Influence of Platelet-rich Plasma on Bone Regeneration: A Histomorphometric Study in Rabbit Calvaria

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Purpose: *The aim of this study was to evaluate whether local application of platelet-rich plasma (PRP) would induce bone regeneration in cranial defects on rabbits.* ***Materials and Methods:*** *Twelve female New Zealand rabbits were used for this study. Two identical 10-mm-diameter bicortical cranial defects were created in each animal. One of the defects was grafted with PRP, while the contralateral was left unfilled as a negative control. Animals were sacrificed at 2, 4, 6, and 8 weeks after surgery, and biopsy specimens were evaluated histologically and histomorphometrically under light microscopy. Analysis of variance was used for statistical analysis.* ***Results:*** *The histomorphometric evaluation showed more regenerated bone after local administration of PRP at 2 weeks (*$P > .05$*), 4 weeks (*$P < .05$*), and 6 weeks (*$P > .05$*). At week 8, new bone formation was comparable in both groups.* ***Conclusion:*** *In this animal model, local application of PRP in bone defects enhances healing significantly at 4 weeks.* Int J Oral Maxillofac Implants 2007;22:563–568

Key words: *bone regeneration, platelet-rich plasma, rabbits*

Bone availability is important to the stabilization of dental implants, which is why many studies have focused on improving bone quality and quantity. Autogenous bone grafts, bone substitutes, membranes, and osteoinductive growth factors have long been studied for bone regeneration purposes.[1–3] Growth factors, such as platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), bone morphogenetic proteins (BMPs), and growth hormone (GH) are of special interest for bone and periodontal regeneration because they stimulate cellular migration, differentiation, and proliferation.[4–15]

Fibrin glue is a blood derivative frequently used as a biomaterial for graft compaction in traumatology.[16] It contains some growth factors with osteoinductive properties, such as PDGF,[17,18] that stimulate osteoblast proliferation and differentiation.[19] In 1994, Tayapongsak et al[20] introduced the idea of applying fibrin glue in combination with an autogenous bone graft for reconstruction of mandible defects. The fibrin network enhanced the graft osteoconduction properties, obtaining faster bone regeneration.

Platelet-rich plasma (PRP) is a blood derivative with a higher amount of platelets than fibrin glue.[21,22] Platelets in PRP contain bone-related growth factors (PDGF, transforming growth factor [TGF]-β1 and TGF-β2), that can be released through platelet activation with bovine thrombin or calcium chloride and stimulate bone regeneration.[22,23] The application of PRP with autogenous bone grafts increases bone mineral density and accelerates bone regeneration and soft tissue healing.[21,22] PRP has been successfully used for mandibular reconstructions, sinus lifting, cleft palates, and dental implant procedures.[22,23] However, few studies have evaluated the effect of PRP alone in enhancing bone regeneration.[24–28] The aim of the present study was to determine whether local application of PRP alone could induce histologic differences in new bone formed on rabbit calvarial defects.

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MATERIALS AND METHODS

Prior to beginning the in vivo animal study, the protocol was approved by the Ethical Committee for Animal Experiments of the Complutense University of Madrid (UCM). Experiments were conducted in accordance with the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC), and adequate measurements were taken to minimize pain and discomfort in the animals.

Twelve healthy 6-month-old female New Zealand rabbits weighing 3.9 to 4.4 kg were used. The animals were accommodated in the official stable for animal assays of the UCM at 22 to 24°C with 55% to 70% humidity, light cycles of 12 hours, and air renewal 15 times per hour. The rabbits were fed with Panlab food (Barcelona, Spain), and water was provided ad libitum.

Preparation of PRP

All rabbits were anesthetized with an intramuscular dose of 0.75 mg/kg ketamine (Imalgene 1000, Rhone Merieux, France) and 0.25 mg/kg xilacine (Rompun; Bayer, Leverkusen, Germany). Immediately before surgery, 10 mL of whole blood was withdrawn from the ear via venous aspiration into 4.5-mL test tubes and mixed with a 3.8% sodium citrate solution at a ratio of 1 mL sodium citrate solution to 5 mL whole blood to achieve anticoagulation through calcium binding. The blood was then centrifuged with a Nahita centrifuge (Navarra, Spain) into 3 basic components: red blood cells (RBCs), PRP (sometimes referred to as "buffy coat"), and platelet-poor plasma (PPP). Because of differential densities, the RBC layer forms at the lowest level, the PRP layer in the middle, and the PPP layer at the top. A pipette (Gilson, Middleton, WI) was used to separate each layer. About 2.25 mL of PPP, 0.9 mL of PRP, and 2.25 mL of RBC were obtained. Platelet counting of the obtained PRP was measured with a flow cytometry device (ADVIA 120, Hematology System; Bayer, Leverkusen, Germany). Before its surgical application, PRP (0.2 mL) was activated with a 30% $CaCl_2$ solution.

