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Case Report

Clinical application of platelet-rich fibrin as the sole grafting material in periodontal intrabony defects

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Abstract *Background/purpose:* Platelet-rich fibrin (PRF) obtained by Choukroun's technique is a healing biomaterial that concentrates in a single autologous fibrin membrane, mostly of platelets and cytokines from blood harvest, and without any artificial biochemical modification. However, no data are presently available concerning the use of PRF for the treatment of periodontal intrabony defects. This report was to present the clinical and radiographic changes of a patient with periodontal intrabony defects treated with PRF.

Material and methods: The left mandibular first molar (#36) and left maxillary second molar (#27) with intrabony defects were filled with PRF as the sole grafting material in a 38-year-old female patient. The primary outcomes evaluated in this study included changes in probing depth, attachment level, and radiographic bone density between baseline and 6 months post-operatively.

Results: The results showed that the application of PRF as the sole grafting material in intrabony defects exhibited pocket reduction and gain in clinical attachment after 3 months and 6 months. Using National institute of health program, the 6 months postoperative radiographic density of images for #27 and #36 showed an increase of 1.6 and 1.3 fold compared with each preoperative radiography, respectively.

Conclusions: From a clinical and radiologic point of view at 6 months after surgery, the use of PRF as the sole grafting material seems to be an effective modality of regenerative treatment for periodontal intrabony defects.

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Introduction

Periodontal regeneration is a multifactorial process and requires an orchestrated sequence of biological events including cell adhesion, migration, proliferation, and differentiation.¹ Significant progress in the understanding of periodontal wound healing has been made. Although complete regeneration of the periodontal attachment apparatus, including bone, periodontal ligament, and cementum is possible, it is still not predictable.²

The most positive outcome of periodontal regenerative procedures in infrabony defects³⁻⁵ and furcations^{6,7} has been achieved with a combination of bone grafting and guided tissue regeneration. Recently, polypeptide growth factors which are biological mediators that have the ability to regulate cell proliferation, chemotaxis, and differentiation have also been investigated. As preliminary evidence for their potential applications in periodontal wound healing, several polypeptide growth factors have been identified in the human periodontal tissue by immunohistochemistry and *in situ* hybridization.¹

Platelet-rich fibrin (PRF) described by Choukroun et al.⁸ is a second-generation platelet concentrate which allows one to obtain fibrin membranes enriched with platelets and growth factors, after starting from an anticoagulant-free blood harvest without any artificial biochemical modification. The PRF clot forms a strong natural fibrin matrix, which concentrates almost all the platelets and growth factors of the blood harvest^{9,10} and shows a complex architecture as a healing matrix, including mechanical properties no other platelet concentrate offers. It is an autologous biomaterial, and not an improved fibrin glue. Some clinical applications have been described in oral maxillofacial surgery¹¹⁻¹³ and in dental implantology.^{14,15}

Recently, we first report that PRF can stimulate cell proliferation of osteoblasts, gingival fibroblasts, and periodontal ligament cells but suppress oral epithelial cell growth.¹⁶ These cell type-specific actions of PRF may be beneficial for periodontal regeneration. However, no data are available on the use of PRF in the treatment of human periodontal intrabony defects. In this report, we present the clinical and radiographic changes of a patient using PRF as the sole grafting material in the treatment of periodontal infrabony defects and furcations.

Case report

A 38-year-old woman was referred to the Department of Periodontics, Chung Shan Medical University Hospital with



Figure 1 Radiographic shadows indicated that the infrabony defects and the furcation invasions might be occurred at teeth of #27 and #36.

a complaint of periodontal disease. The patient was healthy and had not taken any long-term anti-inflammatory medication or antibiotics. After periodontal examination and radiographic evaluation (Fig. 1), the diagnosis was chronic periodontitis.¹⁷ The study protocol was approved by the Institutional Review Board of Chung Shan Medical University Hospital. The surgical procedure and possible alternatives were discussed with the patient.

Initial therapy consisted of oral hygiene instructions, which were repeated until the patient achieved an O'Leary plaque score¹⁸ of 20% or below. Scaling and root planing of the teeth were performed. Trauma from occlusion was evaluated by examining the obvious presence of fremitus in centric occlusion or in working or balancing excursions. Four weeks following Phase I therapy, a periodontal re-evaluation was performed to confirm the suitability of the left mandibular first molar (#36) (Table 1) and maxillary second molar (#27) (Table 2) for this periodontal surgical study.

