Use of Autologous Platelet-Rich Plasma (PRP) in Periodontal Defect Treatment After Extraction of Impacted Mandibular Third Molars

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Purpose: The extraction of mesioangular impacted third molars may cause multiple periodontal defects at the distal root of the second molar. Platelet-rich plasma (PRP) is a material containing many autologous growth factors that may be used in repairing and preventing periodontal complications at the distal root of the second molar adjacent to the extracted third molar.

Patients and Methods: We analyzed the effects of autologous PRP on periodontal tissues after extraction of the third molar in 18 young patients (age, 21–26 years). Inclusion criteria were the presence of a pocket distal to the mandibular second molar with a probing depth ≥7.5 mm and a probing attachment level ≥6 mm.

Results: We observed, at 12 weeks after surgery, a notable reduction in the probing depth and an improvement in the probing attachment level in those cases treated with PRP compared with the controls, as well as formation of new bone tissue in the bone defect.

Conclusion: We showed that PRP is effective in inducing and accelerating bone regeneration for the treatment of periodontal defects at the distal root of the mandibular second molar after surgical extraction of a mesioangular, deeply impacted mandibular third molar.

J Oral Maxillofac Surg 63:766-770, 2005

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This study was supported in part by a grant from the W.W. Smith Charity Trust to P.P.C.

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0278-2391/05/6306-0010$30.00/0
doi:10.1016/j.joms.2005.02.010

The extraction of mesioangular impacted third molars may cause multiple periodontal defects at the distal root of the second molar.[1-3] These complications are even more frequent in older patients and when there are preoperative periodontal defects on the distal surface of the second molar before extraction of the impacted third molar.[4,5]

Mesioversion is among the most damaging positions of impacted mandibular third molars because it results in the growth of a soft osseous defect distally to the second molar and/or the development of periodontal pockets around the latter.[6]

Platelet-rich plasma (PRP) is a material containing many autologous growth factors, such as platelet-derived growth factors (PDGF) and transforming growth factor-β, among others, which may be used in repairing and preventing periodontal complications at the distal root of the second molar adjacent to the extracted third molar.[7-9]

The aim of this clinical study was to assess the efficacy of autologous PRP in bone regeneration techniques to prevent periodontal complications at the distal root of the mandibular second molar following extraction of a mesioangular impacted third molar.

Patients and Methods

SELECTION OF PATIENTS

We selected 18 patients (10 males, 8 females) between the ages of 21 and 26 years; all were nonsmokers, with bilateral soft tissue mesioangular impacted mandibular third molars. All 36 cases of impactions were selected for a split mouth study. In each patient, the cases of dental impaction were treated by using 2 different therapeutic approaches, thereby yielding 2 different study groups, each of which was composed of 18 cases. Inclusion criteria were the presence of a pocket that was located distally to the mandibular second molar with a probing depth (PD) $\geq$7.5 mm and a probing attachment level (PAL) $\geq$6 mm. Furthermore, the postextraction defect nevertheless needed to present the vestibular and lingual cortical bone intact, not damaged during surgery. The exclusion criteria included systemic diseases, possible compromised immune system, platelet count $<$150.000/mm^3, and allergies or hypersensitivity to drugs, antibiotics, and anti-inflammatory and cortisone medication for the 12-month period preceding surgery.

All patients signed an informed consent before participating in the study, which was reviewed and approved by the University Institutional Review Board.

PREOPERATIVE THERAPY AND PREPARATION OF PRP

Before surgery, all patients received oral hygiene instructions to reach an O'Leary plaque index that was $\leq$25%.

Forty milliliters of blood was drawn from each patient and collected in 4 glass tubes (Vacutainer, Becton & Dickinson, Rutherford, NJ) containing a 10% trisodium citrate anticoagulant solution. The tubes containing the blood were placed in a centrifuge at 1,200 rpm for 15 minutes, after which we obtained the separation of 3 fractions: platelet-poor plasma on top, PRP in the middle, and red blood cell fraction at the bottom. Two milliliters of the top layer corresponding to platelet-poor plasma were aspirated for each of the 4 tubes with a Pasteur pipette and discarded. The PRP was collected from each tube together with 1–2 mm of the red blood cell fraction to ensure that the largest and newest platelets were collected.