Surgical Procedure

Animals were placed in sternal recumbency, the head was shaved, and the cutaneous surface was disinfected with povidone-iodine solution prior to the operation. The calvaria was exposed through a skin incision approximately 4 cm in length over the median line. A pair of tweezers was used to lift the skin before the periosteum was also incised in the same place. A periosteal elevator was used for separating the periosteum from the bone surface. Two similar circular bicortical defects 10 mm in diameter were made in the parietal bone using a trephine on a slow-speed electric handpiece with 0.9% physiologic saline irrigation. A defect was made on each side of the median sagittal suture. One defect was filled with PRP (experimental group), while the contralateral defect was left empty as a negative control (control group). Closure of the periosteum and subcutaneous tissues was done with resorbable Dexon 3-0 sutures (North Haven, CT), while the skin was relocated with 3-0 silk continuous sutures (Apositos Sanitarios Aragoneses, Huesca, Spain). Postoperative antibiotics were administered (Terramicina [Pfizer, Alcobendas, Spain] in water for 7 days. Rabbits were sacrificed using an overdose of sodium pentobarbital IV (Dolethal; Vetoquinol, Lure, France) at 2, 4, 6, and 8 weeks after surgery (3 rabbits at a time).

Postmortem, a surgical bur attached to a slow-speed electrical handpiece was used to harvest the bone blocks containing the defects from the animal's calvariae. Samples were then preserved fixed in formaldehyde 10% buffer solution at pH 7.0, decalcified with EDTA, and included in paraffin as reported elsewhere.[29] Serial and parallel coronal cuts 6 µm thick were made and then stained by hematoxylin-eosin for study under light microscope by an observer who was blinded to group assignment.

Histologic evaluation of bone neoformation was carried out by means of optical microscopy. For histomorphometric analysis, light micrographs of the biopsy slices at magnification of 6× were captured with a digital camera and analyzed with histomorphometry software MIP-4 (Digital Image System, Barcelona, Spain).[30] Six randomly selected slices were analyzed for each biopsy sample. In each section, the area inside the defect was included for histomorphometric evaluation, while the original cortical bone outside the defect was excluded. The already-existing bone was lamellar, while the regenerated bone was woven and grew inside the defects. Thus, the 2 types of bone could be easily differentiated in the histologic observations.

For each defect, the total sample volume and the volume of newly formed bone were obtained. From these data, the average regenerated bone volume formed in the defect was calculated:

$$BV = \frac{\text{Newly formed bone volume}}{\text{Total defect volume}} \%$$

Statistical Analysis

A statistical software package (Statgraphics 5.0; Statistical Graphics, Rockville, MD) was used for analysis of variance (ANOVA) of the histomorphometric and densitometry measurements. Significance was set at $P < .05$.

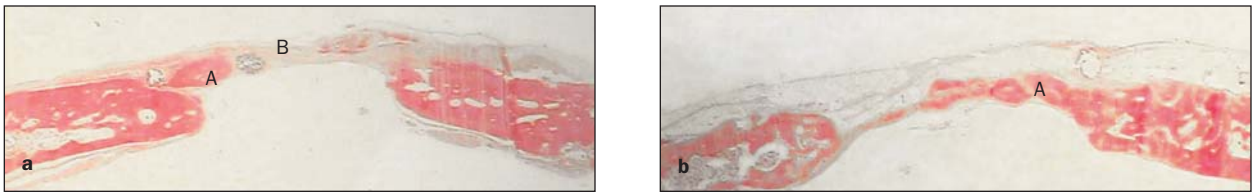

Fig 1 At week 4, connective tissue and slightly newly formed bone could be seen in the control defects *(a)*, while in the experimental defects *(b)*, a complete newly formed bone bridge could be seen linking both sides of the defect. A = bone; B = fibrous tissue.