Blood samples were taken according to the PRF protocol with a PC-02 table centrifuge and collection kits provided by Process (Nice, France).^{13,15} Briefly, blood samples were taken from the patient without an anticoagulant in 10-mL glass-coated plastic tubes (Becton Dickinson Vacutainer, Franklin Lakes, NJ, USA) and immediately centrifuged at 3000 rpm for 12 minutes. A fibrin clot formed in the middle part of the tube, whereas the upper part contained acellular plasma, and the bottom part contained red corpuscles (Fig. 2A). The fibrin clot was easily separated from the lower part of the centrifuged blood (Fig. 2B). The PRF clot was gently pressed into a membrane with sterile dry gauze. PRF membranes were minced as graft materials (Fig. 2C) and trimmed as membranes (Fig. 2D).

Table 1 Changes in clinical parameters of #36.

	Tooth mobility	Probing depth (mm)						Gingival recession (mm)						Attachment gain (mm)						
		DB	B	MB	DL	L	ML	DB	B	MB	DL	L	ML	DB	B	MB	DL	L	ML	
Baseline	II	4	5	8	6	9	8	2	2	3	2	1	2							
Presurgery	II	4	5	7	5	8	7	2	2	3	2	2	2	0	0	1	1	0	1	
Postoperation (3 mo)	I	3	3	4	3	4	4	2	2	3	2	2	2	1	2	3	2	4	3	
Postoperation (6 mo)	0	3	2	3	3	3	3	2	1	2	2	2	2	1	2	3	2	5	4	

Changes in probing depth as measured from gingival margin. Changes in gingival recession and attachment level as measured from cemento-enamel junction.

B = buccal; DB = distal buccal; DL = distal lingual; L = lingual; MB = mesial buccal; ML = mesial lingual.

Table 2 Changes in clinical parameters of #27.

	Tooth mobility	Probing depth (mm)						Gingival recession (mm)						Attachment gain (mm)						
		DB	B	MB	DL	L	ML	DB	B	MB	DL	L	ML	DB	B	MB	DL	L	ML	
Baseline	II	7	6	9	8	5	10	0	2	1	0	3	1							
Presurgery	II	6	5	8	7	5	9	1	3	1	0	3	2	0	0	1	1	0	0	
Postoperation (3 mo)	II	4	3	5	5	3	5	2	4	2	1	4	2	1	1	3	1	1	5	
Postoperation (6 mo)	I	3	3	4	5	3	4	2	3	1	1	4	2	2	2	4	1	1	5	

Changes in probing depth as measured from gingival margin. Changes in gingival recession and attachment level as measured from cemento enamel junction.

B = buccal; DB = distal buccal; DL = distal lingual; L = lingual; MB = mesial buccal; ML = mesial lingual.

The procedures were performed using identical techniques by one investigator. An intrasulcular incision was made on the buccal and lingual aspect of the tooth of left mandibular sextants being treated (Fig. 3A). A full thickness flap was raised and the inner surface of the flap was curetted to remove the epithelium and granulation tissue (Fig. 3B). Root surfaces were thoroughly planed using hand instruments and ultrasonic scalers for furcation areas. The

left mandibular first molar demonstrated mesial intrabony defect and a through-and-through furcation invasion (Fig. 3B). After removing granulation tissue thoroughly, mesial intrabony defect was found to extend to buccal and lingual aspects (2–3 wall infrabony defect). Treatment of the periodontal defects was shown in Figs. 4 and 5. Briefly, minced PRF was applied to the defect walls and root surfaces. The PRF was then tightly packed in the furcation