PREOPERATIVE MEASUREMENTS

We performed an orthopantomogram and intraoral radiography using the Rinn alignment system on all patients. We evaluated the plaque index[10] as well as the gingival bleeding index[11] immediately before surgery. The plaque and gingival indices were then evaluated at 12 and 18 weeks after surgery to allow for proper healing at the treated sites.

Measurements of PD, PAL, and gingival recession were made using cold resin occlusal stents taken from a model obtained from an alginate cast for all patients to be treated. The stents were made so as to cover the occlusal surface, the third vestibular and lingual coronal site of the mandibular second molar adjacent to the site to be treated. Grooves were made in the resin stents such that it was possible to perform measurements before and after surgery by using the same position and the same angulation of the periodontal probe. Measurements were made using a Marquis periodontal probe in 3 different positions for all the mandibular second molars (distovestibular, distal, distolingual). PD, PAL, and gingival recession were assessed at 12 and 18 weeks so as not to interfere with the healing of the treated sites.

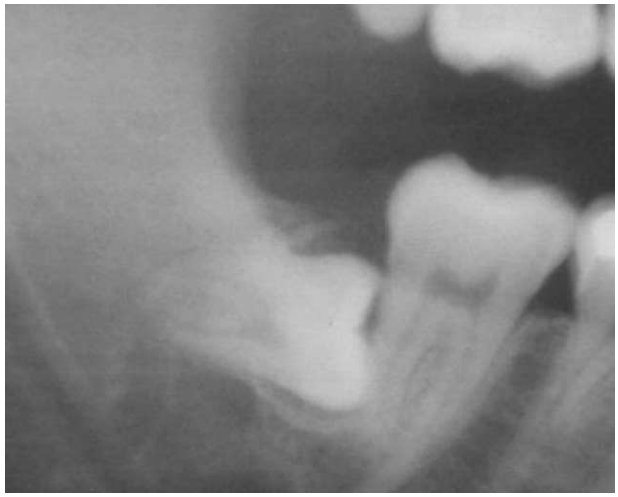

FIGURE 1. Preoperative X-ray showing the inferior third molar deeply impacted.

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SURGICAL PROCEDURE

After locoregional anesthesia was administered, a full-thickness muco-periosteal flap was raised for extraction of the third molar. The flap incision extended from the vestibular side of the retromolar trigon to the marginal periodontal portion of the second molar, corresponding to its distolingual cusp. The incision continued vestibularly around the intrasulcular surface of the second molar. We then proceeded by making a vestibular releasing incision to the papilla between the first and second molars, on a 45° angle. We first performed an osteotomy using a Lindemann burr in conjunction with constant irrigation, followed by an odontotomy using a diamond burr.

After extraction of the impacted third molar (Fig 1), we prepared the residual bone cavity and proceeded with scaling the root surface of the second molar, which was carefully planed using manual instruments.

The bone cavity was filled using 2 different therapeutic approaches: in 18 cases (sides), PRP was ap-

plied while the residual bone cavity was left empty in the remaining 18 sides (controls).

Coagulation of the PRP (Fig 2A) was obtained by adding 1 mL batroxobine (Botropase; Ravizza, Milano, Italy) and 1 mL 10% gluconate of calcium 446 mEq (Fisiopharma, Milano, Italy), which was shaken in a sterile tube for approximately 30 seconds to obtain, within a minute or so, a gel to be applied to the bone defect walls and to the planed root surface of the second molar (Figs 2B,C). In the controls, we simply performed scaling and planing of the root surface of the second molar using hand curettes. In all cases, we used 4-0 Ethilon monofilament sutures to stitch the flap and removed them after 8 days. Patients were given antibiotics (amoxicillin and clavulanic acid every 12 hours for 8 days), oral anti-inflammatory treatment (ibuprofen 800 mg every day for 3 days) and 0.12% chlorhexidine gluconate rinses every 12 hours for 10 days. Oral hygiene was assessed and supportive periodontal therapy was provided for all patients at 2, 4, and 6 weeks after surgery.

