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Effect of platelet-rich plasma on bone healing of autogenous bone grafts in critical-size defects

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Abstract

Aim: This study histologically analysed the effect of autogenous platelet-rich plasma (PRP), prepared according to a new semiautomatic system, on healing of autogenous bone (AB) grafts placed in surgically created critical-size defects (CSD) in rabbit calvaria.

Material and Methods: Sixty rabbits were divided into three groups: C, AB and AB/PRP. A CSD was created in the calvarium of each animal. In Group C (control), the defect was filled by blood clot only. In Group AB (autogenous bone graft), the defect was filled with particulate autogenous bone. In Group AB/PRP (autogenous bone graft with platelet-rich plasma), it was filled with particulate autogenous bone combined with PRP. All groups were divided into subgroups ($n = 10$) and euthanized at 4 or 12 weeks post-operatively. Histometric and histologic analyses were performed. Data were statistically analysed (ANOVA, *t*-test, $p < 0.05$).

Results: Group C presented significantly less bone formation compared with Group AB and AB/PRP in both periods of analysis ($p < 0.001$). At 4 weeks, Group AB/PRP showed a statistically greater amount of bone formation than Group AB ($64.44 \pm 15.0\%$ *versus* $46.88 \pm 14.15\%$; $p = 0.0181$). At 12 weeks, no statistically significant differences were observed between Groups AB and AB/PRP ($75.0 \pm 8.11\%$ *versus* $77.90 \pm 8.13\%$; $p > 0.05$). It is notable that the amount of new bone formation in Group AB/PRP at 4 weeks was similar to that of Group AB at 12 weeks ($p > 0.05$).

Conclusion: Within its limitation, the present study has indicated that (i) AB and AB/PRP significantly improved bone formation and (ii) a beneficial effect of PRP was limited to an initial healing period of 4 weeks.

Key words: bone grafting; bone regeneration; growth factors; in vivo model; platelets

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Researchers continuously strive to improve bone-grafting techniques and to provide the means to obtain a faster and denser bony regeneration. Marx et al. (1998) have proposed the use of platelet-rich plasma (PRP) as a viable technique to obtain a high concentration of growth factors (GFs). These authors observed that autogenous bone grafts matured faster when combined with PRP to treat mandibular defects resulting from the removal of benign and malignant tumours.

PRP is a blood derivative, generated by differential centrifugation, in which platelets are concentrated in a small plasma volume (Roussy et al. 2007). The use of PRP is based on the premise that platelets constitute a reservoir of critical GFs, such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), insulin-like growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF), which, once released, may positively regulate the wound-healing process (Marx 2001, 2004, Roussy et al. 2007).

According to Whitman et al. (1997) and Marx et al. (1998), PRP is prepared by the discontinuous cell separation method from a blood volume of approximately 450 ml. This protocol for PRP

Conflict of interest and sources of funding statement

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preparation involves sophisticated technology and a large blood volume, limiting its use to blood transfusion medical centres or hospital facilities (Tözüm & Demiralp 2003). Therefore, equipment capable of producing small amounts of PRP has become available commercially. Several simplified protocols using either common laboratorial centrifuges (Anitua 1999, Landesberg et al. 2000, Sonnleitner et al. 2000, Efeoglu et al. 2004) or semiautomatic systems (Weibrich et al. 2002, 2003, Eppley et al. 2004, Marlovits et al. 2004, Tamimi et al. 2007) for the preparation of PRP have been developed to facilitate its clinical application. The Platelet Concentrate Collection System (PCCS, BIOMET 3i Inc), a semiautomatic system, has been evaluated in clinical (Wiltfang et al. 2003, Raghoebar et al. 2005), animal (Wiltfang et al. 2004, Thorwarth et al. 2005, Roussy et al. 2007) and in vitro (Marx 2004, Weibrich et al. 2005, Leitner et al. 2006) studies. The in vitro studies have shown that the PCCS can create a high concentration of platelets, works with a small blood volume and does not damage the platelets. PCCS II™, an improved version of PCCS, presents the advantage of providing an easier protocol to be used than its first version with regard to the number of centrifugations and less steps in the PRP preparation. In addition, it provides a better platelet recovery (%) and a constant volume of PRP produced (10% of the total volume of blood drawn). The platelet number and GFs released of PRP prepared according to PCCS II™ have been evaluated in an in vitro study conducted by Eppley et al. (2004). They concluded that a variety of potentially therapeutic GFs were detected and released from the platelets in significant levels in PRP preparations.

The main rationale for adding PRP to bone grafts is that high concentrations of platelets in a bony wound will increase the local concentration of secreted GFs and subsequently enhance the initial bone healing response (Plachokova et al. 2008). Later on, the direct influence of PRP will fade away and physiological mechanisms of bone repair will continue to work on an accelerated level (Jakse et al. 2003).

