Scintigraphic Evaluation of Osteoblastic Activity in Extraction Sockets Treated With Platelet-Rich Fibrin

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Purpose: To evaluate the effect of platelet-rich fibrin (PRF) on the early bone healing process with bone scintigraphy based on technetium-99m methylene diphosphonate uptake in third molar extraction sockets.

Patients and Methods: Fourteen patients with bilaterally soft tissue impacted third mandibular molars were included in the study. The right and left impacted third molars were surgically extracted in the same session. PRF was randomly administered into one of the extraction sockets, whereas the contralateral sockets were left without treatment. Four weeks after surgery, scintigrams were obtained to evaluate scintigraphic differences between PRF-treated and non–PRF-treated sockets. After completion of the clinical study, PRF samples were evaluated by light and scanning electron microscopy.

Results: The average increase in technetium-99m methylene diphosphonate uptake as an indication of enhanced bone healing did not differ significantly between PRF-treated and non–PRF-treated sockets 4 weeks postoperatively ($P > .05$). Abundant fibrin and inflammatory cells were observed by light microscopic examination of PRF samples. Scanning electron microscopic analysis of PRF revealed the existence of platelet aggregates in a fibrin network and crystalline particles on the outer surface of PRF.

Conclusions: PRF might not lead to enhanced bone healing in soft tissue impacted mandibular third molar extraction sockets 4 weeks after surgery. PRF exhibits the potential characteristics of an autologous fibrin matrix. However, whether the presence of crystal-like particles on the outer surface of PRF alters bone healing should be investigated further.

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The initial process in reaction to tissue injury is the prevention of bleeding through platelet aggregation and activation of hemostasis cascade that results in the release of platelet growth factors, cytokines, and hemostatic factors.[1] In the existence of thrombin and Ca^{2+}, the coagulation cascade leads to conversion of soluble fibrinogen into a network of insoluble fibrin fibers, which stabilizes the platelet plug. This fibrin network also comprises activated platelets binding to it by $\alpha_{IIb}\beta_3$ receptors.[2,3] In conjunction with fibronectin and vitronectin, fibrin provides a provisional matrix for migration of cells involved in wound healing. Molecules from the fibrin matrix can modulate the response of fibroblasts to cytokines and the expression of integrins by endothelial cells.[2] Fibrin plays a crucial role in the recruitment of neutrophils and monocytes, endothelial cells, and fibroblasts to the wound site. In addition, the intrinsic characteristics of

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fibrin determine the cellular and humoral processes involved in epithelialization, granulation tissue formation, and angiogenesis.[2-4]

The plasma-derived homologous and autologous fibrin adhesives that mimic the last step of coagulation cascade are used in orthopedic, periodontal, oral, and maxillofacial surgeries to provide topical hemostasis, tissue sealing, and enhanced wound healing. Autologous fibrin adhesives are considered more advantageous than homologous types because they decrease the risk for transmission of viral diseases (eg, acquired immunodeficiency syndrome, hepatitis B).[4-6] In contrast, the low fibrinogen concentration renders autologous products less resistant to mechanical stresses such as pressure and tearing.[7] In addition, depending on production methods, rheologic characteristics of autologous fibrin adhesives are less reproducible,[5] their bovine thrombin content might lead to postoperative coagulopathies,[6] and the need for blood donation several days before surgery can be time-consuming.[7,8] In contrast to biodegradable and biocompatible plasma-derived adhesives, synthetic products (eg, cyanoacrylate derivatives) can lead to side effects such as inflammation, foreign body reactions, tissue necrosis, and scar formation.[6]

Some clinicians suggest that wound healing can be promoted through integration of growth factors (eg, platelet-derived growth factor [PDGF], transforming growth factor-β [TGFβ], fibroblast growth factor, vascular endothelial growth factor [VEGF], and epithelial growth factor) originating from α granules of platelets into autologous fibrin.[8-10] For this purpose, platelet concentrates, commonly referred to as platelet-rich plasma, have been introduced to replace fibrin adhesives.[11] The existence of native fibrinogen, fibronectin, factor XIII, high platelet and growth factor concentrations distinguishes platelet concentrates from fibrin adhesives.[7] However, the preparation of platelet concentrates can be time-consuming and less reproducible owing to the wide range of preparation protocols, kits, and centrifuges. In this sense, a distinction between different production methods and end-products cannot be validated.[11]