RE-ENTRY PROCEDURES

On all patients, we performed an orthopantomogram and control intraoral radiography using the Rinn alignment system after 12 weeks (Fig 3A) and after 18 weeks (Fig 3B) to assess the healing of the treated sites. In those cases in which we had applied PRP, after 12 weeks we performed a second surgery to obtain an osseous biopsy, which measured 0.5 mm at the center of the treated area; constant irrigation was maintained during the procedure. The extracted sample was then fixed in 10% buffered formalin, demineralized with chloridric acid/formic acid for 48 hours, decalcified in nitric acid, routinely processed, and embedded in paraffin. Sections of the sample measuring 5-μm thick were then stained with hematoxylin-eosin. In this manner, it was possible to assess the degree of bone regeneration that was present at the treated site. All surgeries were performed by 1 surgeon, while a second surgeon performed the clinical measurements without being aware of what therapeutic approach was used for the different sites of treatment.

Intraexaminer calibration was achieved by examination of 6 patients twice (48 hours from the surgery and immediately before the beginning of the study). Calibration was accepted if the 2 clinical measurements at baseline and at 48 hours were similar to the whole millimeter and were greater than the 92% level.

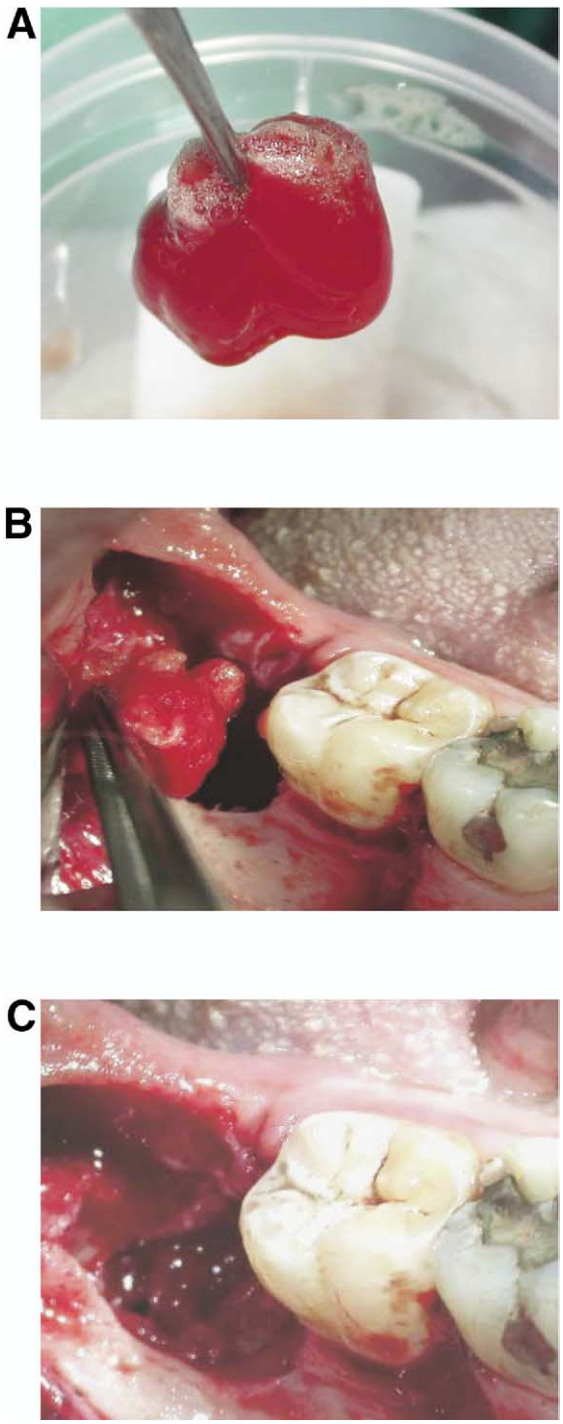

FIGURE 2. *A*, Preparation of the PRP gel. *B*, The PRP gel is inserted into the post-extractive bone defect. *C*, Complete filling of the postoperative bone defect with the PRP gel.