Clinical (Shanaman et al. 2001, Lekovic et al. 2003, Oyama et al. 2004, Simon et al. 2006, Trombelli & Farina 2008) and animal (Aghaloo et al. 2002, Choi et al. 2004, Fennis et al. 2004, Schlegel et al. 2004, Wiltfang et al. 2004, Thorwarth et al. 2005, Gerard et al. 2006, Klongnoi et al. 2006, Thorwarth et al. 2006) studies have evaluated the effects of combining PRP with bone grafts. However, these studies have yielded contradictory results regarding bone formation and maturation. Because there are only a few controlled studies, with many variations (type of graft, anatomic site, PRP preparation protocol), there is still significant disagreement as to whether or not PRP enhances the healing of bone grafts.

Recent literature reviews have concluded that there are insufficient data to recommend the clinical use of PRP (Wallace & Froum 2003, Plachokova et al. 2008), while others have indicated that well-designed controlled studies are needed to provide evidence of PRP efficacy in bone-regenerative procedures (Sánchez et al. 2003, Anitua et al. 2006, Hallman & Thor 2008). Whether the extra cost and time spent on the PRP procedure is justified remains a topic for further study (Hallman & Thor 2008).

The purpose of this study was to histologically analyse the effect of autogenous PRP, prepared according to a new semiautomatic system, on the healing of autogenous bone grafts placed in surgically created critical-size defects (CSD) in rabbit calvaria.

Material and Methods

Experimental model

The experimental protocol was approved by the São Paulo State University – UNESP, Dental School of Araçatuba Institutional Animal Care and Use Committee. Sixty 4 ± 0.3-month-old male white New Zealand rabbits weighing 3.5 ± 0.4 kg (UNESP, Dental School of Araçatuba, Animal Care Unit) were used in this study. The animals were randomly assigned to one of three experimental groups: Group C (control), Group AB (autogenous bone graft) and Group AB/PRP (autogenous bone graft with platelet-rich plasma).

Surgical procedure

Twenty-four hours before the surgical procedure, the animals received an intramuscular (i.m.) injection of enrofloxacin (0.1 ml/kg of body weight – Baytril®, Bayer S.A., São Paulo, SP, Brazil). They were anaesthetized by an i.m. injection of xylazine (0.25 ml/kg body weight) and ketamine (0.35 ml/kg body weight). After an aseptic preparation, a ''U-shaped'' incision was made in the skin over the top of the cranial vault and a cutaneous flap was raised and reflected in a posterior direction to expose the periosteum. The parietal and the frontal bones were exposed via a similar incision in the periosteum, followed by a gentle subperiosteal dissection (Vikjaer et al. 1997). A 15-mm-diameter CSD was made with a trephine used in a low-speed hand piece under continuous sterile saline irrigation. The defect included a portion of the sagittal suture (Hollinger & Kleinschmidt 1990).

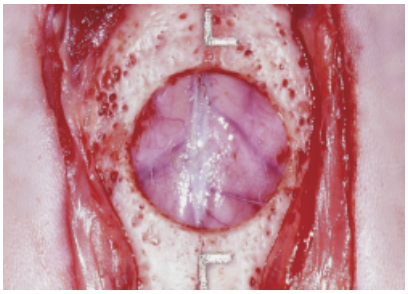

Fig. 1. Critical-size defect (15 mm diameter) and the two reference marks created on the calvarium.

One L-shaped mark was made 2 mm anterior and one 2 mm posterior to the margins of the surgical defect using a small tapered carbide fissure bur and a surgical stent. The long axes of the L-shaped marks were located on the longitudinal axis bisecting the surgical defect. The marks were filled with amalgam (Fig. 1). Their purpose was to allow identification of the centre line of the original defect during laboratory processing and also to be used as references to locate the original bone margins of the surgical defect during histometric analysis (Messora et al. 2008a).

In Group C, the surgical defect was naturally filled with blood only. In Group AB, the surgical defect was filled with approximately 0.30 g of particulate autogenous bone. In Group AB/PRP, the surgical defect was filled with approximately 0.30 g of particulate autogenous bone combined with 0.5 ml of PRP. The amount of bone graft was enough to fill the defects up to the level of their original bone margins. The autogenous bone was obtained from the calvarium during the surgical defect creation and ground with a bone mill (Quentin Bone Mill, Quentin Dental Products, Leimen, BW, Germany).

The periosteum was repositioned and sutured with absorbable sutures (5-0 Vicryl, Ethicon, São Paulo, SP, Brazil). The skin was sutured with non-absorbable sutures (4-0 Silk, Ethicon). Post-surgi-

cally, each animal received daily i.m. injections of enrofloxacin (0.1 ml/kg of body weight – Baytril®, Bayer S.A.,) for 5 days and tramadol chloride (0.04 ml/kg of body weight – Tramal®, Pfizer Ltda., São Paulo, SP, Brazil) for 3 days.

