

Three-Dimensional Architecture and Cell Composition of a Choukroun's Platelet-Rich Fibrin Clot and Membrane

David M. Dohan Ehrenfest,*† Marco Del Corso,† Antoine Diss,† Jaafar Mouhyi,*‡ and Jean-Baptiste Charrier†§

Background: Platelet-rich fibrin (PRF; Choukroun's technique) is a second-generation platelet concentrate for surgical use. This easy protocol allows the production of leukocyte- and platelet-rich fibrin clots and membranes starting from 10-ml blood samples. The purposes of this study were to determine the cell composition and three-dimensional organization of this autologous biomaterial and to evaluate the influence of different collection tubes (dry glass or glass-coated plastic tubes) and compression procedures (forcible or soft) on the final PRF-membrane architecture.

Methods: After centrifugation, blood analyses were performed on the residual waste plasmatic layers after collecting PRF clots. The PRF clots and membranes were processed for examination by light microscopy and scanning electron microscopy.

Results: Approximately 97% of the platelets and >50% of the leukocytes were concentrated in the PRF clot and showed a specific three-dimensional distribution, depending on the centrifugation forces. Platelets and fibrin formed large clusters of coagulation in the first millimeters of the membrane beyond the red blood cell base. The fibrin network was very mature and dense. Moreover, there was no significant difference in the PRF architecture between groups using the different tested collection tubes and compression techniques, even if these two parameters could have influenced the growth factor content and biologic matrix properties.

Conclusions: The PRF protocol concentrated most platelets and leukocytes from a blood harvest into a single autologous fibrin biomaterial. This protocol offers reproducible results as long as the main production principles are respected. *J Periodontol* 2010;81:546-555.

KEY WORDS

Blood platelets; fibrin; leukocytes; scanning electron microscopy.

The use of fibrin glue¹ or platelet concentrate (often named platelet-rich plasma [PRP])^{2,3} during surgical procedures is a current treatment concept used to accelerate wound healing and tissue maturation.⁴ Choukroun's platelet-rich fibrin (PRF), a second generation platelet concentrate,⁵ was defined as an autologous leukocyte- and platelet-rich fibrin biomaterial.⁶⁻⁸ PRF was developed in France by Choukroun et al.⁹ in 2001. Unlike other platelet concentrates, this technique does not require any anticoagulants or bovine thrombin or any other gelling agent. This open protocol is very simple and inexpensive: blood is collected in dry glass tubes or glass-coated plastic tubes and immediately softly centrifuged. Three layers are formed: a red blood cell (RBC) base at the bottom, acellular plasma (platelet-poor plasma [PPP]) as a supernatant, and a PRF clot in the middle (Fig. 1A). This clot combines many healing and immunity promoters present in the initial blood harvest. It can be used directly as a clot or after compression as a strong membrane (Fig. 1B).

Potential clinical indications of PRF in oral and maxillofacial surgery are numerous, including, for example, the improvement of soft tissue healing¹⁰⁻¹² and bone graft protection and remodeling.¹³⁻¹⁵ It is also useful for Schneiderian membrane protection¹⁶ or as a sole osteoconductive filling material during a sinus-lift

* Department of Biomaterials, Institute for Clinical Sciences, The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden.

† The LoB5 Foundation for Research, Paris, France.

‡ Department of Periodontology, University of Southern California, Los Angeles, CA.

§ Department of Ear-Nose-Throat, Head, and Neck Surgery, AP-HP Bicêtre Hospital, Paris Sud University, Le Kremlin Bicêtre, France.

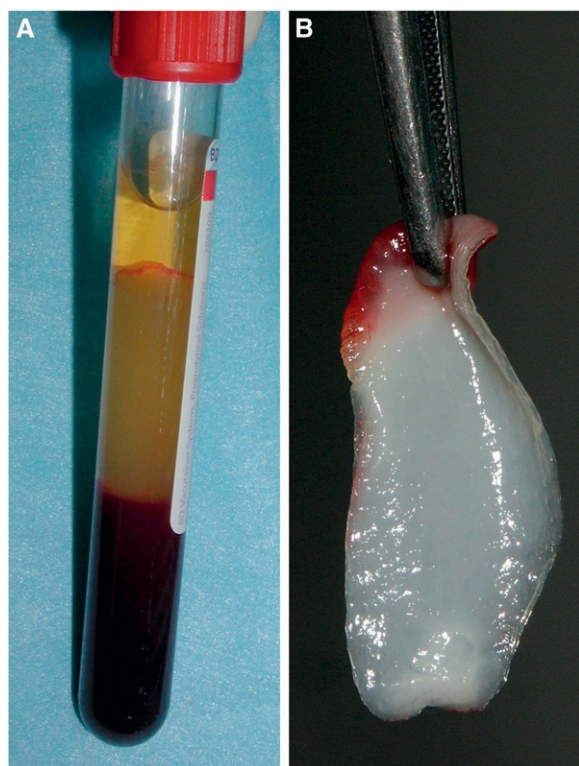


Figure 1.