Most recently, Choukroun et al developed platelet-rich fibrin (PRF), an autologous fibrin product belonging to a new generation of platelet concentrates.[12] They claimed that PRF is a slowly and naturally polymerizing fibrin matrix in which growth factors (PDGF-$\beta\beta$, TGFβ-1, VEGF, and insulin-like growth factor-1), leukocytic cells, and their cytokines (interleukin [IL]-1β, IL-6, IL-4, and tumor necrosis factor-α) are enmeshed.[13,14] After the use of PRF as a membrane in conjunction with bone graft or alone as a fibrin matrix, the favorable effects of this agent on the soft tissue and bone healing has been demonstrated. According to reports, PRF can promote wound epithelialization, bone filling of residual cyst cavities, and bone maturation after sinus lifting operations.[15-17]

The stimulatory effects of fibrin sealants and platelet concentrates, alone or in combination with bone grafts, on soft tissue healing has been well documented. However, their direct effect on bone regeneration is controversial.[7] The effects of PRF on bone healing has been evaluated through re-entry, histologic and radiographic methods,[15-17] but not by bone scintigraphy. Bone scintigraphy is a very sensitive radionuclide imaging technique for detecting osteoblastic activity in the bony skeleton and the maxillofacial region.[18,19] Bone healing in extraction sockets in rats[20] and humans[21] has been studied by bone scintigraphy and the stimulation of osteoblastic activity in human extraction sockets filled with freeze-dried bone has been shown previously.[22]

In this study, the aim was to investigate the difference in the early bone healing process within extraction sockets, treated with PRF alone or not, of soft tissue impacted mandibular third molars by static-phase bone scintigraphy in postoperative week 4.

Patients and Methods

PATIENT SELECTION

The main criterion for inclusion of patients in the study was the presence of bilateral soft tissue impacted mandibular third molars in the vertical position. The reason for this criterion was to assure primary wound closure of extraction sockets after surgery. The presence of bilateral or unilateral impacted mandibular third molars that would necessitate bone removal during surgery was the main criterion for being excluded from the study, because the removal of bone during surgery would complicate the standardization of surgical procedures and observations on the physiologic bone healing process in extraction sockets.

From September 2008 to January 2009, 297 patients who were referred to the Department of Dentistry, Section of Oral and Maxillofacial Surgery, Gulhane Military Medical Academy, Haydarpasa Training Hospital (Istanbul, Turkey) with pain or discomfort in the mandibular third molar region were examined for their possible recruitment in the study. In accordance with the inclusion and exclusion criteria, 32 of 297 patients underwent further radiographic and oral examinations. Twenty systemically healthy patients were consecutively included in the study with an indication for surgical extraction of the bilateral mandibular third molars due to pericoronitis or prophylactic reasons (Fig 1).

All patients signed an informed consent form, prepared in accordance with the Declaration of Helsinki

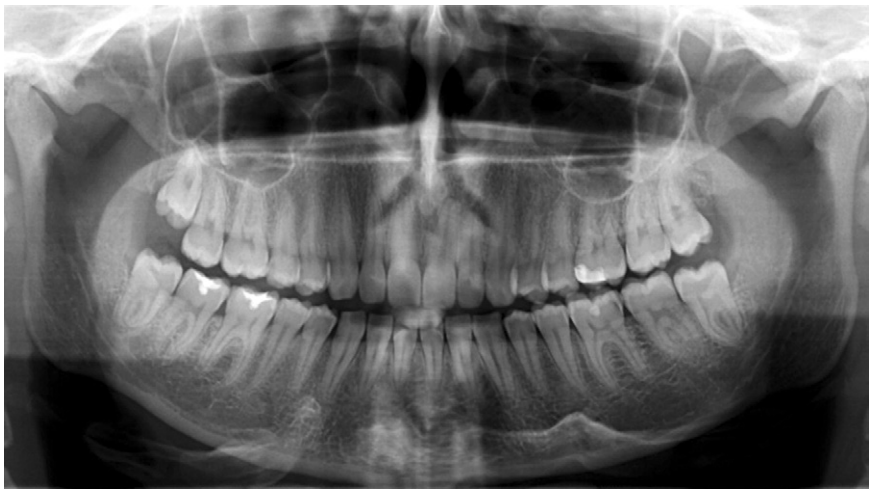

FIGURE 1. Panoramic radiograph of a patient with bilateral soft tissue impacted mandibular third molars in a vertical position.

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and reviewed and approved by the local ethics committee of Gulhane Military Medical Academy.