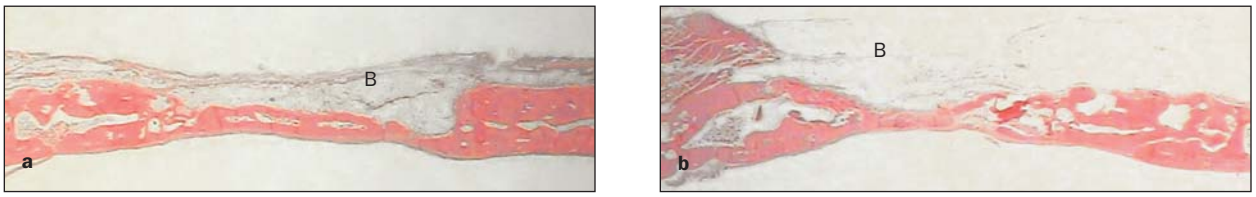

Fig 2 At week 8, the amount of newly formed bone was similar in both groups, with a complete osseous bridge between the healing sides. No differences in bone quality could be seen. B = fibrous tissue.

Table 1 Mean Percentage of Regeneration in Control and Experimental Groups at 2, 4, 6, and 8 Weeks After Surgery

	Control		Experimental	
	Mean	SD	Mean	SD
2 weeks	4	1.51	13	2.41
4 weeks	11	0.71	39	8.86*
6 weeks	37	0.57	50	4.78
8 weeks	50	2.80	49	7.15

*Statistically significant difference relative to control group.

RESULTS

PRP Counts

Platelet counts confirmed that the PRP preparation technique used in this study produced a highly concentrated source of platelets. The average peripheral blood platelet count was 153,000 platelets/mm^3, with a range of 72,000 to 255,000 platelets/mm^3. The average PPP count was 47,000 platelets/mm^3, with a range of 19,000 to 87,000 platelets/mm^3. The average PRP platelet count was 657,000 platelets/mm^3, with a range of 444,000 to 957,000 platelets/mm^3.

Surgery

No complications were registered intraoperatively. The animals recovered without problems, and no macroscopic indications of infection were observed postmortem. No animals were lost during the study.

Histology

The histologic samples at week 2 showed poor bone formation in both experimental and control defects. At week 4, new bone was slightly formed in the control defects, while in the PRP grafted defects, a complete bone bridge linking both sides of the defect could be seen (Fig 1). At week 8, the amount of newly formed bone was similar in both defects, with a complete bone bridge between the healing sides (Fig 2).

Histomorphometry

The results of the histomorphometry study are shown in Table 1. At week 2, augmented bone volume (BV) ranged from 4% to 13% in all defects (Fig 3) but was slightly higher for experimental defects, although the difference was not significant ($P = .14$). At week 4, BV mean values were 39% for the experimental defects and only 11% for the control defects (Fig 4), and differences between the 2 groups were significant ($P = .025$). At week 6, BV mean values were 50% for the experimental defects and 37% for the control defects (Fig 5), although the difference between groups was not significant ($P = .12$). At week 8, BV mean values were 49% to 50% in both groups (experimental and control; $P = .67$; Fig 6).

DISCUSSION

The use of growth factors is very promising for implant and oral surgery.[8,11,12] The application of a combination of platelet growth factors, such as PDGF and IGF-I, increases bone regeneration in periodontal or peri-implant defects.[5,6,7,9,10]

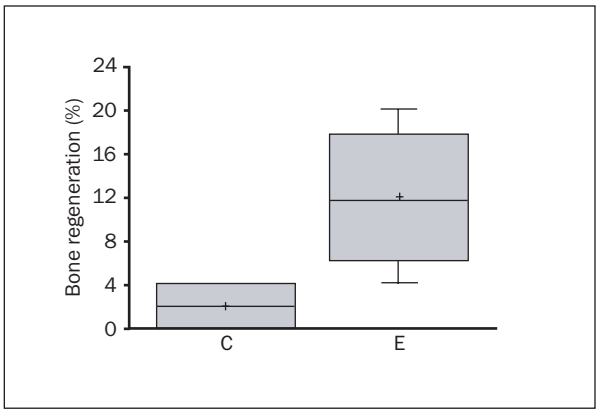

Fig 3 BV values at week 2. Bone regeneration was slightly higher in the experimental group (E) than in the control group (C) (13% vs 4%), although the difference was not significant ($P = .14$).