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Results

All subjects completed the study. For all cases, we averaged the 3 results obtained by probing at the level of the distal surface of the second molar. All results were calculated using the mean value ± standard deviation for each of the parameters considered. The Student t test was used to compare the differences between the 2 groups.

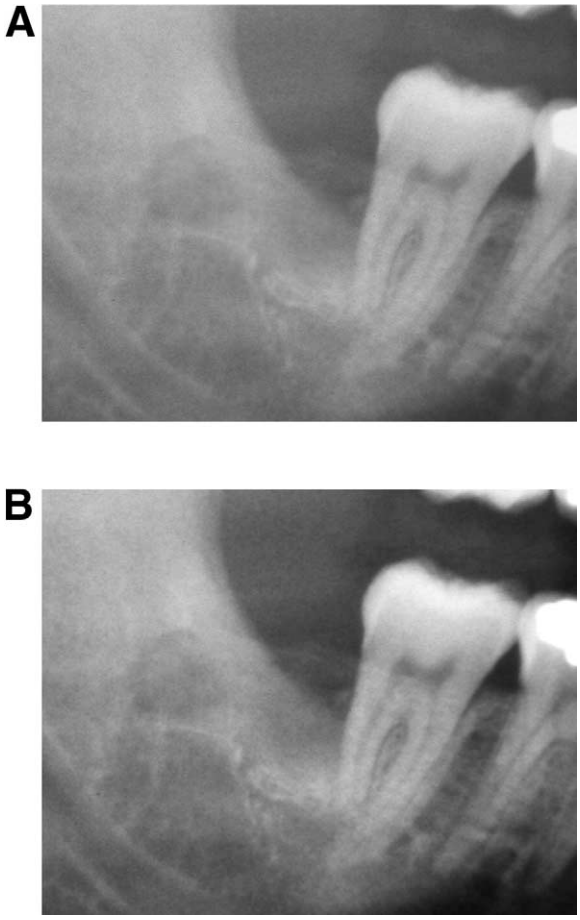

FIGURE 3. *A*, X-ray showing bone regeneration at 12 weeks from the intervention. *B*, X-ray showing the conserved osseous level 18 weeks after the intervention.

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We observed a notable reduction in the PD and an improvement in the PAL in those cases treated with PRP compared with controls.

The decrease in PD corresponding to the distal surface of the mandibular second molar in the cases treated with PRP was 4.13 ± 1.34 mm at 12 weeks and 3.65 ± 1.30 mm at 18 weeks. On the other hand, in the control cases, the decrease in PD was 7.37 ± 0.91 mm at 12 weeks and 7.18 ± 0.95 mm at 18 weeks (Table 1).

The attachment gain for the 18 cases treated with PRP was 2.91 ± 1.39 mm at 12 weeks and 2.42 ± 1.38 mm at 18 weeks. In the controls, the attachment gain was 5.99 ± 0.89 mm at 12 weeks and 5.84 ± 0.90 mm at 18 weeks. As such, we can state that the results were clearly more satisfactory in the 18 cases treated with PRP compared with the control group (Table 1).

Concerning the PD, the difference between the 2 groups was 3.24 ± 1.65 mm at 12 weeks and 3.53 ± 1.53 mm, favoring the PRP-treated group, at 18 weeks (Table 1). Furthermore, the PRP-treated group presented a significant difference in clinical advantage compared with the control group (3.08 ± 1.49 mm at 12 weeks; 3.42 ± 1.43 mm at 18 weeks) (Table 1). With respect to the gingival recession, we observed at both 12 weeks and 18 weeks that there were no statistical differences between the 2 groups considered (Table 1).

Finally, no statistically significant differences were observed between the 2 groups in terms of plaque index or gingival bleeding index (Table 1).

During histologic examination of the sites treated after 12 weeks from surgical dental extraction, following staining with hematoxylin-eosin, we observed the presence of newly formed bone tissue with a high osteoblast population, along with a small amount of fibrous, highly cellular tissue. Overall, we could observe considerable bone regeneration in 17 cases treated with PRP. Only in 1 treated case was there scarce formation of bone tissue while there was a large amount of fibrous tissue present; the clinical results corresponding to this histology were not acceptable.

