Platelet-rich plasma plus bioactive glass in the treatment of intra-bony defects: a study in dogs

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Received: May 22, 2009 - Modification: August 20, 2009 - Accepted: October 17, 2009

ABSTRACT

Objective: This study was designed to evaluate, histomorphometrically, the association of platelet-rich plasma (PRP) and bioactive glass (BG) in the treatment of periodontal intrabony defects. Material and Methods: Nine mongrel dogs were included in the study. Three-wall intrabony defects were surgically created at the mesial and distal aspect of first mandibular molar and exposed to plaque accumulation for 1 month. The defects were randomly assigned to the groups: control, BG, PRP, PRP+BG. Dogs were sacrificed 90 days after the surgeries. The histometric parameters evaluated were: length of sulcular and junctional epithelium, connective tissue adaptation, new cementum, new bone, defect extension and area of new bone filling the defect. Results: A superior area of new bone was observed in PRP+BG and BG (13.80±2.32 mm^2 and 15.63±2.64 mm^2, respectively) when compared to the other groups (8.19±1.46 mm^2 and 8.81±1.47 mm^2 for control and PRP, respectively). No statistically significant differences were observed in the remaining parameters. Conclusions: Within the limits of this study, it may be concluded that PRP failed to provide statistically significant improvements in the histometric parameters.

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

INTRODUCTION

The ultimate goal of periodontal therapy includes not only the arrest of progressive periodontal disease but also the restitution of those parts of the supporting apparatus which have been destroyed by disease. Regenerative procedures have been evaluated in several studies using grafting materials, root surface conditioning, guided tissue regeneration and application of growth factors[3,5,6,9].

Different materials have been introduced for bone defect filling, i.e., autografts, xenografts, allografts, and alloplasts. Among the various subgroups of alloplastic bone grafts, bioactive glass (BG) is a type of bioactive ceramic with the ability to improve bone formation that has been assessed in experimental models[13,27].

Previous studies have shown that BG particles, when implanted *in vivo*, incorporate into the connective tissue and undergo chemical transformation increasing the pH of the site to approximately 10[7,8]. This process leads to the formation of a silica gel on the surface of the particles and subsequently an amorphous calcium phosphate layer is formed by the interaction between calcium and phosphate from the BG and the surrounding medium[7]. Hench and Polak[8] (2002) have shown that the release of ions (Na, Ca, and Si) from BG materials controls the cell cycle leading to the differentiation and proliferation of bone cells, modulating the expression of genes that regulate osteogenesis, and the synthesis of growth factors.

Growth factors are vital modulators during

this process which can induce the migration, attachment, proliferation and differentiation of periodontal progenitor cells. Platelet-rich plasma (PRP) is a preparation of autologous plasma that contains a higher platelet concentration, allowing it to deliver a greater concentration of autologous growth factors such us platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF-I), and epithelial growth factor (EGF), that regulate cell proliferation, chemotaxis and differentiation[17].

Some specific platelet growth factors, like PDGF and TGF-β, 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[29]. Other interesting feature of PRP is its sticky consistency due to its high fibrin content. The fibrin component of PRP may work as a haemostatic agent aiding in stabilizing the graft material and the blood clot[22]. Blood clot immobilization has been suggested as an important event in the early phases of wound healing of periodontal regenerative procedures[22].

Studies using histological techniques suggested that PRP preparations may enhance local bone formation[17], while others[10] did not confirm these findings. Clinical studies have reported that treatments with a combination of PRP and demineralized freeze-dried bone graft allograft (DFDBA) improve clinical parameters in intrabony periodontal defects[21]. In the other hand[4] didn't find additional benefit in the reduction of pocket depth, clinical attachment gain and defect filling using PRP with BG in the treatment of intrabony defects.

The use of PRP associated with biomaterials is an alternative treatment approach that may favor the process of bone tissue regeneration and should be investigated. Therefore, the goal of this study was to evaluate, histomorphometrically, the healing of surgically created intrabony defects in dogs treated with PRP+BG.