PREPARATION OF PLATELET-RICH FIBRIN

PRF was prepared according to the technique described by Choukroun et al.[12] Twenty minutes before starting surgery, 10 mL of venous blood was collected in a sterilized dry, neutral glass tube (16 × 100 mm; Isolab, Wertheim, Germany) without an anticoagulant. After immediate centrifugation (Universal 320, Hettich, Tuttlingen, Germany) at 400*g* (2,030 rpm) for 10 minutes, the platelet-poor plasma, which accumulated at the top, was discarded. PRF was dissected approximately 2 mm below its connection to the red corpuscle beneath to include remaining platelets, which have been proposed to localize below the junction between PRF and the red corpuscle[14] (Figs 2A, C, and D).

After completing the clinical work, PRF preparations were also obtained from 3 volunteering authors (B.G., L.P., and M.T.) for cytologic and ultrastructural evaluations. The samples taken from the upper, middle, and lower thirds of PRF were separately placed into tubes containing carbowax solution. The cytospin preparations from these samples were stained with Giemsa and the cytologic evaluation was carried out by light microscopy (E600; Nikon, Tokyo, Japan).

For scanning electron microscopic examination, PRF samples were cut into pieces approximately 3 × 4 mm and fixed in 2.5% phosphate buffered glutaraldehyde solution (0.1 mol/L, pH 7.4) for 2 hours. Subsequently, the samples were postfixed in 1% phosphate-buffered osmium tetroxide solution and passed through an increasing alcohol and amyl acetate series. After drying with a Bio-Rad critical-point dryer and gold coating (Bio-Rad SC 502; Pleasanton, CA), the samples were examined by a scanning electron microscope (JEOL 5,200 JSM; Tokyo, Japan).

SURGICAL PROCEDURE

To standardize the surgical operations, all patients were operated on by the same surgeon (B.G.). The mandibular third molars of which the extraction sockets would receive PRF treatment were selected randomly before surgery and these molars were operated on first. After regional anesthesia of the inferior alveolar nerve and infiltration anesthesia of the buccal mucosa on both sides of the mandible, soft tissue impacted right and left molars of the same patient were extracted in the same session. An incision starting from the trigonum retromolare was continued sulcularly through the buccal aspect of the second molar. No releasing incision was made; instead, the sulcular incision was extended to the distal aspect of the first molar when necessary. After elevation of the full-thickness envelope flap, the third molar was extracted and the soft tissue remnants were removed as required. After bleeding control, PRF was immediately administered into the socket (Fig 2B) and the wound was primarily closed with 3.0 silk sutures. Subsequently, the contralateral impacted molar was extracted by the same surgical procedure; however, PRF was not applied into this socket. In this manner, the non–PRF-treated extraction sockets constituted the controls and thus the patients served as their own controls. Because only the bilateral mandibular third molars similarly impacted in the vertical direction were selected for the study, there was no significant difference in the time and surgical trauma associated with surgical extractions.

