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Basics of Platelet-Rich Fibrin Therapy

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Effective Date: 04/01/2017 Expiration Date: 04/01/2020

Learning Objectives: After reading this article the individual will learn: (1) the history and current status of platelet-rich fibrin (PRF), and (2) clinical uses for PRF in dentistry.

About the Authors

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Disclosure: Dr. Miron reports no disclosures.

Dr. Bishara has a practice focused on but not limited to implants, bone grafting material regeneration, and tissue regeneration in Bowmanville, ON, Canada. He is an active member of the International Association of Dental Research and collaborates with members of the Miron Lab, with a focus on tissue regeneration utilizing PRF. He can be reached at bbishara6@gmail.com.

Disclosure: Dr. Bishara reports no disclosures.

Dr. Choukroun is both a trained anesthesiologist and pain management specialist. His research findings led to the development of PRF, which pioneered the field more than 15 years ago as autologous platelet concentrates that could be utilized without anticoagulants. He is currently the president of Syfac, which holds one of the world's largest congresses on growth factors and platelet concentrates. He works as chief of staff in his Pain Therapy Center in Nice, France, and lectures internationally on PRF. He can be reached at joseph@a-prf.com.

Disclosure: Dr. Choukroun is a shareholder of the process of PRF.

Wound healing is a complex biological process with 4 overlapping phases including hemostasis, inflammation, proliferation, and remodeling.[1-3] One of the key players during these phases has been the accumulation of platelets, cells that have been shown to be important regulators of hemostasis through vascular and fibrin clot formation.[4] It was proposed in the 1990s that platelet concentrates could be utilized and centrifuged to reach supraphysiological doses to achieve wound healing and tissue regeneration by facilitating angiogenesis. During the past decade, many advancements have been made with respect to the development of a *platelet-rich fibrin* (PRF). This article discusses how further advancements in PRF therapy have provided optimal wound healing at low cost in various indications in regenerative dentistry.

BRIEF HISTORY OF PLATELET CONCENTRATES

Recently, the use of platelet concentrates has gained tremendous momentum as a regenerative autologous source of growth factors utilized in various fields of medicine. It was originally proposed that concentrated platelets derived from autologous sources could be collected in plasma solutions later to be utilized in surgical sites with the potential to release 6 to 8 times supraphysiological doses of growth factors responsible for promoting local healing.[5,6] Further work by Jameson [7] and Marx[8] led to the popular working name *platelet-rich plasma* (PRP). The goal of PRP was to collect the largest and highest quantities of growth factors from platelets to be later utilized for regenerative purposes. The PRP protcol required more than 30 minutes of centrifugation cycles and the use of anticoagulants to prevent clotting. The final composition of PRP contains more than 95% platelets, known cells responsible for the active secretion of growth factors involved in initiating wound healing of various cell types including osteoblasts, epithelial cells, and connective tissue cells.[7,8]

One of the reported limitations to the PRP technique, apart from its longer centrifugation protocols, was the fact that it included the additional use of bovine thrombin or calcium chloride ($CaCl_2$) in addition to coagulation factors. These drastically reduce the healing process during the regenerative phase by preventing coagulation and fibrin clot formation. The entire protocol was technique sensitive, with several phases lasting sometimes upwards of one hour, making it inefficient for everyday dental practice. These limitations later led to the emergence of a second generation of platelet concentrates that takes advantage of the fact that without anticoagulants, a fibrin matrix that incorporates the full set of growth factors trapped within its matrix and slowly released throughout time could be achieved.[9]

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From PRP to PRF

Research pioneered by Dr. Joseph Choukroun in the early 2000s led to the development of a second-generation platelet concentrate without utilizing anticoagulation factors. A platelet concentrate lacking coagulation factors could be harvested from the upper layer of centrifugation tubes following single centrifugation cycles of 12 minutes at 2,700 rpm (750 g). This formulation was termed *PRF*, owing to the fact that it contained a fibrin matrix following centrifugation. PRF (leukocyte-PRF, or L-PRF) additionally contains white blood cells (WBCs), the necessary cells involved in the wound healing process by improving defense immunity and secreting a large quantity of growth factors. As depicted in Figure 1, macrophages and leukocytes are among the 3 key cell types found in PRF derived from the myeloid lineage (WBCs) and secrete a wide range of growth factors including transforming growth factor-beta, platelet-derived growth factor, and vascular endothelial growth factor. These cells, in combination with neutrophils and platelets, are the main players in tissue wound healing and together are able to further enhance new blood vessel formation (angiogenesis), which subsequently leads to new bone and tissue formation.[10]