MATERIAL AND METHODS

Nine adult female mongrel dogs (mean weight=15 kg) were included in the experiment. The study protocol was approved by the Institutional Animal Research Committee, State University of Campinas (Protocol #686-1). The surgical procedures were performed under general anesthesia with intravenous injection of a 3% sodium pentobarbital solution (0.5 mL/kg). The mandibular second and fourth premolars had been previously extracted and the extraction sites had been allowed to heal for 2 months. After this period, buccal and lingual mucoperiosteal flaps were elevated and 3-wall intrabony defects (4x4x4 mm) were created at the mesial and distal aspect of the left and right mandibular first molar (Figure 1)[14]. Following root planning, aiming cementum removal, a reference notch was made with curettes on the root surface at the cement-enamel junction.

Before that the gingival flaps have been positioned and sutured, a tofflemire matrix involved by cotton ligature was adapted over the tooth surface and the remaining defect was filled with gutta-percha (Figure 2). So, the defects were exposed to plaque accumulation for a period of one month before the treatment. After that, 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.

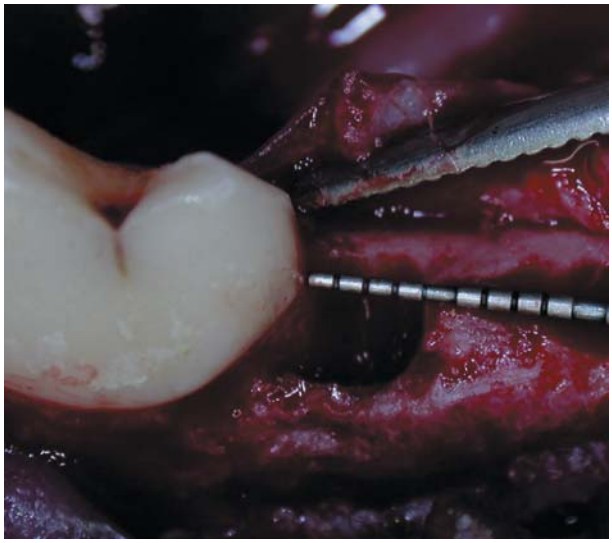

Figure 1- Clinical view of the surgically created 3-wall intrabony defect (4x4x4 mm) at the mesial aspect of the first mandibular molar

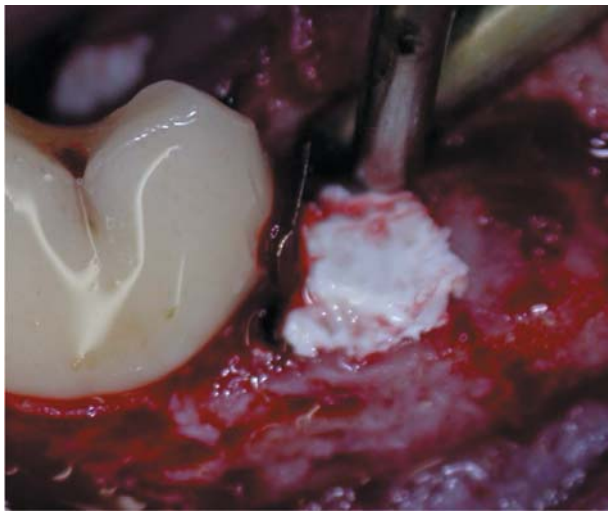

Figure 2- Clinical view of the insertion of gutta-percha in the defect

PRP Preparation

Blood was obtained several min before the administration of anesthesia. Four 5-mL tubes containing 0.5 mL of 3.2% sodium citrate solution (an anticoagulant) were drawn from each dog. Three 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 microliters 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[2,26]. A tube with a sample of whole blood as well as a sample of PRP, retrieved before adding calcium chloride of 10%, was sent to a clinical veterinary laboratory to perform a platelet counting.

Defect Treatment

In the moment of the defect treatment, an apical reference notch was made with curettes on the root surface at the remaining bone level. Each defect in each animal (a total of 4 defects/animal) was randomly assigned to one of the following treatments (the treatments with PRP were located at the same side that was randomly determined): *Control*: the defect was naturally filled with coagulum; *BG (Perioglas*, US Biomaterials, Alachua, FL, USA): BG particles were applied, filling the defect; *PRP*: after obtaining the PRP gel, it was immediately applied on the defect; *PRP+BG*: BG was incorporated to the PRP (1:1) and this assemblage was immediately taken to a water bath at 37ºC for formation of a mixture of the gel and the BG graft.

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) at their presurgical position. Immediately after the surgeries, an intramuscular injection of penicillin (1.5 mL – 150,000 IU) was given to the animal.