PRP preparation

Before the surgical creation of the CSD, 40 ml of blood was drawn from each animal, via a cardiac puncture, into a syringe containing 5 ml of anticoagulant citrate dextrose solution (ACD-A). The same volume of saline (40 ml) was then injected intravenously (i.v.) to maintain the systemic blood volume of the animal. PRP was prepared according to the PCCS II™ (BIOMET *3i*, Inc., Palm Beach Gardens, FL, USA) protocol. The blood sample was centrifuged at 3200 r.p.m. for 12 min. PRP was prepared and stored in a non-activated form at room temperature until its clinical use. The time elapsed between PRP preparation and its placement in the surgical defect was approximately 45 min. A 10% solution of calcium chloride (calcium chloride 10% solution, ScienceLab.com Inc., Houston, TX, USA) was used to activate PRP samples (0.025 ml of calcium chloride for each 0.5 ml of PRP). The PRP samples were then immediately combined with approximately 0.30 g of particulate autogenous bone and placed in the surgical defect.

Platelet counts

Brecher liquid was used to lyse the erythrocytes and dilute the whole blood and PRP samples (1:100 dilution). The platelets in the diluted whole blood and PRP samples were then counted manually in a Neubauer chamber. In addition, non-activated PRP and whole blood smears were stained with ''Panótico Rápido LB'' (LaborClin, Pinhais, PR, Brazil) in order to observe the morphology of the platelets. The platelet counts and the analysis of the platelet morphology were performed by a veterinary haematologist.

Tissue processing

Each group of animals was divided into two subgroups for euthanasia at either 4 or 12 weeks post-operatively. The area of the original surgical defect and the surrounding tissues were removed en bloc. The blocks were fixed in 10% neutral formalin, rinsed with water and then decalcified in 18% ethylenediaminotetraacetic acid solution. After initial decalcification, each specimen was divided longitudinally into two blocks exactly along the centre line of the original surgical defect using the long axis of both L marks as references. Transverse cuts were then made using the short axis of each L mark as a reference. Each specimen then measured 19 mm in length along the longitudinal axis running through the centre of the defect, allowing for identification of the original surgical defect margins during both histologic and histometric evaluations (Fig. 2). After complete decalcification, they were processed and embedded in paraffin. Serial sections 6 μm thick were cut in a longitudinal direction starting at the centre of the original surgical defect. The sections were stained with either haematoxylin and eosin (H&E) or Masson's Trichrome for analysis by light microscopy.

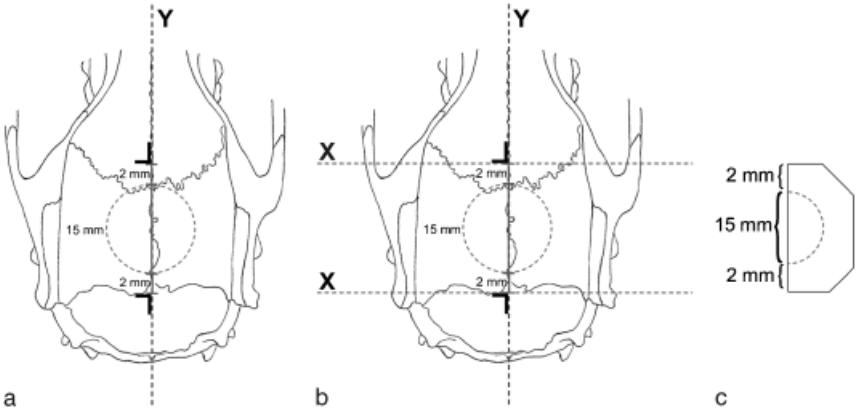

Fig. 2. (a) Longitudinal cut along the centre line (Y) of a critical-size defect; (b) transverse cuts (X); (c) dimensions of specimen to be embedded in paraffin.

Histomorphometric analysis

Three histologic sections, representing the centre of the original surgical defect, were selected for the histologic and histometric analyses. Two histologic sections were stained with Masson's Trichrome for the histometric analysis and one histologic section was stained with H&E for the qualitative histologic analysis, which was also complemented by the sections stained with Masson's Trichrome. The histologic and histometric analyses were performed by an examiner blinded with respect to the treatment rendered. Images of the histologic sections were captured by a digital camera (Olympus DP 10, Olympus Optical, Tokyo, Japan) connected to a light microscope with an original magnification of × 32. The digital images were saved on a computer. A composite digital image was then created by combining four smaller images because it was not possible to capture the entire defect in one image at the level of magnification that was used. The composite image was created based on anatomic reference structures (e.g. blood vessels and bone trabeculae) within each of the histologic sections. The ''ImageLab 2000'' software (Diracon Bio Informática Ltda., Vargem Grande do Sul, SP, Brazil) was used for the histomorphometric analysis.