A) The PRF clot was produced in either dry glass tubes or glass-coated plastic tubes. **B)** The clots were collected and changed into membranes. This autologous biomaterial that was built with fibrin, platelets, and leukocytes showed a specific architecture.

procedure.¹⁷ In plastic surgery, PRF clots are often directly used to fill cavities¹⁸ or mixed with an adipocyte graft during a lipostructure.¹⁹ Membranes could also be useful for small otologic surgery.²⁰

Although platelet growth factors play an important role in the biology of PRF, the fibrin architecture^{21,22} and leukocyte content are two key parameters.^{6,23} However, most studies⁶ on platelet concentrates only highlight the platelet and growth factor concentrations, rarely assess the leukocyte content, and almost never analyze the fibrin structure of each product. Nevertheless, the fibrin architecture directly influences the biology of all fibrin-based biomaterials.²⁴⁻²⁶ The PRF clot is yielded by a natural polymerization process during centrifugation, and its natural fibrin architecture seems responsible for a slow release of growth factors and matrix glycoproteins during ≥ 7 days.²⁷ Such a slow release is impossible to point out in most PRP techniques because of their brutal platelet activation, immediate release of growth factors, and very light fibrin network produced to sustain the concentrate injection.²⁸

In the field of hematologic sciences, some authors²⁹ examined the clot structure in the whole blood

and in transfusion PRP. Using scanning electron microscopy (SEM), they²⁹ observed structural changes of the forming clot related to different thromboelastography variables. However, to our knowledge, no study has been conducted for a structural analysis of platelet gels for surgical use like PRP or PRF.

The main objective of this study was to perform a detailed examination of the composition and architecture of the Choukroun's PRF clot (particularly the distribution of the platelets and leukocytes within the fibrin clot) using hematologic counts, photonic microscopy, and SEM. The secondary objective of this work was to point out the structural and morphologic differences between PRFs commonly produced with two different kinds of collection tubes (dry glass tubes and glass-coated plastic tubes) and using two different methods for the compression of the PRF clot into the membrane (forcibly or softly).

MATERIALS AND METHODS

Preparation of PRF

Blood samples were collected at the Jules Ferry Institute (Cannes, France) in May 2005 from 10 healthy male volunteers (age range: 20 to 55 years; mean age: 35 years) with no history of aspirin intake or other medications that might interfere with coagulation over the previous 2 weeks. The volunteers provided oral informed consent and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. For each volunteer, the blood sample (11 tubes of 9 ml each) was obtained from an antecubital vein (in two stages: five tubes harvested on the right arm and six tubes harvested on the left arm). One tube contained an anticoagulant for current whole blood analysis (control group). The 10 other tubes were taken without anticoagulant for PRF production (test groups): five were taken in dry glass tubes^{||} (series 1) and five in glass-coated plastic tubes[¶] (series 2).³⁰

The blood collection was performed quickly, and the tubes were immediately centrifuged at 3,000 rpm for 10 minutes with a specific table centrifuge[#] at room temperature. After centrifugation, the PRF clot was removed from the tube using sterile tweezers, separated from the RBC base using scissors, and placed in a sterile metal cup. Each PRF clot started to release its serum (PRF-clot exudate) and was ready for compression into the membrane. In each series (dry glass or glass-coated plastic tubes), two clots were emptied from their serum by compressing them with a metal spoon (forcible exudate extraction; method 1), and two clots were left aside to release their serum slowly during 20 minutes into a metal

^{||} Process protocol, Process, Nice, France.

[¶] Process protocol, Process.

[#] Process protocol, Process.

cup (soft exudate extraction; method 2). Each PRF clot exudate was transferred back to its original preparation tube for hematologic analysis. Finally, in each series, the fifth clot was processed for SEM evaluation and fixed in 2.5% glutaraldehyde directly with its serum content without compression.

All membranes underwent a final compression on sterile woven gauzes to flush out a maximum of fluids; the dehydration facilitated fixation and processing for histology. In each series, the four emptied membranes were sent for histologic examination; in each method group (forcible or soft exudate extraction), one membrane was analyzed using light microscopy, and one membrane was analyzed using SEM.

In summary, for each volunteer, 11 blood samples were collected: one sample for direct blood analyses (control group) and 10 samples for PRF production (test groups). For the test groups, two PRF clots (from different series) were gathered for SEM analyses, and eight membranes were collected, each membrane being different and defined by a series (dry glass or glass-coated plastic tubes), a method (forcible or soft exudate extraction), and a microscopy analysis (light or SEM).

Leukocyte and Platelet Counts

For each volunteer, three types of harvesting were analyzed using an automatized counter** at the hematologic laboratory (Jules Ferry Institute) after isotonic dilution:

- whole blood with anticoagulant (control group): one sample per volunteer;
- RBC base put back into solution using the PPP supernatant and the PRF clot exudate obtained by strong compression (method 1): four samples per volunteer (two in dry glass tubes and two in glass-coated plastic tubes);
- RBC base put back into solution using the PPP supernatant and the PRF clot exudate obtained without compression (method 2): four samples per volunteer (two in dry glass tubes and two in glass-coated plastic tubes).

The hemogram was performed by an impedance measurement. The leukocyte formula was evaluated by flow cytometry. The mean platelet volume was also measured.

Histologic Procedures for Light-Microscopy Evaluation

PRF membranes were dehydrated in increasing gradients of alcohol (70%, 95%, and 100%) and placed in toluene before paraffin inclusion. After complete dehydration, the membrane was ~0.5 mm thick. For each PRF membrane, a series of 20 successive 7- μ m sections was performed according to the long axis of the membrane; i.e., ~140 μ m of the mem-

brane thickness could be analyzed in a longitudinal and reliable manner. These 20 sections were stained using two different specific protocols: 10 sections with hemalaun and eosin and 10 sections with Masson's trichrome (modified by Goldner) (Fig. 2).

