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Effect of platelet-rich plasma on bone regeneration in autogenous bone graft

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Abstract. In this study, we evaluated the effect of platelet-rich plasma (PRP) on bone regeneration in an autogenous bone graft in a canine model. The mandibular premolar teeth had been bilaterally extracted previously, and the ridges had been allowed to heal for 3 months. After this period, continuity resection was performed on both sides of the mandible. One defect (the PRP group) was reconstructed with the original particulate bone mixed with PRP. As a control, the contralateral defect (non-PRP group) was reconstructed with the original particulate bone alone. Biopsies after 6 weeks showed lower levels of bone formation in the PRP group than in the non-PRP group, and fluorescence microscopy revealed a delay in the remodelling of grafts loaded with PRP. These findings suggest that the addition of PRP does not appear to enhance new bone formation in autogenous bone grafts.

Key words: PRP; bone regeneration; bone graft.

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Introduction

There is currently great interest in oral and maxillofacial bone grafting procedures, which involve the use of platelet-rich plasma (PRP) to enhance bone formation, and specifically to increase the rate of bone graft healing. Previous clinical studies have shown that a combination of PRP and autogenous bone graft can increase the rate of osteogenesis and enhance bone formation qualitatively[7,14]. The use of PRP is based on the premise that the large numbers of platelets in PRP release significant quantities of growth factors that aid bone graft maturation[3,4,7,9,13]. However, the amount of basic research that endorses PRP's ability to promote bone healing is limited. In a review of the current literature, we found no animal study that substantiates PRP's ability in this respect. Therefore, we decided to examine the ability of PRP to enhance bone formation in critically sized defects in the dog mandible.

Materials and methods

Eight mongrel dogs, each weighing more than 15 kg, were used in this experiment. All surgical procedures were performed under systemic (ketamine, 5 mg/kg and xylazine, 2 mg/kg i.m.) and local (2% lidocaine with 1:80 000 epinephrine) anaesthesia. The mandibular premolar teeth of each dog had been bilaterally extracted previously, and the ridges had been allowed to heal for 3 months. After this period, continuity resection was performed on both sides of the mandible to create bilateral 15 mm defects. These defects are large enough to be critically sized[2], and cannot heal naturally, without some form of treatment or intervention. Before resection, two miniplates (Martin Medizin Technik, Tuttlingen, Germany) were adapted in order to maintain the mandible in the correct position, and the neurovascular bundle entering the mandible was ligated in order to control bleeding after resection. After resecting the segment, the mandible was stabilized by fitting two miniplates. The resected segment was then ground in a bone mill (Leibinger, Germany) to a uniform particle size. This particulate bone was then used for the reconstruction of the defect site from which the bone had been obtained. The particulate bone mixed with PRP was implanted into the bone defect on one side of the mandible (PRP group). As a

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control, an identical defect was reconstructed on the contralateral side of the mandible with particulate bone alone (non-PRP group). To prepare PRP, 45 cc of autologous blood was withdrawn before surgery, and treated using a technique described previously[14]. Briefly, the blood was centrifuged at 5600 rpm to separate the platelet-poor plasma from the erythrocytes and PRP. After discarding the platelet-poor plasma, the centrifuge speed was reduced to 2400 rpm to separate the PRP from the red blood cells. A 1-ml volume of PRP together with the top 1 mm of the red blood cell layer was then collected. To confirm the concentration of platelets in the PRP, platelet counts were performed on each dog's PRP in Table 1. The mean platelet count of the PRP was 1 120 000 with a range of 910 000 to 1 804 000. This was activated just before application with a 10% calcium chloride solution and 5000 units of bovine thrombin to form a gel, which was mixed with the particulate bone, as described by WHITMAN & BERRY[15].