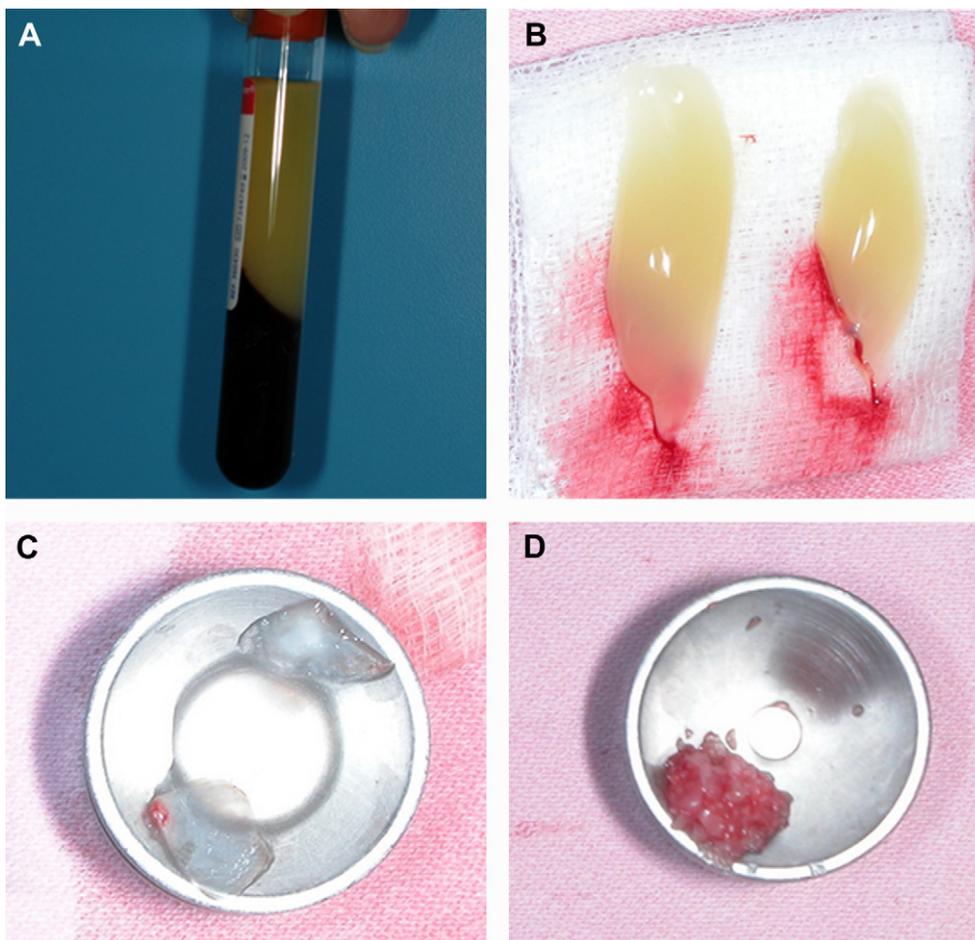


Figure 2 Preparation of platelet-rich fibrin (PRF). (A) PRF formed in the middle part of the tube. The upper part contained acellular plasma and the bottom part contained red corpuscles. (B) The fibrin clot was easily separated from the lower part of the centrifuged blood. (C) The PRF clot was gently pressed between 2 layers of sterile dry gauze to form a membrane (D) PRF can be minced as a grafting material.

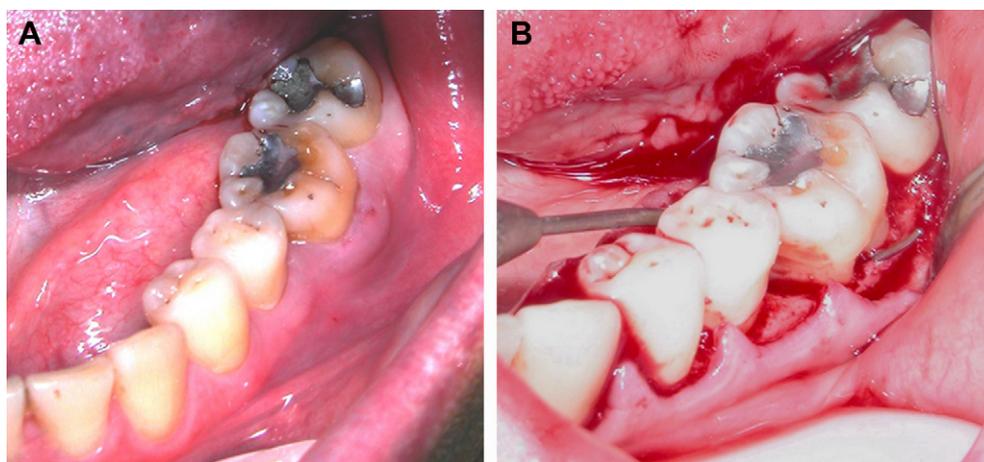


Figure 3 The clinical pictures of left mandibular sextant. (A) Before surgery. (B) After flap reflection, mesial infrabony defect and a through-and-through furcation invasion were noted at #36.

area using amalgam condensers to the vertical level corresponding to the roof of the furcation. In the horizontal direction, the PRF was flushed with the bone present on the most apical area of the furcation. The PRF membrane was trimmed and adapted over the grafted defect and above the cemento enamel junction. The flaps were repositioned

to their presurgical levels and sutured with silk utilizing an interrupted technique (Fig. 6A).