Histologic Procedures for SEM Evaluation

A morphologic evaluation of the PRF clot and membrane was conducted with a scanning electron microscope.^{††} The PRF clot and membrane were fixed in 2.5% glutaraldehyde for 1 hour and treated for desiccation. To observe the fibrin matrix, the PRF clot was cut longitudinally in its center, and the membrane was cut at each end (Fig. 3). Specimens were sputter-coated with 20 nm gold and subsequently examined in a scanning electron microscope. Photographs were taken at 15 to 25 kV using 15 to 3,500 magnifications. SEM was used to complete the observations of the photonic microscopy concerning the identification of the cell bodies trapped in the matrix (leukocytes, platelets, and RBCs) and to analyze the overall architecture of the fibrin network.

Analyses of Platelet and Leukocyte Distribution

Each series of stained longitudinal sections observed by light microscopy was analyzed by counting the violet spots in the different areas of the membrane. These spots represented platelet aggregates and leukocytes. The distinction between platelet aggregates and leukocytes was only possible by morphologic examination in the microscope and, thus, was very operator-dependent. The detailed examination of these successive sections allowed us to obtain an approximate charting of the platelet/leukocyte colonies clustered in the PRF network.

Statistical Analyses

This study was mainly descriptive. However, results of blood countings were analyzed statistically. Results obtained in dry glass tubes (series 1) and glass-coated plastic tubes (series 2) were compared to each other globally and within each method group and finally compared to the control group. Moreover, in each series, the two methods were compared to one another. Statistical analyses were performed by one-way analysis of variance, and when there was a significant difference, the Tukey test was used. *P* values <0.05 were considered statistically significant.

RESULTS

Platelet and Leukocyte Counts

Blood analyses are usually difficult to interpret because of large interindividual variations. In the present study, it was not possible to demonstrate either a significant difference (*P* >0.05) in residual blood

** HMX Beckman Coulter Automat, Beckman Coulter, Fullerton, CA.

†† JEOL JSM-5310 LV, Jeol, Tokyo, Japan.

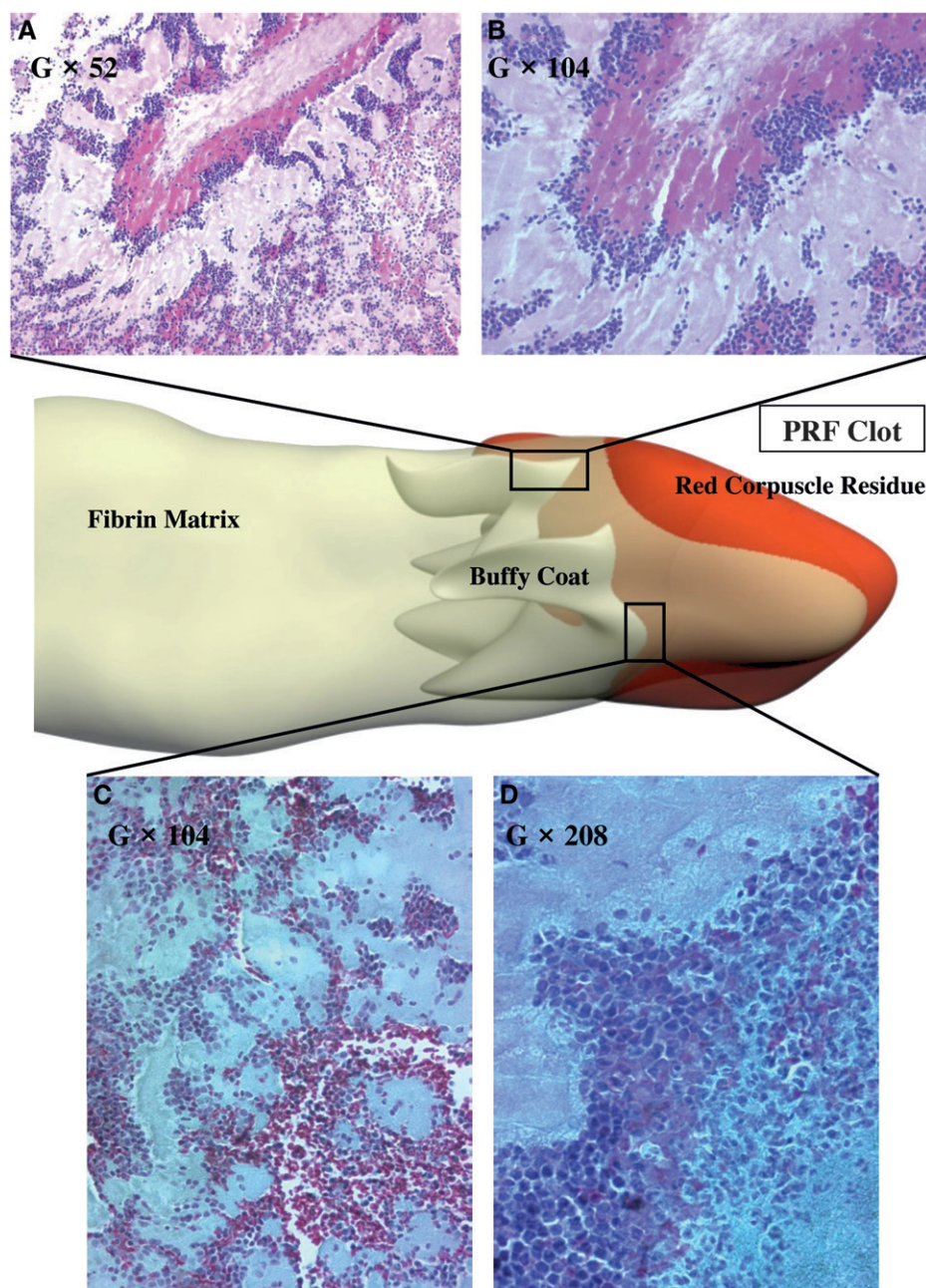


Figure 2.