Discussion

Extraction of mesioangular impacted mandibular third molars when they provoke periodontal damage at the distal root of the adjacent second molar has been practiced for some time.[12] The use of PRP has shown to be a valid technique for promoting bone regeneration at the level of the distal surface of the mandibular second molar following extraction of a mesioangularly inclined, deeply impacted third molar (preoperative PD ≥7.5 mm).

This method has yielded satisfactory results both clinically and histologically. The use of PRP alone to fill the osseous defect has shown to be more valid for periodontal regeneration compared with the control sites, because of both the decrease in the PD and the attachment gain.

The mechanism by which PRP can influence periodontal regeneration is ascribed to the presence of PDGF and TGF-β, although this is not yet completely acknowledged. Some in vitro studies[13] have suggested that PDGF acts principally on osteoblastic proliferation and that, on the other hand, morphogenetic proteins (which are part of the TGF-β superfamily) act as a cellular differentiation agent favoring the expression of markers of mineralization when they are incubated with preosteoblastic cells. This suggests that TGF-β could favor the differentiation of osteoblasts and cementoblasts and the production of fibronectin, a molecule involved in the adhesion of fibroblasts to the radicular surface and in the angiogenic process.[14-16] As the result of its fibrin content, the PRP gel permits stabilized coagulation of the blood, thereby favoring regeneration of the osseous defect, particularly in the early stages.[17,18]

Table 1. DESCRIPTIVE STATISTICS OF THE OVERALL SERIES

	Platelet-Rich Plasma	Control	*P* Value	Difference Between Groups
Variations in the probing depth in millimeters corresponding to the distal surface of the mandibular second molar (n = 18 bilateral cases)				
Initial	8.89 ± 1.01	8.90 ± 0.71	>.05 NS	
At 12 wks	4.13 ± 1.34	7.37 ± 0.91	<.05*	3.24 ± 1.65
At 18 wks	3.65 ± 1.30	7.18 ± 0.95	<.05*	3.53 ± 1.53
Variations in clinical advantage in millimeters (n = 18 bilateral cases)				
Initial	6.89 ± 1.01	6.80 ± 0.78	>0.05 NS	
At 12 weeks	2.91 ± 1.39	5.99 ± 0.89	<0.05*	3.08 ± 1.49
At 18 weeks	2.42 ± 1.38	5.84 ± 0.90	<0.05*	3.42 ± 1.43
Variations in gingival recession in millimeters (n = 18 bilateral cases)				
Mean variation at 12 wks	0.74 ± 0.39	0.77 ± 0.48	>0.05 NS	0.03 ± 0.51
Mean variation at 18 wks	0.80 ± 0.37	0.82 ± 0.51	>0.05 NS	0.01 ± 0.61
Variations in plaque index and gingival bleeding index (n = 18 bilateral cases)				
Initial plaque index	0.68 ± 0.42	0.76 ± 0.32	>0.05 NS	
At 12 wks	0.54 ± 0.33	0.61 ± 0.44	>0.05 NS	
At 18 wks	0.52 ± 0.35	0.57 ± 0.53	>0.05 NS	
Initial gingival bleeding index	0.73 ± 0.45	0.81 ± 0.39	>0.05 NS	
At 12 wks	0.65 ± 0.31	0.72 ± 0.36	>0.05 NS	
At 18 wks	0.61 ± 0.27	0.68 ± 0.29	>0.05 NS	

Abbreviation: NS, not statistically significant.
*Statistically significant.

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The present study shows that the use of PRP is certainly a valid method which is effective in inducing and accelerating bone regeneration for the treatment of periodontal defects at the distal root of the mandibular second molar after surgical extraction of a mesioangular, deeply impacted mandibular third molar.

Our results have shown that as early as 12 weeks after surgery, this method caused a satisfactory reduction in the PD and attachment gain, as well as the formation of new bone tissue in the bone defect.

Acknowledgment

The authors thank Marie Louise Basso for editing the manuscript.

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