Bone formation was labelled using a sequence of fluorescent dyes, i.e., at 2 weeks after operation with tetracycline (12 mg/kg body weight, Bayer, Korea), at 3 weeks with alizarin red (30 mg/kg body weight, Sigma), and at 4 weeks with calcein green (20 mg/kg body weight, Sigma). After 6 weeks, the defects and the adjacent host bone were obtained en bloc, fixed in 70% alcohol and embedded in methylmethacrylate. Undecalcified serial sections of approximately 10 µm were then taken perpendicularly to the long axis of the grafts. Sections 1, 3, 5, etc. were then surface-stained by using the Masson–Goldner trichrome method, while even numbered sections were not stained and used for fluorescence microscopy. Computer-assisted histomorphometric measurements of newly formed bone were obtained by using an image analysis system (IBAS, Contron, Erching, Germany). The regenerated bone was distinguished from its histologic features, i.e., by chroma staining and by the morphology of the bone cells and matrix (Fig. 1). The perimeter of the newly formed bone was traced, and the enclosed area was determined in mm^2 by using image analysis software. The percentages of newly formed bone within the former bone defect outline were calculated. The quantitative results obtained were tested for statistical differences using the Wilcoxon's test at a significance level of 5%.

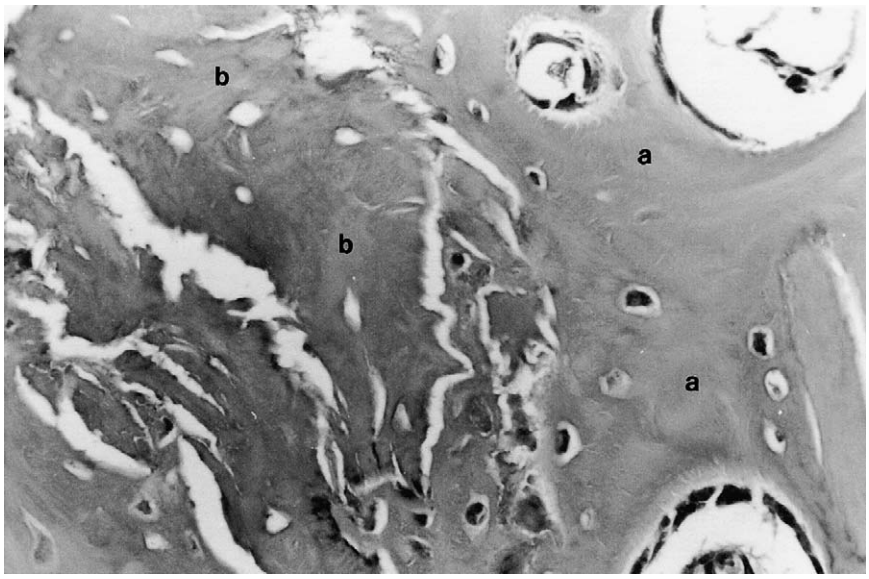

Fig. 1. Representative histologic section reveals areas of newly formed bone (a) and residual bone particles (b). Note the numerous osteocytes within the lacunae and osteoblasts lining the surface of the bone (original magnification × 150).

Results

Healing was uneventful in all animals except one. In this one animal, the graft became infected and was lost on both sides due to wound dehiscence; no histologic examination was performed on the lost grafts. At the time of harvesting, neither plate fractures nor screw loosening were noted. The bone gaps appeared to be bridged by newly formed bone in all cases except one. In this one case, the PRP-treated site showed a fibrous union, and did not show solid bone bridging on gross examination, whereas solid bone bridging was observed at the matching non-PRP treated site. Figure 2 shows a typical example of frontal sections of the defects. The non-PRP group showed extensive bone formation throughout the defect. In contrast, the PRP group showed large islands of either fibrous tissue or residual nonvital bone particles in the defects. The results of the histomorphometric analysis are shown in Table 2. Percentage calculations for areas showing bone regeneration within the former defect outline were 56.7% for the non-PRP group and 36.8% for the PRP group. The difference between these two groups was statistically significant, and indicated significantly better results on the non-PRP side. In both the PRP and non-PRP groups, the surface of the new bone was lined with osteoids and osteoblasts, indicating active bone formation. Fluorescence microscopy showed deposition of fluorochrome stains in their order of application (Fig. 3). The 2-week label (tetracycline) was more frequently found in the non-PRP group than in the PRP group. This label was present across the whole cross-section of the graft in areas adjacent to the recipient bone, but decreased gradually in the centre of the graft where it was replaced by the subsequently administered fluorochrome stains, i.e., alizarin red (the 3-week label) or calcein green (the 4-week label).