Light-microscopy analysis of the PRF clots. **A and B)** The hemalaun and eosin stainings were not sufficient to correctly distinguish the various cell bodies trapped in the fibrin matrix. **C and D)** Using Masson's trichrome staining, it was possible to more easily separate platelet aggregates and leukocytes (dark blue) from RBCs (red). Magnifications (G) are indicated in each panel.

contents between method groups (forcible or soft exudate extraction) or any difference between the tube series (dry glass tubes and glass-coated plastic tubes), globally or within each method group (Tables 1 and 2).

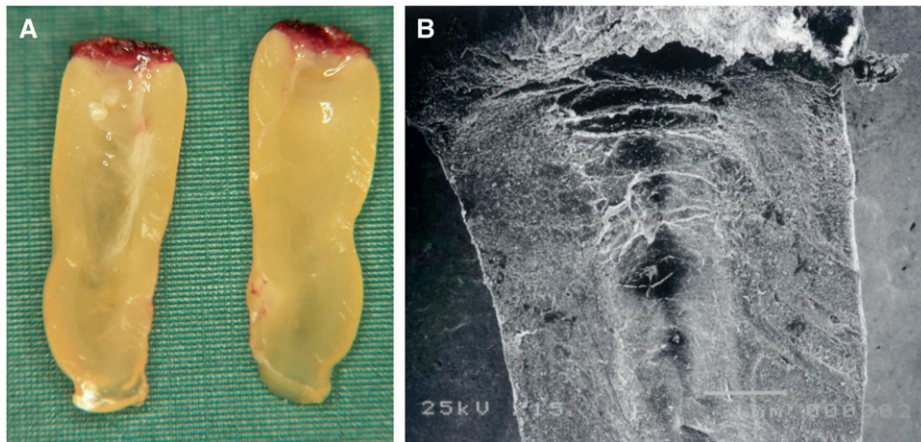
On the other hand, we noted significant differences between the test groups and the control group ($P < 0.01$). The concentration effect due to membrane

extraction from the PRF tube was taken into account: the fibrin volume that was removed from the tube was about 1 ml, indicating that cell concentrations might be higher in the test groups compared to the control group. This was what we observed for the RBCs.

Inversely, almost all platelets (>97%) were absent from the test group tubes after PRF-membrane extraction. In the test groups, the leukocyte levels dropped significantly compared to the control group ($P < 0.01$); taking into account the concentration effect due to PRF collection, more than the half of the leukocytes seemed to have disappeared (Table 1). The missing platelets and leukocytes remained trapped in the PRF matrix when using the described collection method with scissors. The absence of a difference between the two method groups ($P > 0.05$) seems to indicate that the brutal compression of the PRF clot did not influence the possible release of cell bodies trapped within the fibrin matrix.

Moreover, the leukocyte formula was again significantly different between the control group and test groups (Table 2). In the test groups, lymphocyte proportions were significantly lower, and neutrophil-leukocyte proportions were significantly higher, than in the control group ($P < 0.01$). This result indicated that lymphocytes were more likely to be trapped in the PRF matrix than the other leukocytes, which tended to be eliminated with the residual RBC base.

Finally, the mean platelet volume decreased significantly between the control and test groups ($P < 0.01$): it dropped from $9 \mu\text{m}^3$ (range: 8 to $11 \mu\text{m}^3$) in whole blood to $4.7 \mu\text{m}^3$ (4.5 to $5.1 \mu\text{m}^3$) in the test groups (for the residual platelets that remained in the tube after PRF-clot collection). This phenomenon would normally be due to the increase of the plasmatic osmolarity in the tube after the activation cascades of coagulation.



and a red portion located at the end of the clot (full of RBCs). Between these two areas, a whitish layer called the “buffy coat” (similar to the whitish layer in PRP technologies) can be observed with the naked eye and concentrates cell corpuscles requiring identification (Fig. 2).

With the hemalaun and eosin staining, the fibrin matrix appeared homogeneous in light pink, and platelet aggregates were dark blue/violet (Fig. 2A). RBCs and leukocyte cytoplasm were not easily detectable: they were darker pink. The leukocyte nuclei were stained in dark blue with the hemalaun, but they looked like platelet aggregates.

Therefore, it was very difficult to distinguish them from the platelet aggregates (Fig. 2B).

With Masson’s trichrome (modified by Goldner) staining, platelet aggregates were still dark blue, but

Figure 3.

A) Preparation of the PRF clot for SEM analysis. **B)** The first pictures at low magnification ($\times 15$) demonstrated fibrin-clot shrinkage due to fixation and the artifact concavity in the center of the PRF clot (after sectioning in two parts along its long axis).

Light-Microscopy Study

The PRF clot can be described as composed of two main parts observable with the naked eye (Fig. 1B): a fibrin yellow portion, constituting the main body,

Table 1.

Number of Leukocytes, RBCs, and Platelets in the Whole Blood (control group) and Residual RBC Base After Collection of the PRF Membrane (test groups)

Sample	Leukocytes/ μ l		RBCs/ μ l		Platelets/ μ l	
	Average	Range	Average	Range	Average	Range
Control	6,900	6,100 to 7,800	5.19 (10^6)	5.01 to 5.52 (10^6)	2.66 (10^5)	2.18 to 3.09 (10^5)
Series 1	3,500	3,000 to 3,800	5.89 (10^6)	5.75 to 6.08 (10^6)	6,000	4,000 to 8,000
Series 2	3,600	3,300 to 4,000	5.84 (10^6)	5.78 to 5.91 (10^6)	7,000	6,000 to 9,000

Table 2.