The following criteria, based on the work of Messora et al. (2008a), were used to standardize the histomorphometric analysis of the digital images:

(1) The total area (TA) to be analysed corresponded to the entire area of the original surgical defect. This area was determined by first identifying the external and internal surfaces of the original calvarium at the right and left margins of the surgical defect, and then connecting them with lines drawn following their respective curvatures. Considering the total length of the histologic specimen, 2 mm were measured from the right and left edges of the specimen towards the centre in order to determine the margins of the original surgical defect. The bone area (BA) was delineated within the confines of the TA.
(2) TA was measured in millimetre square and was considered 100% of the area to be analysed. BA was also measured in millimetre square and calculated as a percentage of TA.

The type of newly formed bone, the characteristics of the connective tissue, the presence of osteoid matrix and remnants of bone graft, as well as the type of inflammatory infiltrate were evaluated in the qualitative histologic analysis. In addition, the healing of the sagittal suture was also evaluated according to the studies by Mardas et al. (2002) and Kostopoulos & Karring (2000). For this evaluation, the presence or absence of the sagittal sinus, as well as of a suture-like connective tissue displayed in a continuous form or interrupted by areas of bony union (synostosis) were considered.

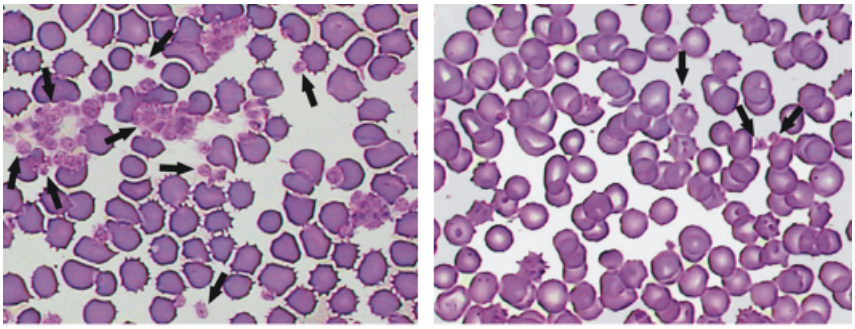

Fig. 3. Platelet-rich plasma (a) and whole blood (b) smears showing platelets (arrows) with a normal morphology. Panótico Rápido LB staining. Original magnification × 1000.

Statistical analysis

The values of BA for each animal were represented by the mean percentage of the two histologic sections. These percentage data were transformed into square root for the statistical analysis. The significance of differences between groups in relation to BA was determined by an analysis of variance, followed by a post hoc Student's *t*-test when the analysis of variance suggested a significant difference between groups ($p < 0.05$).

Pearson's correlation coefficient (r_p) was used to demonstrate the relationship between the BA and the platelet counts from the PRP samples, as well as between the platelet counts from the PRP and whole blood samples.

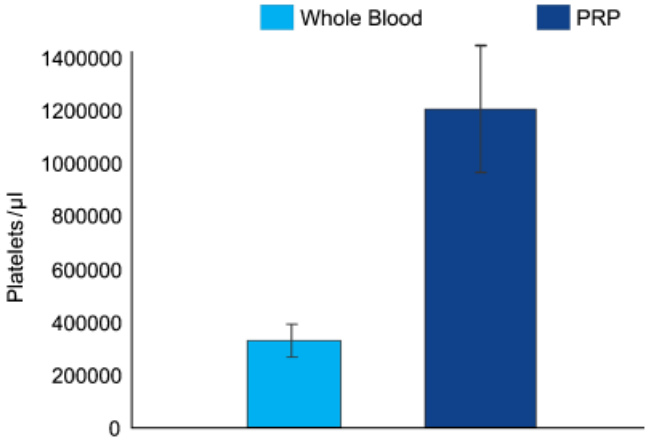

Fig. 4. Mean number of platelets per microlitre (µl) and standard deviations in the samples of platelet-rich plasma (PRP) and whole blood.

Results

All animals tolerated the surgical procedures well and were healthy during the entire experimental period.

One specimen of Group AB/PRP (euthanized at 4 weeks post-operatively) was lost because of problems encountered during lab processing.

Platelet count study

The PRP smears showed higher concentrations of platelets than the whole blood smears. Platelets exhibited a normal morphology in the whole blood and PRP smears (Fig. 3). Platelet counts confirmed that the PRP preparation technique used in this study produced samples of highly concentrated platelets. The average whole blood platelet count was $327.83 \pm 73.61 \times 10^3/\mu l$, while the average PRP platelet count was $1204.03 \pm 369.88 \times 10^3/\mu l$. Thus, the concentration of the platelets in PRP was increased by almost fourfold (Fig. 4).

