration was 227.02 % (mean of 463,015 platelets/µL) in relation to blood platelet count.

The technique to produce PRP used in this study was previously described by Suaid, et al.[26] (2008). This technique enables to produce a platelet concentration generally four times greater than the whole blood. Lacoste, et al.[12] (2003) performed a study that measured the concentrations of bFGF, VEGF, PDGF-BB and TGF-β1 released from platelet concentrate and whole blood, before and after the addition of calcium alone, thrombin alone and various concentrations of calcium and thrombin. They observed that calcium chloride, regardless of the use of thrombin, released platelet growth factors. In this study, the concentration of growth factors that was released was sufficient to promote endothelial cell proliferation *in vitro*[12]. Since the use of thrombin in this type of investigation is not allowed in our country, instead of thrombin, 10% calcium chloride was added to PRP and heated in a water bath at 37°C.

CONCLUSIONS

Within the limits of this animal study, it was concluded that PRP in association with GTR and BG may enhance the amount of new cementum and provide a more mineralized bone (mature) in a shorter period of time when applied to the treatment of class II furcation lesions in dogs. Future studies are required to clarify the mechanism of action of PRP in the periodontal regeneration process and the ideal platelet and growth factor concentrations to adequately promote periodontal regeneration.

ACKNOWLEDGEMENTS

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