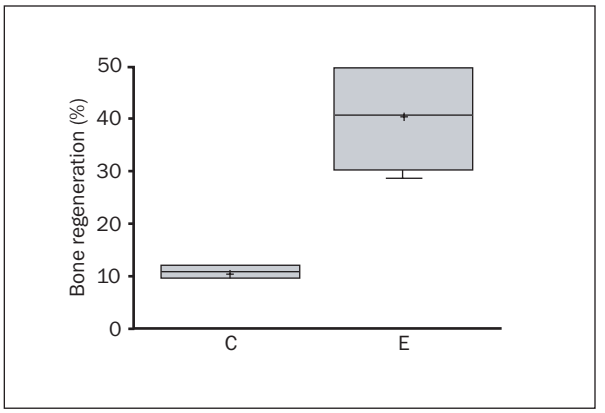

Fig 4 BV values at week 4. Bone regeneration was significantly higher in the experimental group (E) than in the control group (C) (39% vs 11%; $P = .025$).

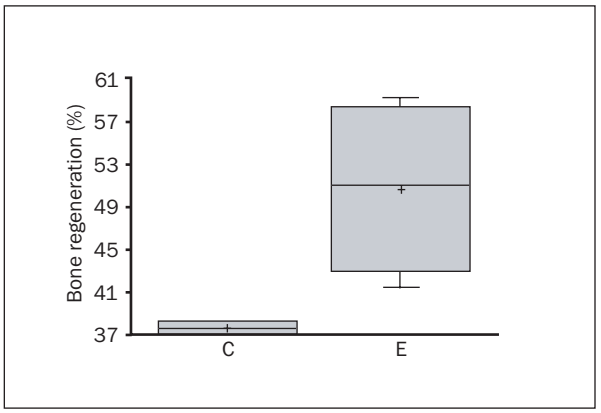

Fig 5 BV values at week 6. Bone regeneration was significantly higher in the experimental group (E) than in the control group (C) (50% vs 37%; $P = .12$).

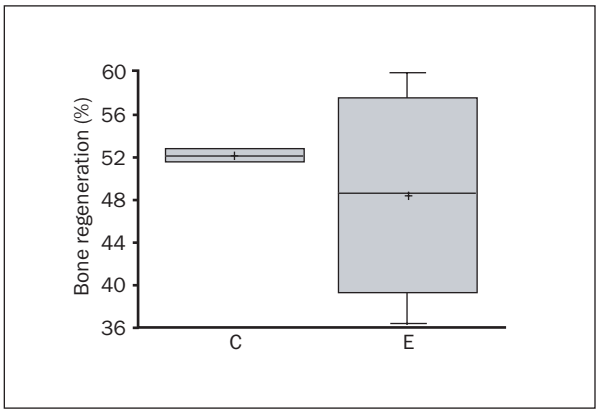

Fig 6 Percentage of bone regeneration at week 8. Bone regeneration was slightly higher in the control group (C) than in the experimental group (E; 50% vs 37%), although the difference was not significant ($P = .12$).

PRP has been widely used, in combination with autologous bone grafts or bovine hydroxyapatite, as an easy and inexpensive source of growth factors for oral and maxillofacial procedures.[22,23,31,32] Several studies suggest that PRP, in combination with bovine hydroxyapatite (Bio-Oss) or in association with mesenchymal stem cells, is able to increase bone regeneration.[25,33] Some authors claim that guided bone regeneration techniques, combined with PRP, are able to obtain vertical and horizontal bone regeneration of defects in humans,[34] and that local application of PRP alone in implant surgery may improve bone-implant contact.[28,35]

Platelet concentration in PRP is an important factor for bone regeneration. Concentrations below 1,000,000/μL offer optimal results, while higher concentrations may have inhibitory effects.[36] In this study, the platelet concentration was suitable for bone regeneration, because it had a mean value of 657,000/μL.

In this study, the influence of PRP alone in the regeneration of cranial defects was evaluated, and the results were in agreement with other studies claiming that PRP accelerates early stages of bone regeneration.[27,28,31,32] PRP was able to accelerate the bone regeneration process during the first 4 weeks after the intervention. The results obtained were similar to those reported elsewhere in the literature,[27,31,32] as several authors suggest that PRP effect is more important during the first phase of bone healing. However, although some authors have reported that the application of PRP improves the quality of the newly formed bone,[22,23] PRP did not appear to affect the quality of newly formed bone.

Other authors claim that there are no benefits from using PRP in bone regeneration. For instance, Schlegel et al[37] reported that PRP does not affect bone regeneration, and Aghlaloo et al[24] observed reduced bone formation and mineral density in rabbit cranial defects treated with PRP.

In this study, the application of PRP on cranial defects was shown to be beneficial. However, further studies are necessary to confirm these results.

CONCLUSION

Local administration of PRP in bicortical cranial defects on rabbits calvaria stimulates the first phases of bone regeneration.

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