Qualitative histologic analysis

A bony union was always observed between the newly formed bone in the defect and original bone at the margins of the defect in all histologic specimens. A suture-like connective tissue and the sagittal sinus were not encountered in the defect area of any specimen.

Group C (control)

At 4 weeks, almost all of the surgical defect was occupied by connective tissue with collagen fibres parallel to the wound surface and a moderate number of fibroblasts. Newly formed bone surrounded by a small number of osteoblasts was restricted to areas close to the borders of the surgical defect (Fig. 5A). Areas of osteoid matrix were observed in most specimens. At 12 weeks, most specimens presented similar bone formation when compared with the 4-week specimens (Fig. 5B) while only two specimens showed increased bone formation. The connective tissue presented a moderate number of fibroblasts and many collagen fibres.

Group AB (autogenous bone graft)

At 4 weeks, no surgical defect completely regenerated with bone (Fig. 5C). However, an almost complete bone regeneration of the surgical defect was seen in six specimens. The surgical defect was occupied by fibrous connective tissue and isolated newly formed woven bone (Fig. 6A). Remnants of bone graft (Fig. 7A) were observed throughout the defect. Areas of provisional connective tissue (Fig. 6B) and osteoid matrix were also seen. A small number of lymphocytes and macrophages were observed in a few specimens. At 12 weeks, all surgical defects completely regenerated with lamellar bone trabeculae (Figs 5D and 7B). Remnants of bone graft were still observed in a few areas. Areas of osteoid matrix were also seen. In most

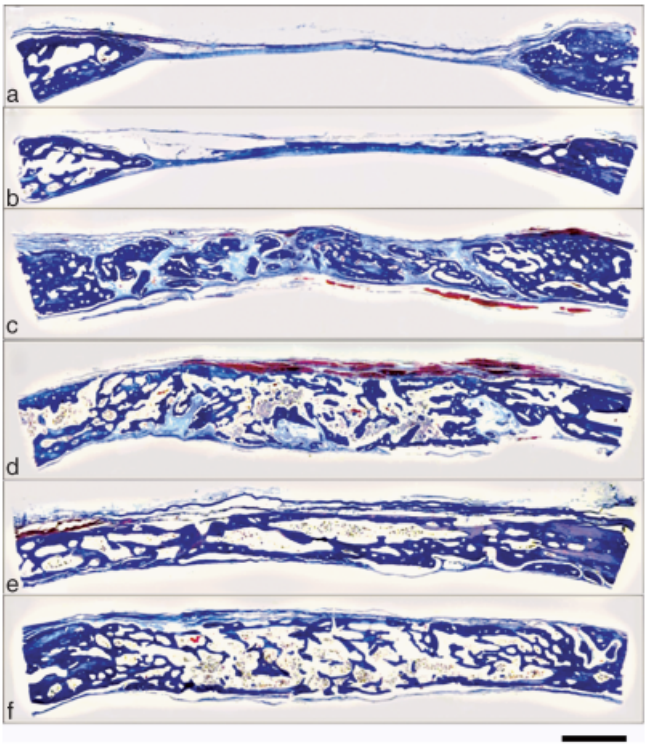

Fig. 5. Panoramic views of the surgical defects. (a) Group C (control)– 4 weeks; (b) Group C – 12 weeks; (c) Group AB (autogenous bone graft)– 4 weeks; (d) Group AB – 12 weeks; (e) Group AB/PRP (autogenous bone graft with platelet-rich plasma) – 4 weeks; and (f) Group AB/PRP – 12 weeks. Masson's Trichrome. Original magnification × 25. Scale bar = 2 mm.

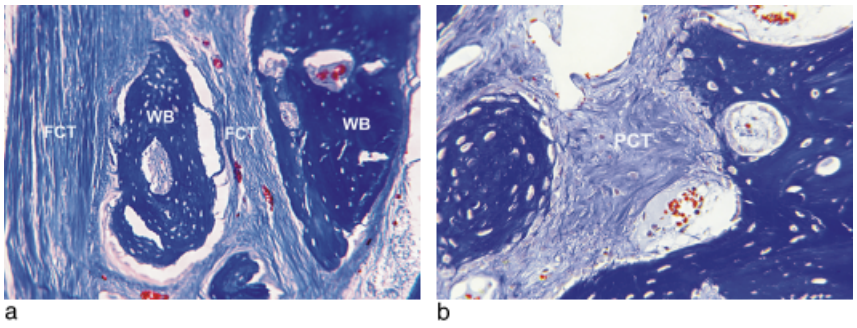

Fig. 6. Group AB (autogenous bone graft) – 4 weeks. (a) Fibrous connective tissue (FCT) and newly formed woven bone (WB). (b) Area of provisional connective tissue (PCT). Masson's Trichrome. Original magnification × 160 (a) and × 400 (b).