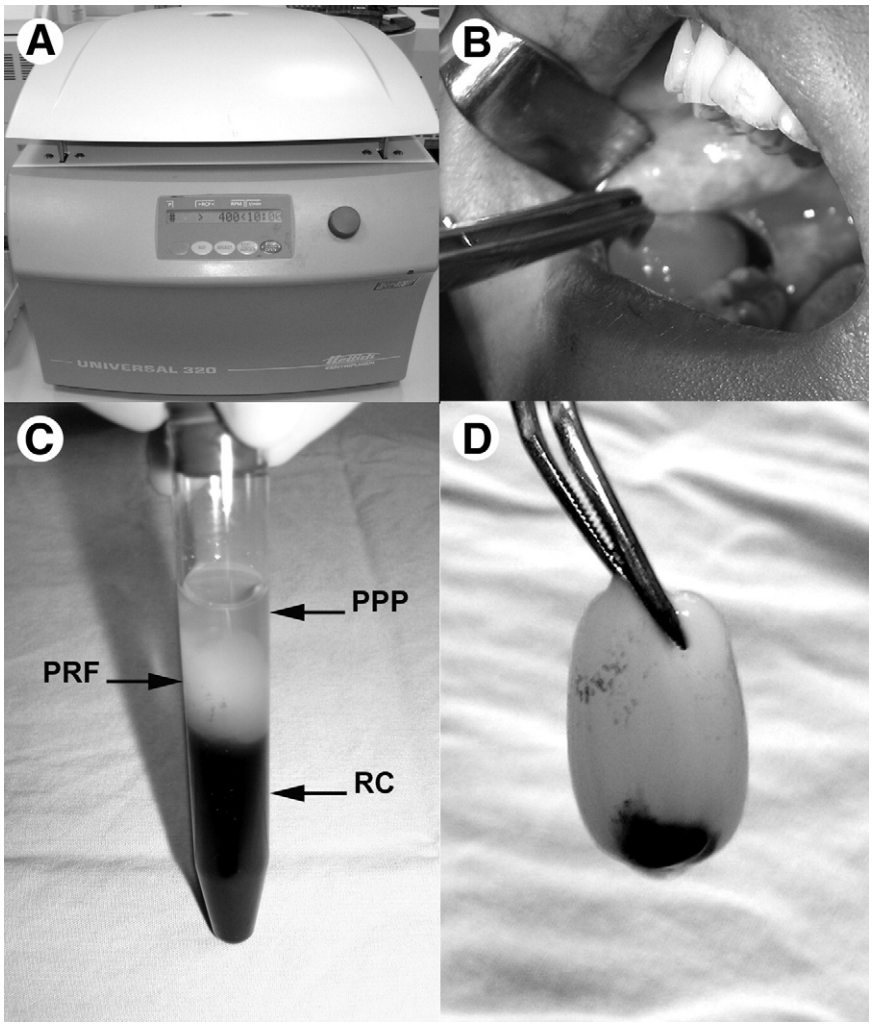

FIGURE 2. The laboratory centrifuge (*A*), application of PRF into an extraction socket (*B*), platelet-poor plasma (PPP), PRF, and red corpuscle (RC) (*C*), and the PRF ready to use (*D*).

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All patients were prescribed amoxicillin-clavulanate 1 g and naproxen sodium 550 mg twice daily for 5 days, and 0.12% chlorhexidine gluconate mouth rinse twice daily for 2 weeks. Nevertheless, the patients were instructed to begin mouth rinsing no sooner than 24 hours after surgery to prevent mobilization of the clot within PRF-treated extraction sockets. Sutures were removed 10 days after surgical operation.

SCINTIGRAPHIC STUDY

The early bone healing in PRF-treated and non–PRF-treated sockets was investigated by bone scintigraphy in postoperative week 4. Static oblique images of left and right extraction sockets on the mandible were obtained using a pinhole collimator 3 hours after intravenous injection of 555 MBq technetium-99m methylene diphosphonate. Regions of interest of equal size were drawn on both operation sites on the mandible to delineate the osteoblastic activity quantitatively. Another region of interest on the temporal bone of calvarium was drawn to define the reference activity of normal bone (Fig 3). In the scintigraphic images, the average increase of osteoblastic activity in PRF-treated and non–PRF-treated sockets to the reference site was calculated by mean pixel values obtained from the related sites. All scintigraphic analyses were made by the same nuclear medicine specialist (MU) who was unaware of which socket was treated with PRF.

STATISTICAL ANALYSIS

The Mann-Whitney *U* test was performed to determine scintigraphic differences in average osteoblastic activity between PRF-treated and non–PRF-treated sockets, using SPSS 15 (SPSS, Inc, Chicago, IL). The level of significance was set at $\alpha = 0.05$.

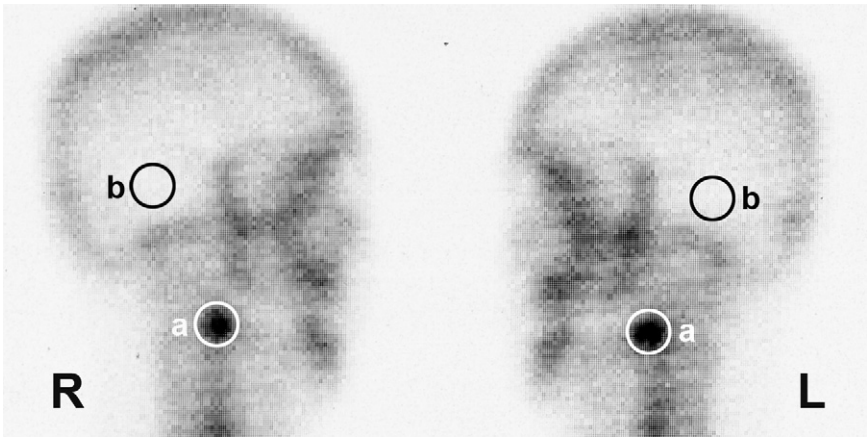

FIGURE 3. Regions of interest drawn on the scintigram to indicate the extraction socket region (*a*) and the temporal bone of calvarium (*b*). *R,* right side, PRF-treated site; *L,* left side, non–PRF-treated site.