Leukocyte Formula Established in the Whole Blood (control group) and Residual Base After Collection of the PRF Membrane (test groups)

Cell Type	Total Blood (%)		Series 1 (%)		Series 2 (%)	
	Average	Range	Average	Range	Average	Range
Neutrophil leukocytes	51.8	49.7 to 53.2	72.1	66.1 to 77.1	66.4	60.9 to 71.4
Eosinophil leukocytes	2.9	2.3 to 3.1	6.1	3.4 to 8.8	5.1	3.9 to 6.1
Basophil leukocytes	0.5	0.3 to 0.8	0.1	0.0 to 0.3	0.4	0.1 to 0.9
Lymphocytes	37.7	35.1 to 39.2	17.5	15.0 to 20.4	24.8	21.4 to 28.0
Monocytes	7.1	6.9 to 7.6	4.2	1.1 to 7.6	3.3	2.5 to 5.0
Total (average) per μ l	6,900 (100%)		3,500 (100%)		3,600 (100%)	

RBCs were stained in bright red and became easily identifiable. Leukocytes were still difficult to separate within the stained platelet aggregates. Nevertheless, the borderline between RBCs and platelet aggregates/leukocytes was very clear (Fig. 2C). In the transition layer, platelet aggregates, leukocytes, and RBCs were mixed together (Fig. 2D).

SEM Evaluation

The observation of the PRF clot at a low magnification ($\times 15$) showed that the clot presented a concavity in its middle part (Fig. 3). This was caused by matrix shrinkage due to fixation (artifact). In the red part of the PRF clot, RBCs were enmeshed in the fibrin network. RBC shapes were normal, but the fibrin-strand network appeared immature (Fig. 4). At the junction between the red and yellow parts of the PRF clot (the buffy coat area), the SEM examination showed leukocytes that clearly appeared as spherical structures with

irregular surface (Fig. 5A). Most of them seemed quite small (between 6 to 8 μm in diameter) and, thus, could have been mainly lymphocytes, as pointed out in the previously investigated leukocyte counts. Platelet aggregates appeared very clearly along the fibrin strands (Fig. 5B).

Beyond the buffy coat base, we distinguished two different areas: the first area was composed of thick fibrin strands and a few scattered RBCs (probably from contamination during clot handling). The fibrin network appeared to be mature (Fig. 6). The second area corresponded to the platelet veins observed by light microscopy. This area contained platelets and fibrin that formed large and dense clusters due to extensive aggregation and clotting (Fig. 7). This aggregate formed a solid and thick mesh. Therefore, platelets seemed to be highly activated during the PRF-preparation protocol.

When observed at a low magnification, the PRF-membrane surface showed the print of the gauze threads. Fibrin is a physiologic glue; therefore, the compression of the fibrin clot into a membrane provided a very compact matrix. To observe the fibrin, we cut one end of the membrane (Fig. 8A). At a higher magnification, fibrin was clearly organized in parallel strands that appeared very thick and dense (Fig. 8B). It was impossible to distinguish cellular elements trapped within this condensed network.

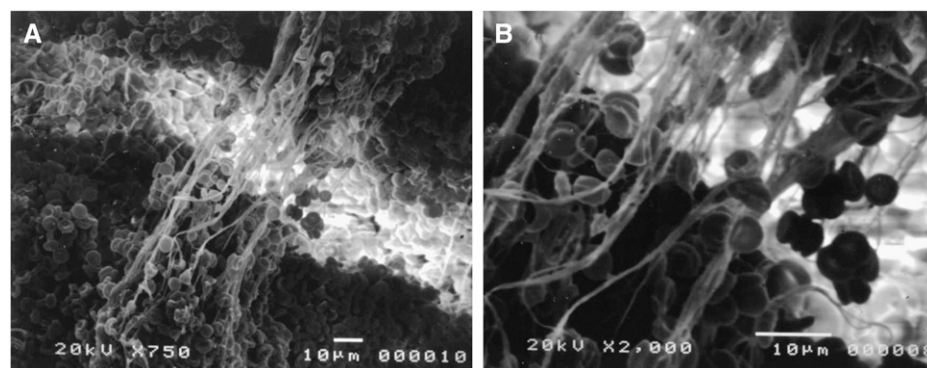


Figure 4.

A and B) The red area of the PRF clot contained many RBCs trapped within an immature and very loose fibrin matrix (SEM; original magnification: A, $\times 750$; B, $\times 2,000$).

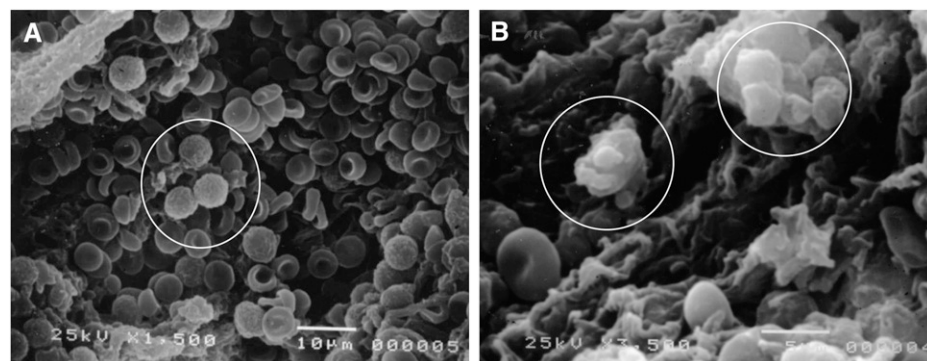


Figure 5.