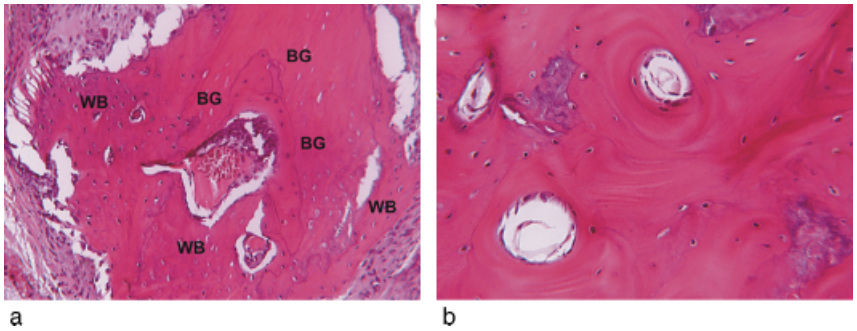

Fig. 7. (a) Newly formed woven bone (WB) and remnants of bone graft (BG) in Group AB (autogenous bone graft) – 4 weeks. (b) Lamellar bone in Group AB – 12 weeks. Haematoxylin and eosin. Original magnification × 200 (a) and × 400 (b).

specimens, at both 4 and 12 weeks, the newly formed tissues in the defect region were thinner than the original calvarium.

Group AB/PRP (autogenous bone graft with PRP)

At 4 weeks, two specimens completely regenerated with bone. An almost complete regeneration of the surgical defect with woven bone (Figs 5E and 8) was seen in the remaining eight specimens. Remnants of bone graft (Fig. 8) and osteoid matrix areas were observed throughout the defect. In most specimens, the newly formed tissues in the defect region were thinner than the original calvarium. At 12 weeks, all surgical defects completely regenerated with bone. In three specimens, an almost complete regeneration of both cortical plates with intervening cancellous bone was seen (Fig. 5F). In others, the defect was occupied with lamellar bone trabeculae. A few remnants of bone graft were still observed in some areas. Areas of osteoid matrix were also seen. In most specimens, the newly formed bone presented a thickness similar to that of the original calvarium.

Histometric and statistical analyses

The data normality and homogeneity of variances were verified. Means and standard deviations of BA for Groups C, AB, AB/PRP, as well as the comparison between the groups, at 4 and 12 weeks post-operatively are documented in Table 1. Group C presented significantly less bone formation compared with Groups AB and AB/PRP during both periods of analysis ($p < 0.001$). At 4 weeks, Group AB/PRP showed a statistically greater amount of bone formation than Group AB ($64.44 \pm 15.0\%$ *versus* $46.88 \pm 14.15\%$; $p = 0.0181$). At 12 weeks, no statistically significant differences were observed between Groups AB and AB/PRP ($75.0 \pm 8.11\%$ *versus* $77.90 \pm 8.13\%$; $p > 0.05$). It is notable that the amount of new bone formation in Group AB/PRP at 4 weeks was similar to that of Group AB at 12 weeks ($p > 0.05$).

A statistically significant correlation was observed between the platelet count from the whole blood and PRP samples ($r_p = 0.66$, $p = 0.0021$). However, no statistically significant correlation was observed between the platelet

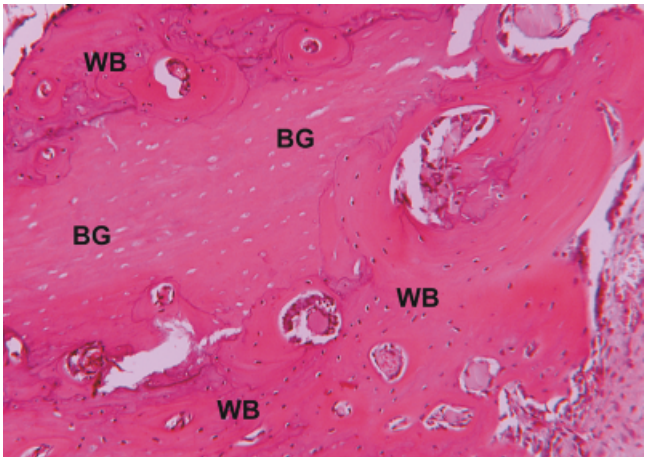

Fig. 8. Newly formed woven bone (WB) and remnants of bone graft (BG) in Group AB/PRP (autogenous bone graft with platelet-rich plasma) – 4 weeks. Haematoxylin and eosin. Original magnification × 160.