After the operation, the patient was prescribed systemic antibiotics (cephalosporin 250 mg, qid. 3 days), non-steroidal anti-inflammatory drugs (cataflam, 25 mg, qid. 3 days), and 0.12 % chlorhexidine rinse (twice a day for

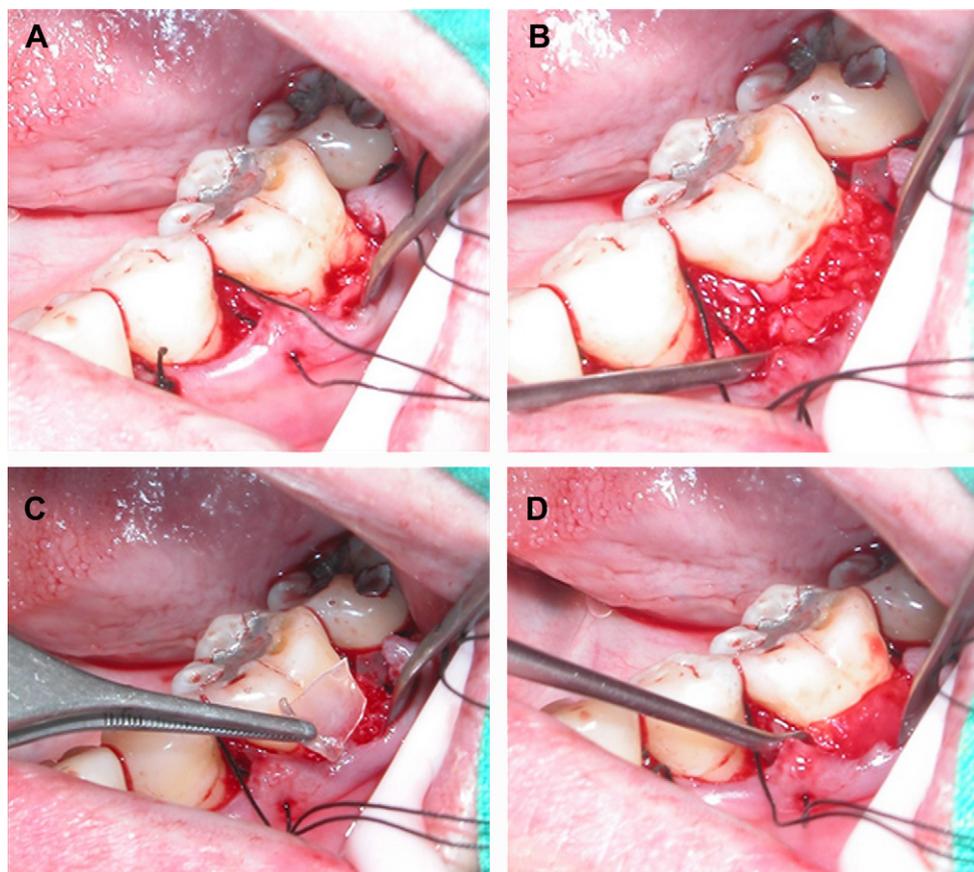


Figure 4 Clinical application of platelet-rich fibrin (PRF) on the buccal aspect of #36. (A) Minced PRF was applied to the defect walls and root surfaces. (B) Minced PRF was tightly packed in the furcation area. (C) PRF membrane was trimmed to cover the osseous defects. (D) PRF membrane was adapted over the grafted defect and above the cemento enamel junction.