A) At the borderline between the red area and yellow clot, clusters of RBCs and leukocytes were found with SEM. Leukocytes appeared like spherical structures with an irregular surface (white circles). Most of them seemed quite small (between 6 to 8 μm in diameter) and, thus, could have been mainly lymphocytes. **B)** Platelets were often enmeshed in the fibrin network but sometimes appeared as aggregates (white circles) that were easily identified. (Original magnification: A, $\times 1,500$; B, $\times 3,500$).

Distribution Analyses

The highest platelet/leukocyte density was found in the first millimeter of the yellow clot, just after the red clot. The platelet/leukocyte distribution became increasingly scarce as we moved away from the clot end, and we did not find anymore platelets or leukocytes beyond the first half of the yellow clot.

In the first 2 mm located beyond the yellow/red border, the platelet/leukocyte distribution was homogeneous throughout the clot width. The more we moved away from the yellow/red border, the more platelets (and leukocytes) were grouped according to central or

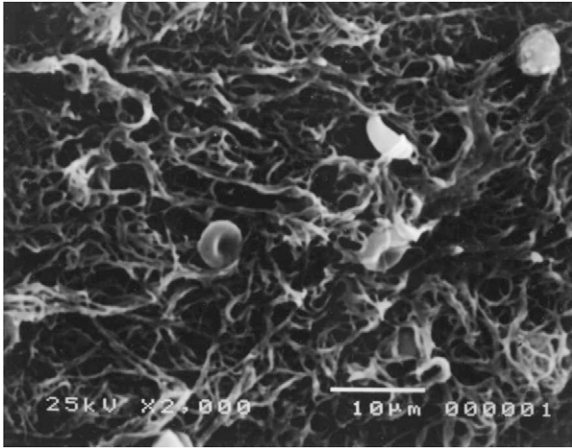


Figure 6. SEM examination of the fibrin yellow clot revealed a dense and mature fibrin matrix with a very low quantity of identifiable bodies (RBCs, leukocytes, or platelet aggregates) trapped inside (original magnification $\times 2,000$).

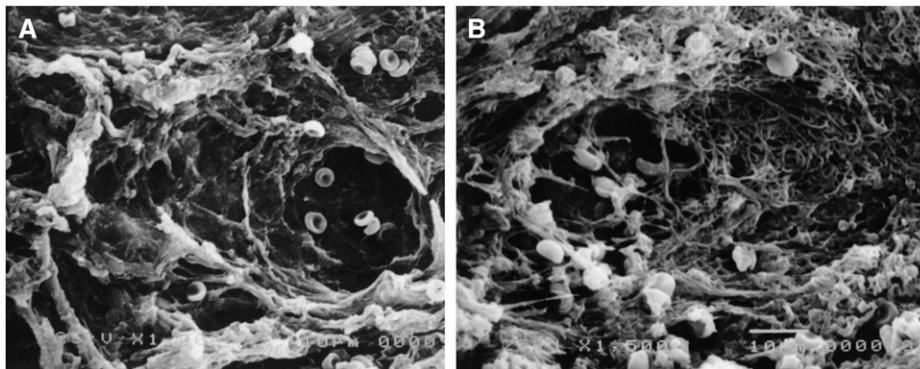


Figure 7. **A and B)** In the whitish veins within the yellow clot, the platelet aggregates were closely merged into a dense and mature fibrin matrix (SEM; original magnification A, $\times 1,000$; B, $\times 1,500$).

centrifugal platelet-concentration veins. These veins offered high platelet/leukocyte densities within a cell-free matrix. This corresponded to what we might observe on a PRF clot with the naked eye: the whitish veins seemed to concentrate on the external surface of the clot.

We observed that this architecture was similar from one clot to another, independently from the patients, collection tubes, or the method of PRF-clot compression. Thus, the Choukroun's PRF protocol was mainly defined by a mechanical concept where platelets and leukocytes were projected within the fibrin clot in formation and seemed very stable, even with slightly modified production variables.

The reconstructed image illustrated in Figure 2 is a stylish extrapolation of the observed platelet/leukocyte distribution.

DISCUSSION

The purpose of this study is to assess platelet and leukocyte amounts and distributions within the PRF clot and membrane, the influence of clot compression on the fibrin network, and the influence of slightly different preparation tubes on the final product.

Platelet counts clearly showed that there was hardly any platelet left within the RBC layer, the PPP, or the exudate provided by compressing the PRF clot. Thus, most of the platelets originating from the whole-blood sample were collected in the PRF membranes. This result was expected because of the close relationship between fibrin and platelets after clotting, and seemed to confirm the first studies^{7,27} on concentrations of platelet growth factor in PRF membranes. Leukocyte counts confirmed that more than half of the leukocytes were trapped in PRF membranes, and small lymphocytes seemed mainly collected, as confirmed by the SEM examination. These leukocytes were already pointed out in cell cultures with PRF and did not seem to be damaged during the PRF preparation.²³

This result has a strong clinical impact because the quantity of leukocytes implanted within each membrane is considerable, and small lymphocytes are particularly efficient in the regulation of inflammatory reactions. Moreover, the cell composition of PRF implies that this biomaterial is a blood-derived living tissue and must be handled carefully to keep its cellular content alive and stable.¹²