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Results

SCINTIGRAPHIC FINDINGS

Of the 20 patients who were operated on, 1 patient was lost to follow-up, 1 female patient did not complete the study due to her pregnancy, and another patient developed postsurgical infection in a non–PRF-treated socket. Three patients were excluded from the study due to an unpredictable necessity for bone removal during surgical operation. Therefore, scintigraphic evaluations were performed in 14 patients (7 male and 7 female; mean age, 24.92 ± 4.69 years; all nonsmokers) 4 weeks after surgery. All the PRF-treated sockets healed uneventfully and postsurgical infection did not occur in any of these sites.

Scintigraphic analysis revealed that the averages of technetium-99m methylene diphosphonate uptake in PRF-treated and non–PRF-treated sockets were 4.544 ± 1.027 and 4.614 ± 1.021 fold of the normal bone metabolism in the reference region, respectively. The mean increase in bone agent accumulation between PRF-treated and non–PRF-treated sockets was statistically nonsignificant ($P = .818$). The mean ratio of technetium-99m methylene diphosphonate uptake in PRF-treated to non–PRF-treated sockets was 0.99 ± 0.12. The results demonstrated that the scintigraphically detectable early bone-formative changes in PRF-treated sockets was almost equal to that in non–PRF-treated sockets 4 weeks after surgery (Fig 4, Table 1).

LIGHT AND SCANNING-ELECTRON MICROSCOPIC FINDINGS FROM PLATELET-RICH FIBRIN SAMPLES

In the cytologic evaluation, the samples from the upper part of PRF were acellular and mainly composed of fibrin (Fig 5A). Cellular elements usually appeared in lower parts of the samples taken from the middle section of PRF, and these parts were hypocellular and had a fibrin-rich appearance. The samples from lowest third, where PRF was connected to the red corpuscle beneath, were hypercellular and had a fibrin-poor appearance. In these samples, cellular components comprised mainly polymorphonuclear neutrophils and lymphocytes, and erythrocyte silhouettes were also observed. However, platelets were rarely seen, most probably due to loss of their cellular integrity upon activation (Fig 5B).

Scanning electron microscopic micrographs of the outer surface and the inner surface of PRF are shown in Figure 6. The thick major fibers, thin minor threads, and platelet aggregates were observed in each part of the PRF. Abundant crystal-like structures were noticed on the outer surface of PRF. In addition, the major and minor threads of fibrin fibers were found to constitute a fibrin network. The crystalline particles were not seen in the inner surface of PRF; however,

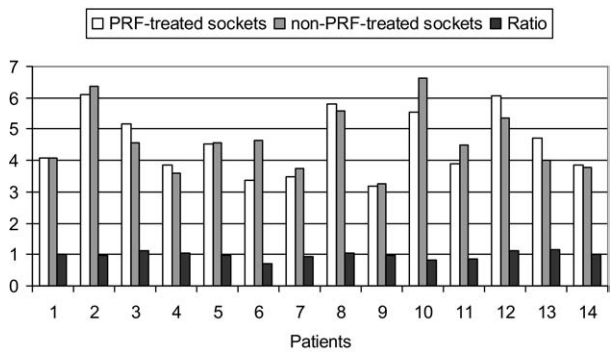

FIGURE 4. Increase in technetium-99m methylene diphosphonate uptake within PRF-treated and non–PRF-treated sockets compared with normal activity 4 weeks after surgery.

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Table 1. OSTEOBLASTIC ACTIVITY IN THIRD MOLAR EXTRACTION SOCKETS 4 WEEKS AFTER SURGERY

Patient No.	Gender	Age	PRF-Treated Sockets	Non–PRF-Treated Sockets	Ratio	Mandibular Jaw Side
1	F	22	4.061	4.065	0.999	Right
2	F	33	6.102	6.349	0.961	Right
3	M	19	5.165	4.561	1.132	Right
4	F	30	3.838	3.601	1.065	Left
5	F	26	4.52	4.564	0.99	Left
6	F	31	3.369	4.65	0.724	Right
7	F	19	3.477	3.742	0.929	Left
8	M	26	5.8	5.568	1.041	Right
9	M	31	3.186	3.24	0.983	Right
10	M	22	5.557	6.617	0.839	Left
11	F	20	3.901	4.503	0.866	Right
12	M	23	6.065	5.356	1.132	Right
13	M	23	4.702	4.012	1.171	Left
14	M	24	3.873	3.767	1.028	Left
Mean ± SD		24.92 ± 4.69	4.544 ± 1.027	4.614 ± 1.021	0.99 ± 0.12	

Abbreviations: F, female; M, male; PRF, platelet-rich fibrin.