Discussion

With respect to the biologic effect of PRP on bone regeneration in a graft, the present results contradict with the findings of MARX et al.[7], who found that a combination of PRP and autogenous bone graft can increase the rate of osteogenesis and enhance bone formation qualitatively. In the present study, the histologic examination found no beneficial effect of PRP on bone formation, nor did the quantitative evaluation of bone-covered portions of the grafts reveal any significant increase in bone formation in the PRP group. In addition, fluorescence microscopy revealed a delay in graft remodelling in the presence of PRP. It is not quite clear why the PRP treated bone graft exhibited decreased bone formation as compared with the non-PRP treated graft. It may be that the explanation is related to the concentration of PRP within the bone graft. Variations in the concentration of platelet-derived growth factor (PDGF) are known to influence bone

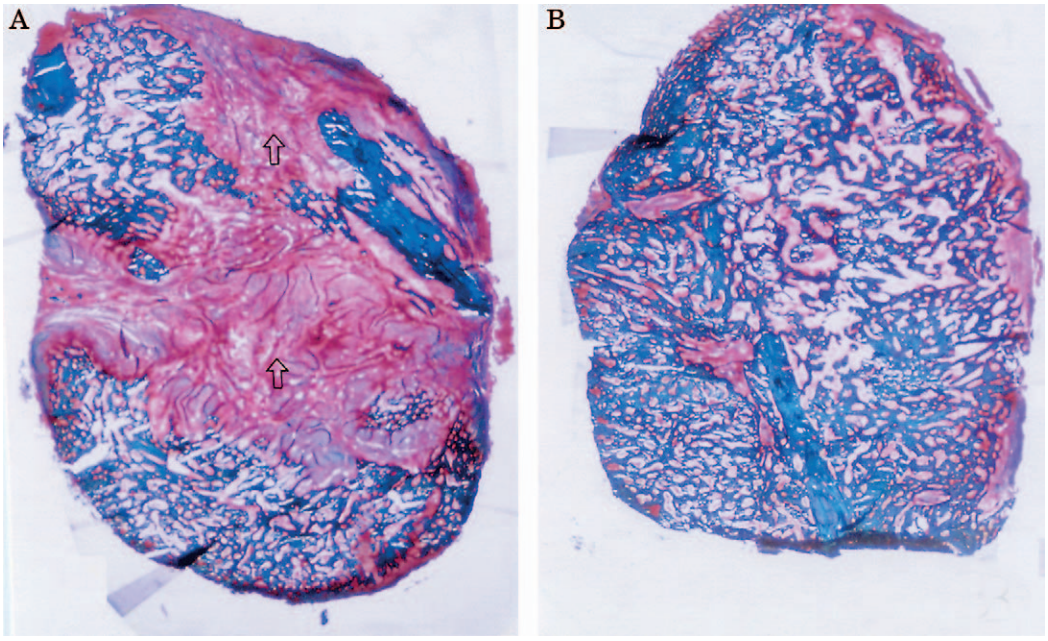

Fig. 2. Frontal sections through the middle of defects. Large islands of connective tissue (arrowed) were seen throughout the defect in the PRP group, whereas the non-PRP group showed extensive bone formation throughout the defect. A: PRP group, B: non-PRP group.

healing[5]. Marden et al.[6] reported that PRP at certain concentrations may inhibit bone regeneration. More basic research into the optimal concentration of PRP within bone grafts is necessary, in order to adequately capitalize on the ability of platelet growth factors to enhance bone formation in a graft.