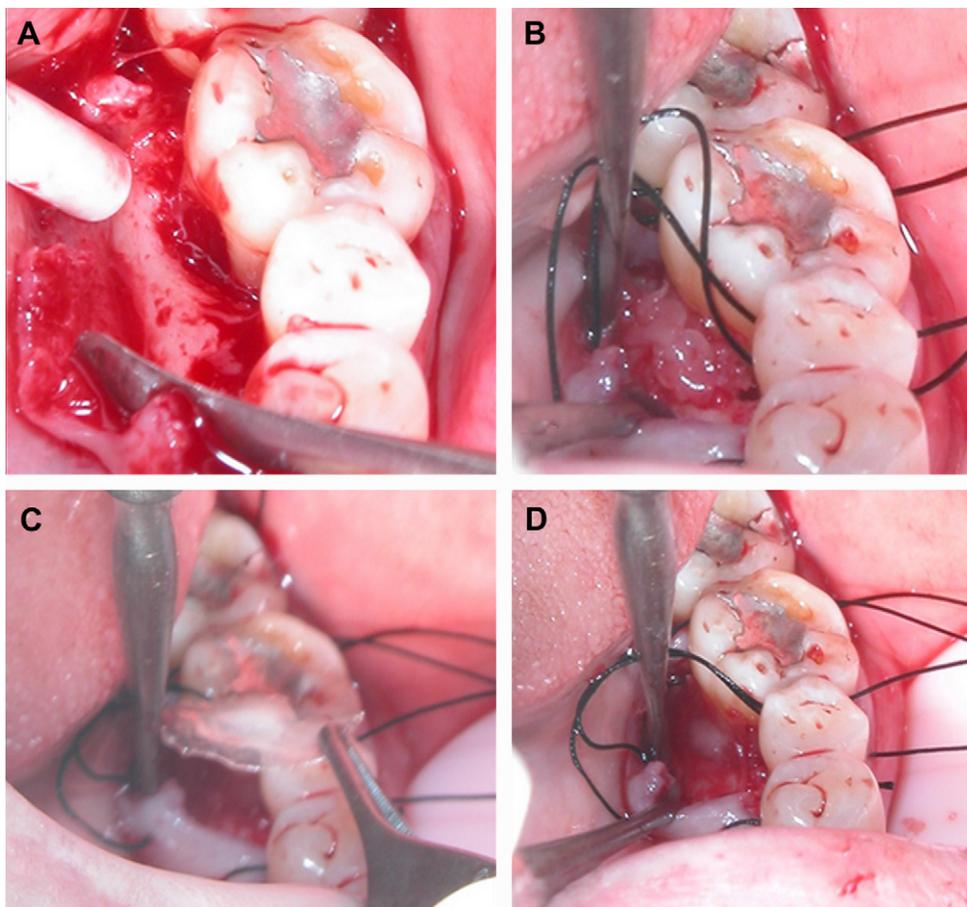


Figure 5 Clinical application of platelet-rich fibrin (PRF) on the lingual aspect of #36. (A) Intrabony defect and furcation invasion were shown. (B) Minced PRF was tightly packed in the intrabony defect and furcation. (C) PRF membrane was trimmed to cover the osseous defects. (D) PRF membrane was adapted over the grafted defect and above the cemento-enamel junction.

4 weeks). Sutures were removed after 7 days. Clinical healing was normal with neither infectious episodes nor untoward clinical symptoms. The patient was seen at 1st week, 2nd week, 1st month, 3rd month, and 6th month (Fig. 6B). During the first 4 weeks, the patient was instructed to brush only the non-involved teeth. Periodontal examinations were

recorded after 3 months and 6 months (Table 1) and periapical radiography was taken after 6 months. The same procedure was performed for #27. Postoperative clinical parameters showed that the application of PRF for periodontal osseous defects achieved probing depth reduction and clinical attachment gain (Tables 1 and 2).