Postoperative plaque control was performed by irrigation with a solution of 1% chlorhexidine gluconate (every other day). After 90 days, under general anesthesia, the animals were sacrificed with a perfusion of 10% neutral formalin solution. The jaws were dissected and the blocks containing the experimental specimens were removed, and 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 and stained with hematoxylin and eosin.

Five sections representing the midbuccal portion of each defect were selected for the histometric procedures. The sections were analyzed by one masked examiner. The following distances were measured:

Total epithelium (sulcular and junctional epithelium): from the gingival margin to the apical border of the junctional epithelium.

Connective tissue adaptation (without cementum): from the apical border of the junctional epithelium to the coronal end of new cementum.

New cementum: from the apical notch to the most coronal part of new cementum.

New bone (bone position): from the apical notch to the most coronal extent of new bone. Negative values would be assigned if the bony crest was located apically to the apical notch.

New bone area: area of newly formed bone filling the defect area (limited by the distance between the two notches in an apico-coronal direction and 4 mm away from the root surface in a lateral direction). The area of new bone was measured with an image analysis system that allowed the placement of a lattice on the image of the created bone defect. The intersection lines localized on bone were marked to calculate the new bone area whereas intersections lines localized on BG particles were not marked and therefore were not included for this purpose.

Defect extension: from the apical notch to the coronal notch.

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, Silver Spring, MD, USA).

Statistical analysis

Mean values for each parameter were obtained per defect. The mean values for all groups were determined using the individual means from the 9 dogs. The hypothesis that there were no differences among the groups was tested by one-way ANOVA. A p value ≤ 0.05 was considered statistically significant. If a statistical difference was detected, Tukey's pairwise multiple comparison test was used.

RESULTS

I - Clinical Observations

Clinically, the healing response was favorable for all treatments. No suppuration or abscess formation was observed during the 90 days.

II - Histological Observations

No undesirable events like inflammation and foreign body reactions were observed. All groups presented a similar healing pattern characterized mainly by the presence of new cementum covering most of the root surface associated with the defect and new bone, up to the top of the defect, with a small amount of connective tissue and epithelium (Figure 3). The periodontal ligament was characterized by the presence of collagen fibers obliquely oriented to the root surface, extending between the new cementum and the newly formed bone.

In the defects treated with BG, it was possible to observe that part of the material seemed to be resorbed and replaced by bone, while some of the remaining BG particles showed dissolution of their central part with newly formed bone inside of them. Some defects presented bone in direct contact and/or surrounding the BG particles (Figure 4). Almost all defects that received BG presented a dense connective tissue surrounding the remaining particles and areas suggesting the formation of new bone. Root resorption and ankylosis were not observed.

III – Histometric Measurements

The histometric results are shown in Table 1. A superior area of new bone was observed in the sites treated with PRP+BG and BG (13.80±2.32 mm^2 and 15.63±2.64 mm^2, respectively). In the other groups, the new bone area was 8.19±1.46 mm^2 (control group) and 8.81±1.47 mm^2 (PRP). Although the difference in the new bone area, no significant difference was found in the linear parameter new bone, e.g. bone position: from the apical notch to the most coronal extent of new bone (4.37±0.44 mm, 4.24±0.68 mm, 4.37±0.46 mm and 4.44±0.42 mm, for control group, BG, PRP and PRP+BG, respectively; p=0.85).

Data analysis showed no significant differences among the groups regarding the initial defect extension (4.55±0.17 mm, 4.48±0.20 mm, 4.48±0.28 mm and 4.57±0.32 mm, for control group, BG, PRP and PRP+BG, respectively; p=0.74). In addition, intergroup analysis demonstrated that no significant difference was observed among the groups in the following parameters: total epithelium extension (2.24±0.58 mm, 1.94±0.37 mm, 1.97±0.37 mm and 1.81±0.61, for control group, BG, PRP and PRP+BG, respectively; p=0.45), connective tissue extension (0.90±0.28 mm, 0.84±0.41 mm, 1.07±0.27 mm and 1.15±0.32 mm, for control group, BG, PRP and PRP+BG, respectively; p=0.59) and new cementum extension (2.63±0.70 mm, 2.56±0.36 mm, 2.37±0.38 mm and 3.10±0.47 mm, for control group, BG, PRP and PRP+BG, respectively; p=0.85).