Table 1. Mean (± SD) of bone area (BA) within the surgically created defect with comparison between the groups (4 and 12 weeks post-operatively)

Group	*n* 4 weeks	BA (%) 4 weeks	SD 4 weeks	*n* 12 weeks	BA (%) 12 weeks	SD 12 weeks
C	10	8.99[§]	± 2.62	10	5.31	± 1.69
AB	10	46.88[*,¶,**]	± 14.15	10	75.00[‡]	± 8.11
AB/PRP	9	64.44[*,†,‖,††]	± 15.00	10	77.90[‡]	± 8.13

Between-group comparisons at weeks 4 and 12:
[*]Compared with Group C ($p<0.001$) at 4 weeks;
[†]Compared with Group AB ($p=0.0181$) at 4 weeks;
[‡]Compared with Group C ($p<0.001$) at 12 weeks;
[§]Compared with Groups AB and AB/PRP at 12 weeks ($p<0.001$);
[¶]Compared with Groups C and AB/PRP at 12 weeks ($p<0.001$);
[‖]Compared with Groups C ($p<0.001$) at 12 weeks.
Within-group comparisons:
[**]Compared with Group AB ($p<0.001$) at 12 weeks;
[††]Compared with Group AB/PRP ($p<0.05$) at 12 weeks.
AB, autogenous bone; SD, standard deviation; PRP, platelet-rich plasma; Group C, control; Group AB, autogenous bone graft; Group AB/PRP, autogenous bone graft with platelet-rich plasma.

count from the PRP samples and the BA ($r_p = -0.245$, $p = 0.3116$).

Discussion

In a recent systematic review, Plachokova et al. (2008) analysed the reported effects of PRP on bone regeneration. The authors concluded that there is currently a lack of scientific evidence in the dental literature either for or against the clinical use of PRP. A substantial heterogeneity among the studies was observed in the review. One of the reasons cited by the authors is that different protocols were used for PRP production.

Qualitative and/or quantitative alterations of the platelets may affect the regenerative potential of PRP. Therefore, selection of an appropriate PRP preparation protocol is fundamental to evaluate the actual biological effects of PRP. In the present study, the PRP was prepared according to the PCCS II™ protocol. The first version of this system (PCCS) has been recommended by Marx (2004) because it can create a ''therapeutic'' concentration of platelets (average percentage increase of approximately 400% in the platelet count). The PCCS II™ used in the present study consistently resulted in such an increase in the number of platelets in the PRP samples when compared with whole blood. The consistency of the increase in platelet concentration achieved by this system was demonstrated by the statistically significant correlation observed between the whole blood and the PRP platelet counts. In addition, the analysis of platelet morphology in the PRP smears demonstrated that the PCCS II™ also seemed to preserve the integrity of the platelet cell membranes during PRP preparation. According to Marx (2004), platelets damaged or rendered non-viable by PRP processing will not secrete bioactive GFs. Thus, their use may result in disappointing clinical outcomes. To the best of our knowledge, the present study is the first to evaluate the in vivo effect of PRP prepared according to the PCCS II™ protocol.

Besides the centrifugation protocol, it is important to note the method used to activate the PRP samples. In the present study, calcium chloride solution alone was used to activate the PRP samples. PRP activated by calcium chloride solution alone has been shown to have a positive biologic effect in clinical (Anitua 1999), animal (Messora et al. 2008a, b) and in vitro (Ferreira et al. 2005) studies. It is important to emphasize that PRP activation by calcium chloride alone avoids the use of bovine thrombin and thus the risk of developing potentially life-threatening coagulopathies (Cmolik et al. 1993, Spero 1993, Landesberg et al. 2000). Another alternative to avoid the use of bovine thrombin to activate PRP is autogenous thrombin. However, independent of the technique used for its preparation, the final thrombin activity is difficult to predict (De Somer et al. 2006). In addition, the traditional technique for preparing autogenous thrombin uses a portion of the PRP in this process, thus reducing the amount of PRP available for clinical use (De Somer et al. 2006).

In the present study, Group AB/PRP showed a significant increase in bone formation compared with Group AB at 4 weeks post-operatively (Table 1). This result corroborates the findings of previous studies that have shown a significant increase in bone formation only in the initial stages of bone healing of CSD created in the skull of domestic pigs (Schlegel et al. 2004, Wiltfang et al. 2004, Thorwarth et al. 2005, 2006) and the mandible of dogs (Gerard et al. 2006). Some studies have demonstrated that PRP may increase the proliferation of the endothelial cells (Fréchette et al. 2005), pre-osteoblasts (Gruber et al. 2006), osteoblasts (Choi et al. 2005, Ogino et al. 2006) and osteoclasts (Gruber et al. 2002) in the surgical site. It could be speculated that these PRP effects might have contributed to the

significant increase in bone formation observed in Group AB/PRP at 4 weeks post-operatively.