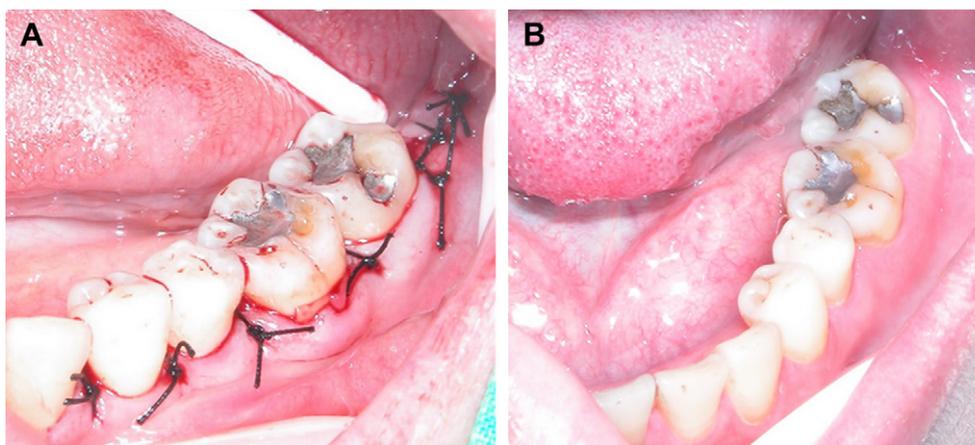


Figure 6 The clinical pictures after platelet-rich fibrin application. (A) The flaps were repositioned to their presurgical levels and sutured with silk utilizing an interrupted technique. (B) The clinical picture of #36 3 months after operation.

Periapical intraoral radiographs were obtained from the periodontal defect sites before and 6 months after surgery (Figs. 7A and 8A). The radiographs were obtained with a paralleling technique using the Rinn system, but no attempts for further standardization were made. The radiographic density of the images was analyzed by means of the mathematical routine developed in the National institute of health (NIH) program environment. For each radiograph, a score from 0 to 100 was created, with the value 0 being conferred on the most radiolucent area and the value 100 on the most radiopaque area. The lesion area and an adjacent bone area in each radiograph were also cropped by means of the NIH program. 6 months after PRF application, radiograph exhibited a significant increase (Figs. 7B and 8B). Using the quantitative measurements by the NIH program, the levels of the postoperative radiographic density were 1.6- and 1.3 fold for #36 and #27 compared with each preoperative radiography, respectively.

Discussion

PRF by Choukroun's technique is prepared naturally without addition of thrombin, and it is hypothesized that PRF has a natural fibrin framework and can protect growth factors from proteolysis.^{19,20} Thus, growth factors can keep their activity for a relatively longer period and stimulate tissue regeneration effectively. PRF can be considered as a natural fibrin-based biomaterial favorable to the development of a microvascularization and able to guide cell migration into wound area.

To the best of our knowledge, this is the first report about the use of PRF as the sole grafting material for periodontal intrabony defects and furcations. In this case report, the reduction in pocket depth and gain in clinical attachment were found after PRF application. Pocket depth reduction is not only a desirable outcome of periodontal regenerative procedures, but may also be the most important parameter in patient care for the clinician, because it directly impacts one's ability to instrument a treated area during maintenance appointments. An important clinical outcome of a periodontal regenerative procedure is gain in clinical attachment. This gain in clinical

attachment might have been a result of true periodontal regeneration by means of new attachment or, alternatively, of healing by repair, which implies the presence of a long junctional epithelium between the newly regenerated tissues and the root surface.²¹

PRF application exhibited the radiographic intensity increase by periapical radiography after 6 months over lesion areas. Radiographic evaluation is a noninvasion examination for bony defects repair. However, bone fill data derived from surgical re-entry are important to substantiate routine postoperative measurement data. Furthermore, histology of the treated periodontal osseous defects is the only reliable method to determine the nature of the periodontal soft and hard tissue interface.

The reason why PRF could improve periodontal osseous defects healing may be explained as follows. Recently, we found that PRF can upregulate phosphorylated extracellular signal-regulated protein kinase expression and suppress osteoclastogenesis by promoting the secretion of osteoprotegerin in osteoblasts cultures.²² PRF was also demonstrated to stimulate osteogenic differentiation of human dental pulp cells by upregulating osteoprotegerin and alkaline phosphatase expression.²³ Furthermore, many growth factors such as platelet-derived growth factor and transforming growth factor are released from PRF.^{9,10,15,16} Recently, studies have demonstrated that the PRF membrane has a very significant slow-sustained release of key growth factors for at least 7days¹⁵ and up to 28 days,¹⁶ which means that the PRF membrane stimulates its environment for a significant time during remodeling. The properties of this natural fibrin biomaterial thus offer great potential during wound healing. It has been clearly demonstrated that fibrin matrix leads directly to angiogenesis.¹⁴ Fibrin constitutes a natural support to immunity and reduce inflammatory process.¹⁰ PRF itself can be recognized as an autologous biomaterial. PRF, as membrane and grafting material, offers an improved spacemaking effect of the barrier, which is conducive to cell events leading to periodontal regeneration, and facilitation of mineralized tissue formation due to osteoconductive and/or osteoinductive properties possibly inherent in PRF.