In comparison with normal bone activity in the reference site, the increase of osteoblastic activity in PRF-treated and non–PRF-treated sockets 4 weeks after surgery (Mann-Whitney test).

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numerous platelet aggregates were present between the fibrin fibers.

Discussion

In this study, the application of PRF into soft tissue impacted mandibular third molar extraction sockets did not lead to scintigraphically detectable enhanced bone healing process 4 weeks after surgery. Bone scintigraphy was carried out to evaluate the difference of bone mineralization within PRF-treated and non–PRF-treated extraction sockets. The bone scintigraphic technique consists of detecting the uptake of technetium-99m–labeled diphosphonates in the mineral component of bone. Although bone scintigraphy is not an alternative to dental radiography, it can be used as an adjunct in diagnosis and treatment of oral diseases.[18,19] The accumulation of technetium-99m–labeled diphosphonates is remarkably correlated with the amount of mineralization by the activity of the osteoblastic cells. However, it has been reported that the uptake of bone agent does not necessarily reflect the number of existing osteoblasts in a given site.[23]

The physical half-life of technetium-99m is short (approximately 6 hours) and the emitted radiation during the bone scanning process is much lower than the pharmacologic levels that are toxic to human cells.[24] Because increased radionuclide uptake could indicate areas of new bone formation,[25] we assumed that bone scintigraphy would be a reliable method for investigation of osteoblastic activity within healing extraction sockets. Although its resolution and specificity are low, bone scintigraphy is a very sensitive method. An approximately 10% increase in osteoblas-

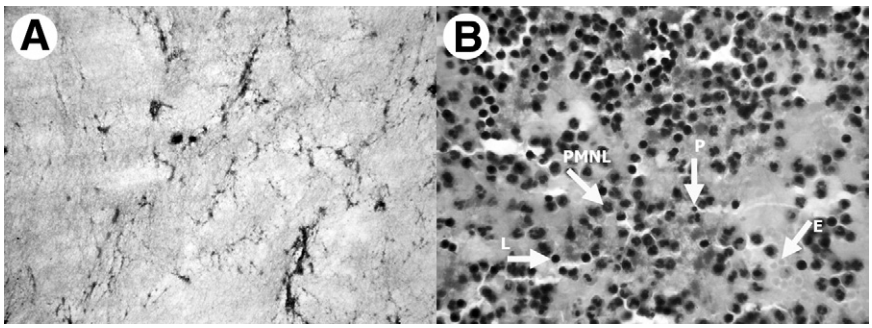

FIGURE 5. *A*, Sample from the upper parts of PRF, comprising completely fibrin and no cellular elements (Giemsa staining, magnification × 200). *B*, Sample from the lowest parts of PRF, comprising mainly inflammatory cells and rare platelets (Giemsa staining, magnification × 400). E, erythrocyte silhouette; L, lymphocyte; P, platelet; PMNL, polymorphonuclear leukocyte.

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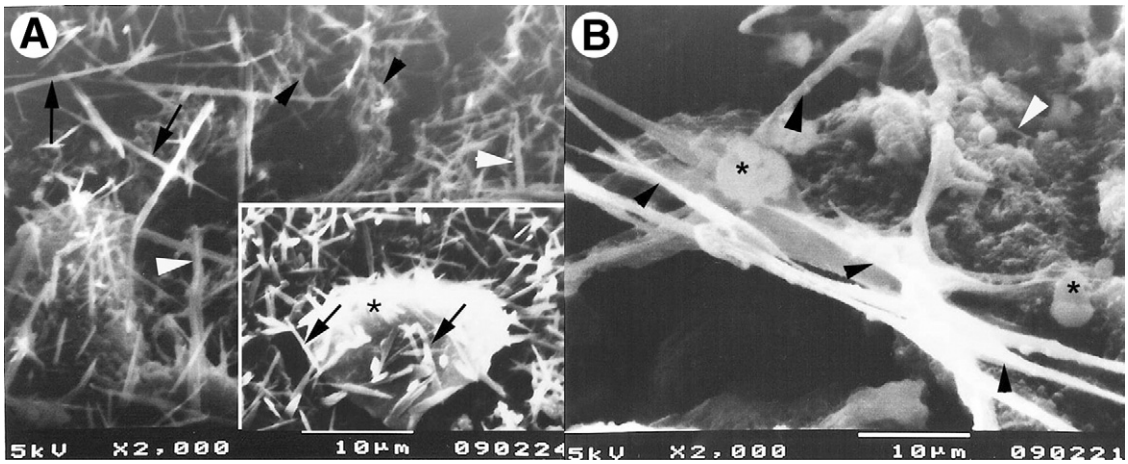