The results from the histometric measurements, *i.e.*, epithelial length (the portion of epithelium located on the root), connective tissue adaptation and new cementum formation, expressed as the

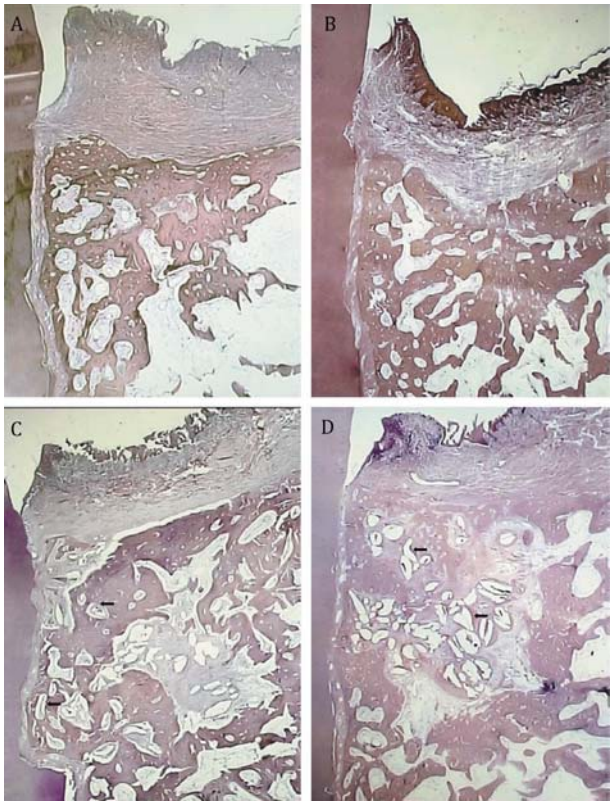

Figure 3- Photomicrographs of the treated 3-wall intrabony defect showing new bone and cementum formation extending coronally to the apical notch (H&E, original magnification x20). A) Control; B) PRP; C) BG; D) BG+PRP. Note the remaining particles of BG surrounded by new bone and connective tissue (C and D). Arrows indicate areas with new bone in contact with BG particles

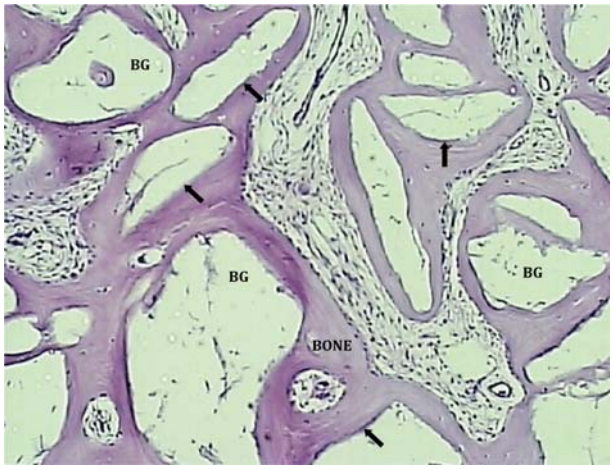

Figure 4- New bone formation observed in the 3-wall intrabony defect treated with BG (H&E, original magnification x100). Arrows indicate bone in direct contact with BG particles

Table 1- Mean and Standard Deviation (SD) for the parameters evaluated after all the treatments (Control, BG, PRP and BG+PRP). Means followed by different letters are statistically different at 5%

Mean ± SD / Parameters	Control	BG	PRP	BG+PRP	p†
Total epithelium	2.24±0.58[a]	1.94±0.37[a]	1.97±0.37[a]	1.81±0.61[a]	0.45
Connective tissue	0.90±0.28[a]	0.84±0.41[a]	1.07±0.27[a]	1.15±0.32[a]	0.59
New cementum	2.63±0.70[a]	2.56±0.36[a]	2.37±0.38[a]	3.10±0.47[a]	0.85
New bone	4.37±0.44[a]	4.24±0.68[a]	4.37±0.46[a]	4.44±0.42[a]	0.85
New bone area	8.19±1.46[a]	15.63±2.64[b]	8.81±1.47[a]	13.80±2.32[b]	<0.0001
Defect extension	4.55±0.17[a]	4.48±0.20[a]	4.48±0.28[a]	4.57±0.32[a]	0.74

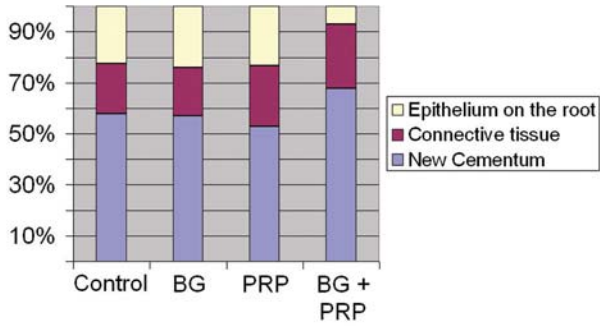

Figure 5- Histometric parameters expressed as a percentage of the defect

percentage of the distance between the coronal and the apical notch are presented in Figure 5.