Several studies have reported upon PRP-enhanced bone regeneration. Whitman et al.[14] reported favourable clinical outcomes following the incorporation of PRP gel in ablative surgical procedures of the maxillofacial region, mandibular reconstruction, the repair of alveolar clefts and fistulas, and implant placement. Marx et al.[7] used PRP in cancellous marrow graft reconstructions of large mandibular continuity defects and reported that PRP induced rapid bone maturation and increased bone density, and Kassolis et al.[3] and Shanaman et al.[12] used PRP in combination with bone allograft to enhance bone regeneration in alveolar ridge defects prior to the placement of implants. In these studies, knowledge of the PRP-enhanced bone regeneration in a graft was based on biopsies in patients who underwent reentry surgery for implant placement. The reentry surgery was performed in the late postoperative stage at 5 to 6 months after bone graft. However, it is generally believed that new bone formation is almost complete in grafted bone in the early postoperative stage[10]. Moreover, platelets and platelet-derived growth factors are known to be likely to act more so during the early stage of bone graft healing[8,11], as the life span of a platelet in a wound and the period of the direct influence of its growth factors is less than 5 days. In addition, the previous studies[1,3,7,12,14] were not designed with matched pairs. Under the conditions of these studies, the evaluation of the true effect of PRP on osseous healing is likely to be complicated by variables, such as genetics, age, hormones, and function. Especially in the late phase, such variables may ultimately influence treatment outcome regardless of the effect of the PRP component. Thus, it is possible that previous studies did not resolve the true effect of PRP on bone formation. On the other hand, in the present study, a histologic examination of grafted bone was undertaken at 6 weeks postoperatively to evaluate new bone formation during the early phase, and both sides of each dog's mandible was used to provide matched pairs. In addition, defect size and bone substitutes were standardized in this

Table 1. Platelet counts

Dog number	Preoperative platelet count	PRP platelet count
1	151 000	1 003 000
2	113 000	970 000
3	108 000	910 000
4	156 000	1 011 000
5	268 000	1 804 000
6	192 000	1 032 000
7	246 000	1 110 000
Mean	176 000	1 120 000

Table 2. Percentages of newly formed bone within the bone defects

Dog number	PRP group	Non-PRP group	*P* value
1	27.1	54.4	
2	46.3	62.4	
3	44.9	56.1	
4	34.7	52.8	
5	36.2	51.2	
6	30.5	59.4	
7	38.1	60.3	
Mean	36.8	56.7	0.018

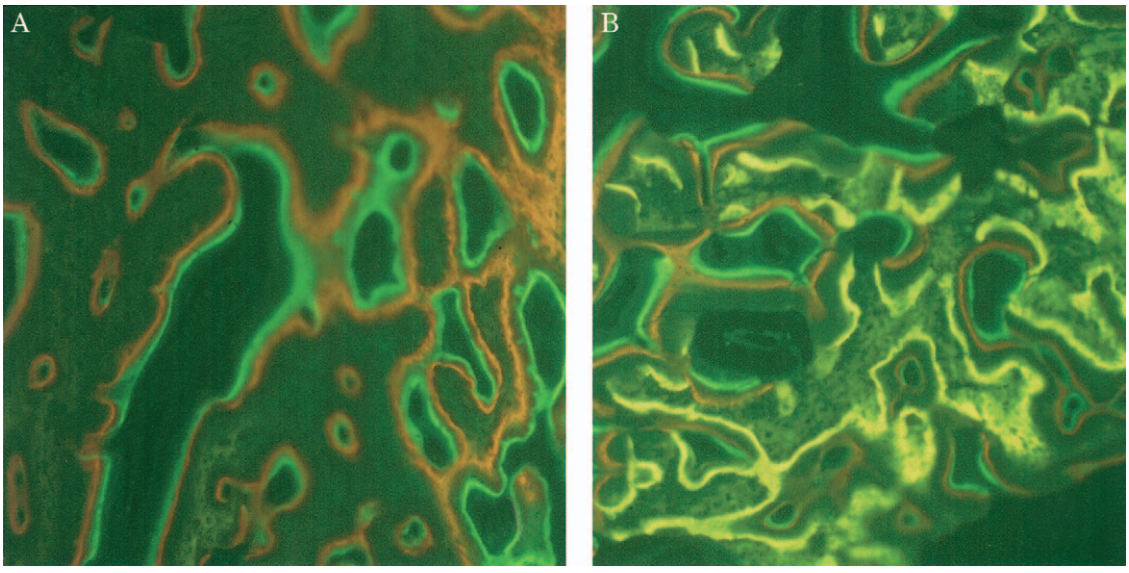

Fig. 3. Frontal sections through the middle of defects showing the deposition of fluorochrome stains (tetracycline: yellow, alzarin red: red, calcein green: green). A: PRP group, B: non-PRP group.

animal model, and all operations were performed by the same surgeon.

In summary, our results demonstrate that the addition of PRP to autogenous bone graft retards new bone formation in mandibular defects. Further controlled *in vivo* and *in vitro* studies are necessary to better understand the effect of PRP on osseous regeneration.

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