The photonic microscopy study showed that the platelet and leukocyte distribution within the clot was not uniform. Platelets and leukocytes were concentrated in an intermediate layer located between RBCs and the fibrin clot and represent a macroscopic buffy coat on the PRF-clot surface. Therefore, when harvesting clots for surgical use, practitioners should collect this intermediate whitish layer. Thus, it is necessary to preserve a small RBC layer at the PRF clot end to collect as many platelets and leukocytes as possible. This part of the procedure is done with scissors and remains operator-dependent, and, thus, an accurate knowledge of the clot architecture is required for adequate PRF preparation. This knowledge is also very important for a reasoned clinical use of PRF, because the exact same biologic and clinical effects from the two extremities of a PRF membrane cannot be expected. Thus, the surgical techniques should be carefully

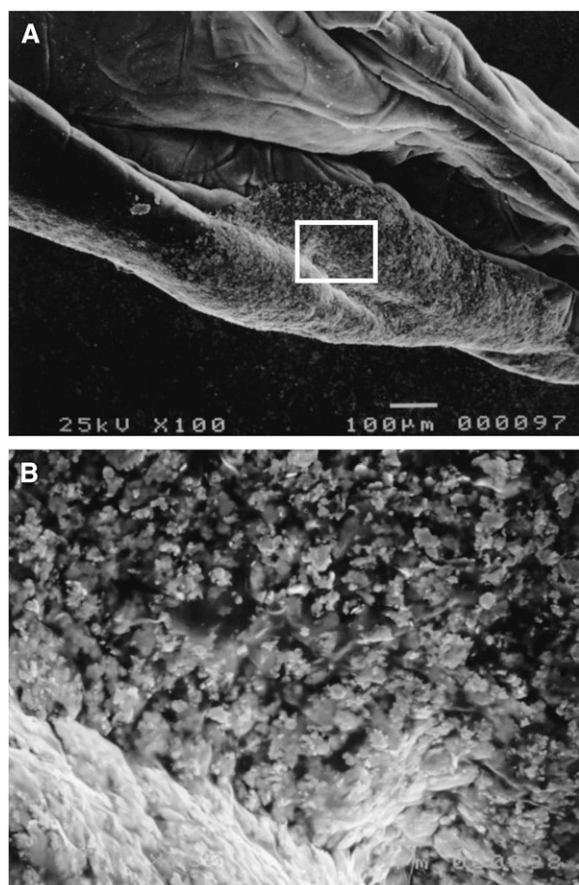


Figure 8.

A) SEM examination of a PRF-membrane surface at a low magnification showed traces of woven gauzes that were used for the final clot compression into a membrane before fixation for histologic analysis. This compression allowed for a very compact fibrin matrix to be obtained. **B)** Enlargement of the rectangle in panel A. Transversal sections of the membrane were observed and clearly showed that the fibrin was organized in very thick parallel bundles. (Original magnification: A, $\times 100$; B, $\times 750$.)

adapted to the membrane composition for some delicate applications such as periodontal surgery.¹²

The SEM evaluation showed that RBCs were widely predominant in the red part of the PRF clot, and the leukocytes were distributed at the junction between the red and yellow parts of the clot. Only a few RBCs were identified in the rest of the clot, which were probably artifacts due to clot handling. Platelet morphology is totally modified by aggregation and clotting processes.³¹ Therefore, it was not possible to identify non-activated platelets (discoid bodies) but rather only a large aggregate of platelet-fibrin polymers. Kawasaki et al.²⁹ obtained the same results with thrombin-activated PRP and showed the contribution of platelets to the structural rigidity of the fibrin network.

The PRF-membrane examination showed the effect of compression on the fibrin matrix: the fibrin strands were condensed and stuck to each other.

PRF membranes were denser than a blood clot or even a common PRP. The effects of this condensation on the fibrin resorption time and healing properties should be investigated. When PRF membranes are used for wound closure in oral surgery, the mean resorption time of these membranes is quite long,¹⁰ following a slow remodeling process of the fibrin matrix into a healing tissue.

Finally, despite the analytic method that we selected, this study remained mainly descriptive. During clot handling, the RBC aggregates often broke, which rendered the microscopy examination of the RBC base (located 3 mm beyond the yellow/red border) very difficult. Moreover, when using the cell counters, the platelets activated during centrifugation may be difficult to detect in the RBC base placed back in suspension in the PPP and PRF exudate. According to the manufacturer of the counting automat, the activated platelets were detectable as long as they were not totally disrupted. It is difficult to assess how many platelets were totally disrupted during the PRF preparation.

Despite these limits, this preliminary study allowed us to define the main cell and matrix characteristics of the PRF clot and membrane. A clear definition of the PRF composition was an essential prerequisite to guarantee the reproducibility of the technique and to allow future investigations on a clearly identified and reproducible standardized protocol. The Choukroun's PRF concept is founded on a mechanical concentration process during clot formation and leads to a specific clot architecture that is very different from a simple fibrin bulk. Not respecting the original protocol might lead to PRF-like clots with inadequate fibrin and platelet and leukocyte concentrations, jeopardize the intrinsic incorporation of growth factors within the fibrin network,²⁷ and yield variations in the clinical results.¹²

CONCLUSIONS

The PRF clot contained most of the platelets and leukocytes from a 10-ml blood sample, and their distribution followed a three-dimensional pattern yielded by the centrifugation process. The correct knowledge of the PRF architecture is very important for a reasoned use of PRF clots and membranes in various clinical situations. This study showed that the type of tested tube (dry glass or glass-coated plastic tubes) and the compression process of the clot (forcible or soft) did not seem to influence the architecture of this autologous biomaterial. Nevertheless, these two parameters could influence the growth-factor content and the matrix properties of the product and should be analyzed carefully.

ACKNOWLEDGMENTS

This work was partially supported by a grant from the LoB5 Foundation for Research. The authors declare no competing financial interests related to this study.