DISCUSSION

The use of different regenerative approaches in the treatment of periodontal defects has demonstrated a variable clinical result[3,6,9]. Some techniques have been used in association, aiming to increase their effect on periodontal regeneration[5,17]. The present study was designed to evaluate, histomorphometrically, the healing process of 3-wall intrabony defects treated with BG, PRP and their association.

PRP has become a focus of current studies due to its potential to accelerate wound healing[11,12]. It has been previously shown that PRP stimulated PDL cells and fibroblastic cell proliferation[11,12] but suppressed epithelial cell division *in vitro*[19]. Consequently, by ordering these cellular responses into a series of related events, PRP may facilitate wound-healing and tissue regeneration. The claimed ability to suppress epithelial cell proliferation seems advantageous for periodontal regeneration by favoring the formation of a new connective tissue attachment and new cementum on the root surface[26]. The results of the present study failed to demonstrate that the use of PRP can promote an additional effect in terms of cementum formation when treating 3-wall intrabony defects. Cementum formation for BG and PRP followed the same trend observed for the control group (BG=2.5 mm/57%, PRP=2.4 mm/53%, control=58%) while the PRP+BG showed a slightly greater cementum formation (PRP+BG=3.10 mm/68%), but the differences among the groups were not statistically significant. In this respect, it should be recognized that a non-contained osseous defect might have favored the discrimination of quantitative differences among the treatments.

In a previous histological study, Kim, et al[14] (2004) evaluated the healing patterns following flap surgery in experimentally induced 1-, 2- and 3-wall intrabony defects. According to the authors, one- and 3-wall intrabony defects can be considered pre-clinical models that are relatively easy to manage and from which homogeneous healing outcomes may be expected. They suggested that these defects appear to be reproducible models to evaluate candidate technologies for periodontal regeneration[14]. Regarding the 3-wall intrabony defect, they reported a mean cementum formation of approximately 70% of the initial defect extension. In the present study, a period of 1 month of plaque accumulation was allowed as a way to obtain some chronification of the defect. The control group showed 2.6 mm (58%) of the original defect covered with new cementum. This result confirms the high regenerative potential of this type of defect that provides favorable anatomic characteristics for clot stabilization and protection.

It is well accepted that healing of intrabony defects is positively correlated to the number of bone walls limiting the defect. Therefore, the 3-wall intrabony defect used in the present study provides ideal extent and location of tissue resources, cells and vascularity circumscribing the defect, which might have favored bone formation. The linear measurement of the new bone height revealed an impressive coronal bone formation for all the groups, with no significant differences among the treatments.

The claimed osteoinductive properties of PRP[18] could not be supported by the results of the present study. It has been suggested that PRP could be used to increase the bone deposition rate and the quality of bone regeneration when augmenting sites prior to or in conjunction with dental implant placement. Nevertheless, more recent animal studies could not find increased bone regeneration when PRP was used[10,13,18].

Recently, bioactive glass has been used as a way to achieve bone and periodontal regeneration based on its osteoconductive properties[3,4,18,27]. The need for an alternative to autologous and allogenic bone grafts have encouraged the search of a material that could be safe, surgically convenient and predictably promote regeneration with a reduced morbidity. In this study, the use of BG was not associated with any foreign body reaction, showing biocompatibility with periodontal tissues. Healing events such as ankylosis and root resorption were not observed. This observation is in agreement with previous studies using BG in animals[7].