FIGURE 6. *A,* Scanning electron microscopic micrograph showing abundant crystals (*arrows*) on the outer surface of PRF. Thick major fibers (*white arrowheads*) and thin minor threads (*black arrowheads*) of fibrin are seen in a network. *Inset,* Crystal-like particles are present on the surface of a platelet aggregate (*). *B,* Scanning electron microscopic micrograph showing thick major fibrin fibers (*black arrowheads*) and thin minor thread fibers (*white arrowheads*) in the inner surface of PRF. Thick major fibrin fibers with a rough appearance (*black arrowheads*) and thin minor fibrin fibers with a smooth appearance (*black arrowheads*) are seen in a network. Platelet aggregates (*) are also seen between the major and minor fibrin fibers.

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tic activity above normal can be determined by scintigrams, which cannot be seen in conventional radiographs.[26] In addition, abnormal changes in bony structures due to pathologic dental, and periodontal conditions can be detected by scintigrams.[27,28] Unlike radiographic and histologic methods, bone scintigraphic images can reveal early bone mineralization during the healing process in extraction sockets.[20]

In a pilot study,[29] it has been shown that bone scintigraphy can be used to investigate the bone mineralization around dental implants. After extraction in rats, positive bone scans indicating increased osteoblastic activity within sockets were seen in 4 to 16 days, and the signs of increased radionuclide uptake were evident in 4 to 42 days.[20] In a human study,[22] bone scintigraphic images showed that a freeze-dried heterograft led to accelerated bone healing within third molar extraction sockets in postsurgical week 4 compared with week 1. Gürbüzer et al[21] also reported significantly increased bone agent accumulation in soft tissue impacted mandibular third molar extraction sockets, treated with platelet-rich plasma or not, 4 weeks after surgery when compared with postsurgical week 1.

Osteoblasts, the cells responsible for bone formation, root from undifferentiated mesenchymal progenitor cells, or osteoprogenitor cells.[30] After tooth extraction in dogs, woven bone formation has been observed on day 14 and the socket completely filled with woven bone on day 30.[31,32] Consequently, in the present study, scintigraphic evaluation 4 weeks after the extraction of mandibular third molars could correspond to a time frame when the healing socket harbored a large amount of woven bone.

Fibrin adhesives, comprising purified fibrinogen and thrombin, are used to seal tissues, achieve hemostasis, and promote wound healing. However, these products may produce a relatively dense architecture, leading to impairment of angiogenesis and overall wound healing. This type of fibrin matrix does not contain growth factors and therefore it cannot bring about active recruitment of undifferentiated cells, which are essential for tissue regeneration, into its scaffolding.[1] In contrast, platelet concentrates enable delivery of growth factors in increased amounts to surgical sites for tissue regeneration.[9] Platelet concentrates also demonstrate bioactive fibrin matrix characteristics upon gelation induced by addition of thrombin and $CaCl_2$. Their effect on tissue regeneration has been attributed to their ability to attract fibroblasts and undifferentiated cells into the matrix in which cell division is triggered through binding of growth factors to cell membranes that in turn leads to intracellular signal transduction.[1]

It has been stated that Choukroun's PRF,[12] an easily and quickly prepared second generation of platelet concentrates and an autologous product with cicatricial properties, is the latest development in bioactive surgical additives for regulation of inflammation and enhanced healing. It has been put forward that PRF would promote wound healing and serve as an immune node that regulates inflammation and provide wound protection due to the presence of growth factors (PDGF-$\beta\beta$, TGFβ-1, VEGF, and insulin-like growth factor-1)[14] and inflammatory cytokines (IL-1β, IL-6, IL-4, and tumor necrosis factor-α).[13] In contrast to the preparation techniques of other platelet concentrates, the venous blood is collected in a dry glass

or glass-coated plastic tubes without anticoagulant while preparing PRF. Upon contact with the glass surface and during the centrifugation, the venous blood coagulates immediately and natural fibrin polymerization occurs without addition of thrombin and $CaCl_2$.[12,14] It has also been theoretically proposed that, because of the relatively slow polymerization process, the homogenous 3-dimensional organization of PRF leads to formation of equilateral junctions, instead of bilateral junctions associated with thick fibrin polymers constituting a rigid fibrin network, between fibrin fibrillae. This enables entrapment of growth factors from platelets and cytokines from leukocytes into fibrin matrix.[14] Most recently, PRF has been shown to be able to release the growth factors TGFβ-1, PDGF-$\alpha\beta$ and VEGF, and thrombospondin-1, an important coagulation glycoprotein of the cellular matrix, for 7 days after its preparation.[33]