REFERENCES

- Gibble JW, Ness PM. Fibrin glue: The perfect operative sealant? *Transfusion* 1990;30:741-747.
- Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; 85:638-646.
- Dohan DM, Choukroun J. PRP, cPRP, PRF, PRG, PRGF, FC... How to find your way in the jungle of platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;103:305-306.
- Man D, Plosker H, Winland-Brown JE. The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery. *Plast Reconstr Surg* 2001;107:229-237; discussion 238-229.
- Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:e37-e44.
- Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol* 2009;27: 158-167.
- Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006; 101:e45-e50.
- Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:e51-e55.
- Choukroun J, Adda F, Schoeffler C, Vervelle A. An opportunity in perio-implantology: The PRF (in French). *Implantodontie* 2001;42:55-62.
- Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:e56-e60.
- Saadoun AP, Touati B. Soft tissue recession around implants: Is it still unavoidable? – Part II. *Pract Proced Aesthet Dent* 2007;19:81-87, quiz 88.
- Del Corso M, Sammartino G, Dohan Ehrenfest DM. Choukroun's platelet-rich fibrin membranes in periodontal surgery: Understanding the biomaterial or believing in the magic of growth factors? (letter to the editor) *J Periodontol* 2009;80:1694-1697; author reply 1697-1699.
- Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:299-303.
- Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM. The relevance of Choukroun's platelet rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part I: A new grafting protocol. *Implant Dent* 2009;18:102-111.
- Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM. The relevance of Choukroun's platelet-rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part II: Implant surgery, prosthodontics and survival. *Implant Dent* 2009;18:220-229.
- Diss A, Dohan DM, Mouhyi J, Mahler P. Osteotome sinus floor elevation using Choukroun's platelet-rich fibrin as grafting material: A 1-year prospective pilot study with microthreaded implants. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;105:572-579.
- Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD, Dohan Ehrenfest DM. Sinus floor augmentation with simultaneous implant placement using Choukroun's platelet-rich fibrin as the sole grafting material: A radiologic and histologic study at 6 months. *J Periodontol* 2009; 80:2056-2064.
- Charrier JB, Monteil JP, Albert S, Collon S, Bobin S, Dohan Ehrenfest DM. Relevance of Choukroun's platelet-rich fibrin (PRF) and SMAS flap in primary reconstruction after superficial or subtotal parotidectomy in patients with focal pleiomorphic adenoma: A new technique. *Rev Laryngol Otol Rhinol (Bord)* 2008; 129:313-318.
- Braccini F, Dohan DM. The relevance of Choukroun's platelet rich fibrin (PRF) during facial aesthetic liposuction (Coleman's technique): Preliminary results (in French). *Rev Laryngol Otol Rhinol (Bord)* 2007; 128:255-260.
- Choukroun JI, Braccini F, Diss A, Giordano G, Doglioli P, Dohan DM. Influence of platelet rich fibrin (PRF) on proliferation of human preadipocytes and tympanic keratinocytes: A new opportunity in facial liposuction (Coleman's technique) and tympanoplasty? *Rev Laryngol Otol Rhinol (Bord)* 2007;128:27-32.
- Clark RA. Fibrin and wound healing. *Ann N Y Acad Sci* 2001;936:355-367.
- van Hinsbergh VW, Collen A, Koolwijk P. Role of fibrin matrix in angiogenesis. *Ann N Y Acad Sci* 2001;936: 426-437.
- Dohan Ehrenfest DM, Diss A, Odin G, Doglioli P, Hippolyte MP, Charrier JB. In vitro effects of Choukroun's PRF (platelet-rich fibrin) on human gingival fibroblasts, dermal prekeratinocytes, preadipocytes and maxillofacial osteoblasts in primary cultures. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; 108:341-352.
- Collen A, Koolwijk P, Kroon M, van Hinsbergh VW. Influence of fibrin structure on the formation and maintenance of capillary-like tubules by human microvascular endothelial cells. *Angiogenesis* 1998;2: 153-165.
- Mosesson MW, Siebenlist KR, Meh DA. The structure and biological features of fibrinogen and fibrin. *Ann N Y Acad Sci* 2001;936:11-30.
- Buchta C, Hedrich HC, Macher M, Hocker P, Redl H. Biochemical characterization of autologous fibrin sealants produced by CryoSeal and Vivostat in comparison to the homologous fibrin sealant product Tissucol/Tisseel. *Biomaterials* 2005;26:6233-6241.
- Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammartino G. Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): A gold standard to achieve for all surgical platelet concentrates technologies. *Growth Factors* 2009;27:63-69.
- He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and

- differentiation of rat osteoblasts in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;108:707-713.
29. Kawasaki J, Katori N, Kodaka M, Miyao H, Tanaka KA. Electron microscopic evaluations of clot morphology during thrombelastography. *Anesth Analg* 2004;99:1440-1444.
30. Dohan DM, Del Corso M, Charrier JB. Cytotoxicity analyses of Choukroun's platelet-rich fibrin (PRF) on a wide range of human cells: The answer to a commercial controversy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;103:587-593.
31. Zilla P, Groscurth P, Rhyner K, von Felten A. Surface morphology of human platelets during in vitro aggregation. *Scand J Haematol* 1984;33:440-447.

Correspondence: Dr. David M. Dohan Ehrenfest, Department of Biomaterials, Institute for Clinical Sciences, The Sahlgrenska Academy at University of Gothenburg, Medicinargatan 8B, 41390 Gothenburg, Sweden. E-mail: lob5@mac.com.

Submitted September 11, 2009; accepted for publication November 22, 2009.