Histological observations after treatment of periodontal defects with BG in nonhuman primates showed a significant increase of new cementum and inhibition of downgrowth of junctional epithelium[27]. When BG is implanted *in vivo*, the pH of the site increases, a layer rich in silica gel is formed on the surface of the particles and subsequently an amorphous calcium phosphate layer is formed by the interaction between calcium and phosphate from the BG and the surrounding medium[7]. Its superficial bioactivity may stimulate a rapid formation of a connective tissue seal that is supposed to have the ability to block the epithelium migration and allow for the repopulation of the previously contaminated area by periodontal ligament cells[7]. In the present study, almost all defects that received BG presented a dense connective tissue surrounding the remaining particles in accordance with observations of previous studies[27].

In the groups treated with BG, the area of new bone was 13.80±2.32 mm^2 (PRP+BG) and 15.63±2.64 mm^2 (BG), respectively. In the groups that did not receive this material, the new bone area was 8.19±1.46 mm^2 (control group) and 8.81±1.47 mm^2 (PRP). Based on the significant differences observed in the area of newly formed bone when groups with and without BG were compared the present study corroborates the hypothesis that this material could act as an osteoconductive graft. In addition, it was observed that some remaining BG granules showed dissolution of their core with newly formed bone, as described in the literature[28].

Aiming to increase the periodontal and bone regeneration, some studies evaluated the additional effect of PRP, combined with different types of grafting materials, on the treatment of intrabony defects. In spite of some clinical results showing a greater reduction of pocket depth and gain in clinical attachment level, other clinical studies failed to demonstrate an additional effect. In 3-wall peri-implant defects in dogs treated with PRP associated to xenogenic bone graft, PRP demonstrated a low bone regenerative potential[25]. The same authors also reported no significant effect with the addition of PRP to xenogenic bone grafts on bone mineral density or graft maturity[24]. These observations are in agreement with the results of the present study, which could not demonstrate an additional effect of PRP in periodontal and bone regeneration of 3 wall intrabony defects. The histomorphometric findings demonstrated that the sites treated with PRP+BG showed a similar amount of new bone (13.80±2.32 mm^2) when compared with the sites that only received BG (15.63±2.64 mm^2).

The ideal platelet and growth factor concentration to promote periodontal regeneration has not yet been established. To the date, there is insufficient information about growth factor interactions and how they influence the activations of gene expression and protein production. In the present study, the achieved percentage mean of platelet concentration was 227.02% (mean of 463.015 platelets/mL) in relation to blood platelets count. Lacoste, et al.[15] (2003) reported that growth factors may act at specific times and at appropriate concentrations and this may be other explanation for the different results obtained in studies evaluating the use of PRP. The great variability in the outcomes of the studies with PRP may also be explained by the diversity of methods to obtain it. The technique to produce PRP in the present study was used in other animal studies by our research group[2,26]. This technique enables the production of a platelet concentration generally four times greater than the whole blood. According to Landsberg, et al.[16] (1998), thrombin promotes the release of antibodies to coagulation factors V and VI, which could be a potentially life-threatening event. These antibodies cross-react with human factor V thereby, causing a factor V deficiency that can be sufficiently severe to induce excessive bleeding and even death. Considering that, the use of thrombin in this type of study is not allowed in our country. In the present study, instead of thrombin, 10% calcium chloride was added to PRP and heated in a water bath at 37°C. Lacoste, et al.[15] (2003) 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. Those authors observed that calcium chloride, regardless of the use of thrombin, released platelet growth factors. In this study[15], the concentration of growth factors that was released was sufficient

to promote endothelial cell proliferation *in vitro*.

Recently, the tissue engineering science has provided a new possibility of cell therapy as a future alternative for the use of conventional regenerative techniques[1]. This strategy that exploits the regenerative capacity of stem cells grown in a three-dimensional scaffold and subsequently implanted into the defect, in the presence of molecule modulators, may help overcoming several limitations of current regeneration modalities[1]. In an *in vitro* study, the application of growth factors found in PRP (TGF-β1 and IGF-I) in cultures of periodontal ligament cells, increased their mitotic activity and their capacity for adhesion on the root fragments surface[20,23]. The use of platelet growth factors associated with cell therapy could be an alternative favoring the process of periodontal regeneration.

CONCLUSIONS

Within the limits of this animal model study, it may be concluded that the association of PRP with BG did not exert an additional effect to periodontal regeneration of 3-wall intrabony defects in dogs. Further studies are necessary to investigate the advantages and limitations of these materials in the treatment of periodontal defects.

ACKNOWLEDGEMENTS

The authors thank the financial support of FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, #04/12428-8) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, #300817/2007-0).

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