With respect to soft tissue healing, PRF promotes the proliferation of human tympanic keratinocytes and preadipocytes under in vitro conditions.[34] It has been stated that PRF could have a beneficial role in cicatrization and consolidation of an adipocyte graft used in facial esthetic lipostructure operations.[35] In 1 case, PRF accelerated wound epithelialization and enabled suture removal in 48 hours when used as a barrier membrane to cover the bone-graft-filled extraction sockets.[16]

In the present study, neither short-term nor long-term observation was made to compare the soft tissue healing between PRF-treated and non–PRF-treated extraction sockets. However, our preliminary results from another pilot study demonstrated that PRF, upon its placement under a coronally repositioned periodontal flap, could be as efficient as a connective tissue graft from the palate in root coverage of the teeth with Miller Class I or II gingival recession defect (unpublished data).

With regard to bone healing, in 1 case, PRF induced complete bone filling of a residual cystic cavity in 2 months 2 weeks, a much shorter period than 6 to 12 months of physiologic healing.[16] In a case series on maxillary sinus lifting operations, 3 cases were treated with PRF and freeze-dried bone allograft mixture and 6 cases with freeze-dried bone allograft alone. Histomorphometric results showed that PRF and allograft mixture accelerated bone regeneration, allowing implant placement in 4 months after maxillary sinus lifting procedure. Furthermore, the amount of newly formed bone was equivalent to that achieved with an allograft alone 8 months after surgery.[15] Diss et al[17] reported promising results after placing PRF instead of bone graft under the sinus membrane during a closed-sinus lifting technique and demonstrated that an average of 3.2 mm bone gain could be obtained in the sinus after 1-year follow-up.

Our light microscopic findings from the PRF samples seemed to be in accordance with some of the above mentioned characteristics of PRF. In these samples, abundant fibrin and inflammatory cells, mainly neutrophils and leukocytes, were observed. In addition, scanning electron microscopic analysis revealed the presence of platelet aggregates among major and minor fibrin threads. These important evidences could provide a basis for considering that PRF application into extraction sockets would promote wound healing and bone regeneration. However, the PRF-treated extraction sockets did not demonstrate increased osteoblastic activity in postoperative week 4. In a similar study, Gürbüzer et al[21] reported that platelet-rich plasma might not bring about promoted activity of osteoblasts in soft tissue impacted mandibular third molar sockets in week 1 or 4 after extraction.

There might be some possible explanations why PRF failed to increase osteoblast activity in extraction sockets. Although beneficial effects of fibrin-based products on soft tissue healing are well documented, their direct impact on osseous cells is still controversial.[7,16] Although growth factors such as PDGF, TGFβ, and insulin-like growth factor stimulate proliferation and chemotaxis of osteoblasts,[36,37] the effects of different combinations of growth factors and their actions may change depending on cell population and culture conditions.[7] In addition, the impact of some growth factor combinations may be synergistic[36,38] and/or antagonistic.[39,40] It has also been mentioned that the growth factor and inflammatory cytokine contents of PRF play a secondary role in its bioactivity, and PRF does not appear to enhance proliferation of osseous cells, but instead to provide graft vascularization through angiogenesis.[15] It should also be noted that, although PRF is considered a naturally polymerizing physiologic product, the fibrin formation occurs under in vitro conditions during PRF preparation. Namely, the glass lining vicinity of a centrifuge tube may not actually provide a biological environment during fibrin polymerization. In this context, whether the presence of crystalline particles, which were suspected to be silica, on the outer surface PRF alters bone healing or not seems to be controversial. It has been suggested that the use of blood collection tubes containing silica activators should be avoided for the preparation of PRF.[41] However, it has been demonstrated that the silica content of PRF exerts no cytotoxic effect on cultured gingival fibroblasts, preadipocytes, keratinocytes, and osteoblasts and even leads to improved mitochondrial respiration of preadipocytes and keratinocytes. Furthermore, silica is of importance in obtaining PRF because it acts as a clot activator upon direct contact of ve-

nous blood with the glass lining of the centrifuge tube.[42]

In conclusion, according to the results obtained from the present study, PRF does not seem to increase scintigraphically detectable enhanced bone healing within the extraction sockets of soft tissue impacted mandibular third molars 4 weeks after surgery. Further studies are needed to evaluate the impact of PRF or its combinations with bone grafts on the remodeling phase of the bone healing process. Further, whether the presence of crystal-like particles on the outer surface of PRF alters bone healing or not should be investigated further.

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