The acrylic stent, surgical re-entry, and digital radiography are necessary to evaluate for further assessment of PRF outcome for periodontal regeneration. A limitation of

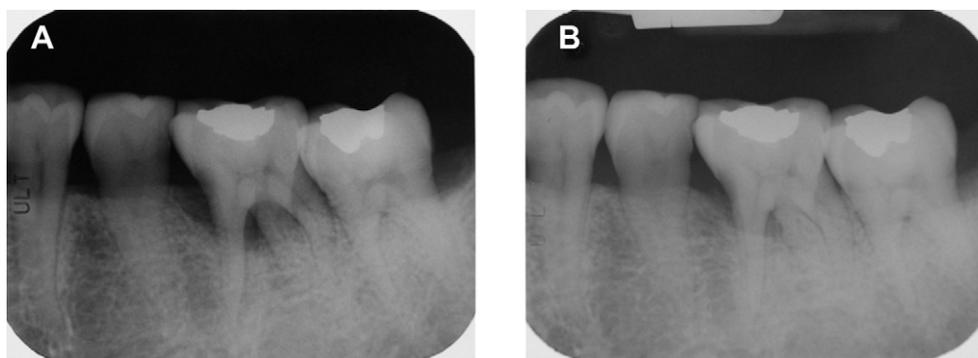


Figure 7 The periapical radiography of #36. (A) preoperatively and (B) 6 months postoperatively. The quantitative measurement by the National institute of health program, the level of the postoperative density was 1.6 fold compared with preoperative radiography.

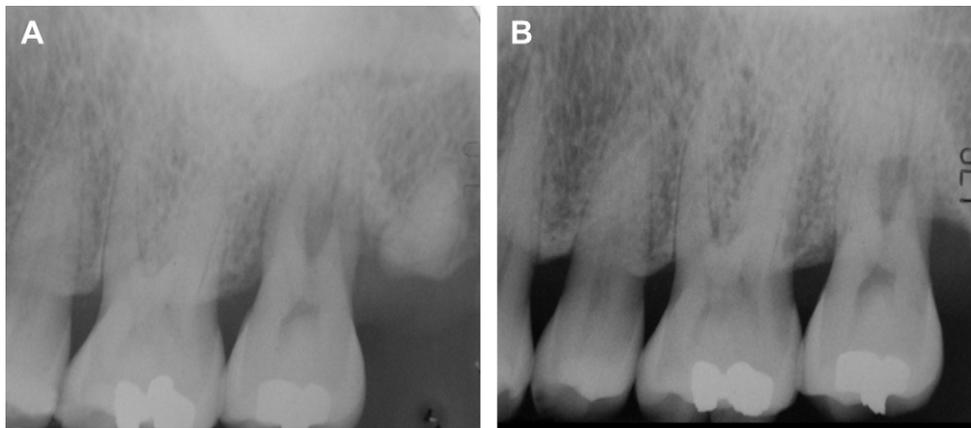


Figure 8 The periapical radiography of #27. (A) preoperatively and (B) 6 months postoperatively. The quantitative measurement by the National institute of health program, the level of the postoperative density was 1.3 fold compared with preoperative radiography.

the present study is the 6-month follow-up time, which could be regarded as rather short, especially for the evaluation of osseous changes. Furthermore, because case report(s) and case series were performed without a control group, the interpretation is only based on observation of the relevant case(s). The parallel observation on contralateral periodontal intrabony defects without PRF as a control should be performed in future to specify the advantages of this method in periodontal regeneration.

PRF by Choukroun's technique is a simple and inexpensive technique, and the systematic use of this biomaterial for periodontal regeneration seems a very promising option. This case report demonstrates that the use of PRF, as the sole grafting material in periodontal osseous defects, is an effective modality in promoting a clinical resolution. The practical aspect of PRF use in periodontal osseous defects may be clinically relevant. Because PRF preparation utilizes the patient's own blood, the risk of human to human/animal disease transmission is virtually eliminated, making it a safe treatment modality. The application of PRF for periodontal osseous defects achieves probing depth reduction, clinical attachment gain, and intensity increase of periapical radiography over a 6-month period. Thus, PRF, as a natural and optimized blood clot, seemed the adequate adjuvant to secure this technique and to improve the guided tissue regeneration.

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