At the later period of observation (12 weeks post-operatively), Groups AB and AB/PRP showed similar amounts of newly formed bone (Table 1) with complete bone regeneration of the surgical defects in all specimens, corroborating the findings of an animal study conducted by Gerard et al. (2006). However, it is important to note that Group AB/PRP, at 4 weeks post-operatively, showed similar bone formation when compared with Group AB at 12 weeks (Table 1). This means that the use of PRP significantly accelerated the healing of the autogenous bone graft, corroborating the findings of Marx et al. (1998). The accelerated healing of the autogenous bone grafts in Group AB/PRP was also observed in the qualitative histologic analysis of the specimens at 12 weeks post-operatively. An almost complete regeneration of both cortical plates with intervening cancellous bone was seen in three specimens. Contributions of muscle and brain have been shown to impair bone healing in surgically created CSD in rat calvaria (Bosch et al. 1995, Verna et al. 2002). Thus, it has been suggested that the use of occlusive expanded polytetrafluoroethylene membranes to isolate endo and exocranial tissues may increase bone formation and maturation in the calvarial skeleton (Verna et al. 2002). It is possible that increased bone regeneration of the surgical defects may have been obtained if guided bone regeneration (GBR) therapy had been used. Nevertheless, it is important to point out that in the histomorphometric analysis of the present study, no herniation of brain tissue in the defect area was observed in any of the specimens.

Grageda (2004) suggested a standardized protocol for future studies evaluating the biological effects of PRP. As part of this protocol, Grageda (2004) suggested assessing the correlation between the histormorphometric analysis and the number of platelets in the PRP. In the present study, no significant correlation could be found between the platelet count in the PRP samples and the BA. Because the regenerative potency of PRP undoubtedly depends on its GFs levels, it could be inferred that this result supports the findings of Weibrich et al. (2002), who demonstrated that neither whole blood nor PRP platelet counts are reliably predictive of the resultant GFs levels in PRP. These findings are also corroborated by recent studies that have observed differences in GFs levels in PRP samples with the same concentration of platelets (Martineau et al. 2004, Fréchette et al. 2005).

One of the drawbacks of the present study is the lack of a group utilizing PRP prepared according to the original PCCS protocol. Because PCCS II™ is purportedly an improved version of PCCS, the inclusion of such a group may have helped to better demonstrate the benefits of the new PRP preparation protocol evaluated in this study. Another limitation of this study is that the levels of GFs in the PRP samples were not measured. Therefore, it was not possible to establish a correlation between the levels of GFs and BA. So far, very few studies have attempted to correlate the effects of GFs released by PRP with bone regeneration (Roussy et al. 2007). The literature lacks information regarding the biological significance of different proportions of GFs within the PRP preparations in humans and large animals (Grageda 2004). In a recent study, Roussy et al. (2007) determined the effects of activated human PRP, prepared according to PCCS, on early and mature bone formation in rat calvaria. PDGF-BB, VEGF, TGF-β and interleukin-1β (IL-1β) levels were measured by ELISA following PRP activation. Positive correlations were observed between VEGF levels and bone formation. A negative correlation was also found between PDGF and BB concentration and bone formation. These new data reinforce the importance to better understand the molecular composition of PRPs and the physiological effects of its constituents on bone formation (Roussy et al. 2007).

It is important to point out that the Consensus Report of the Sixth European Workshop on Periodontology concluded that the use of GFs for localized alveolar ridge augmentation remains overall at an early stage of development as compared with other bone-regenerative technologies used clinically (GBR, bone grafting, biomaterials, etc.). The information available for the GFs has demonstrated encouraging early evidence for regeneration. Most of these results are confined to lower level animal models. The refinement of relevant intra-oral animal models is needed to better study GF-mediated alveolar ridge repair. Clinical and animal studies should address the questions regarding the clinically effective doses required, the adequate carrier materials needed, and the optimal release kinetics for the clinical applications of GFs (Jung et al. 2008, Tonetti & Hämmerle 2008).

Within the limits of this study, it can be concluded that PRP improved the early healing of AB grafts in CSD in rabbit calvaria. However, this effect was no longer significant after 12 weeks. The early improved healing occurred by increasing the amount of new bone that was formed.

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Clinical Relevance

Scientific rationale for the study: The clinical benefits of combining PRP with bone grafts remain controversial. A major reason for this is probably due to the use of different PRP preparation protocols. In this study, PRP was prepared according to the PCCSII™ protocol. It provides a better platelet recovery (%) and a constant volume of PRP produced. In addition, it is easy to use and requires only one centrifugation.

Principal findings: PRP, prepared according to the PCCS II™, improved the early healing of the autogenous bone graft. However, this effect was no longer significant after 12 weeks.

Practical implications: Autogenous bone grafts combined with PRP may result in faster healing than grafts without PRP.