The following 3 components are essential to improve tissue repair: (1) a 3-D matrix capable of supporting tissue ingrowth; (2) locally harvested cells capable of influencing tissue growth; and (3) bioactive growth factors capable of enhancing cell recruitment and differentiation within the biomaterial surface. With respect to PRF, all 3 of these properties are met whereby (1) fibrin serves as the scaffold surface material; (2) cells including leukocytes, macrophages, neutrophils, and platelets attract and recruit future regenerative cells to the defect sites; and (3) fibrin serves as a reservoir of growth factors that may be released during 10 to 14 days.[11]

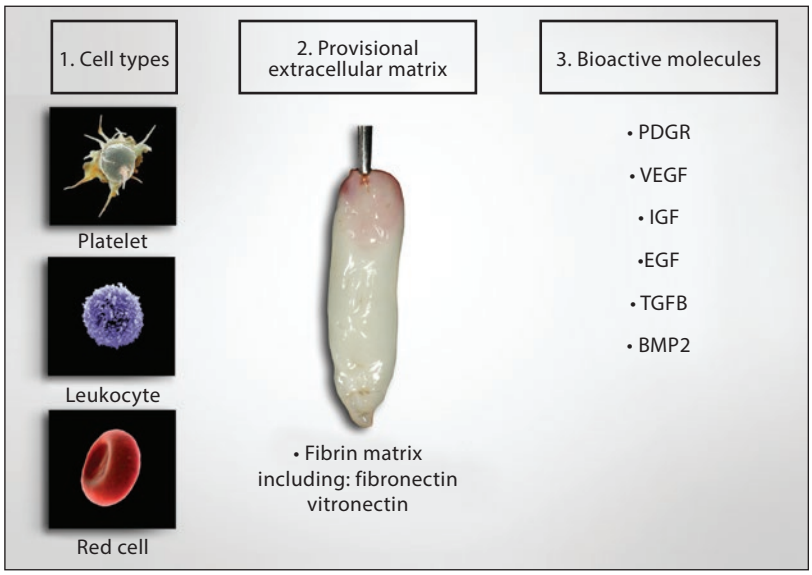

Figure 1. Natural components of platelet-rich fibrin (PRF) include **(1)** cell types: platelets, leukocytes, and red blood cells; **(2)** a provisional extracellular matrix 3-D scaffold fabricated from autologous fibrin, including fibronectin and vitronectin; **(3)** a wide array of more than 100 bioactive molecules, including most notably platelet-derived growth factor (PDGF), vascular endothelial GF (VEGF), insulin-like GF (IGF), epidermal GF (EGF), transforming growth factor-beta (TGF-ß), and bone morphogenetic protein-2 (BMP2). (*Reprinted with permission from Miron et al, 2016.*)

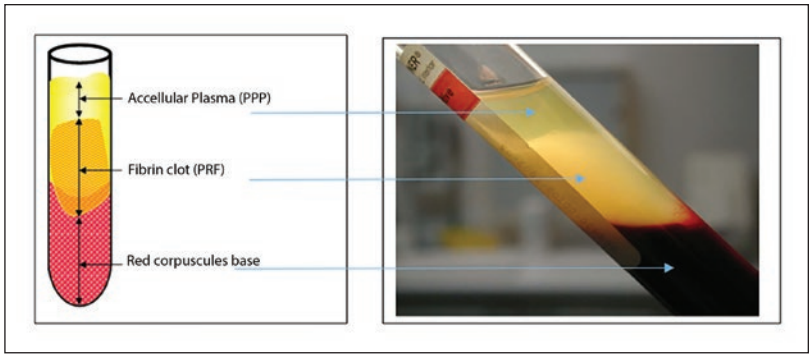

Figure 2. Fibrin clot in the tube after centrifugation.

PRF: A Natural Fibrin Matrix and Its Biological Properties

While PRF was first developed by Choukroun et al in 2001,[12] the lack of an anticoagulant meant that the fibrin clot begins to form during the centrifugation process. When centrifugation tubes are removed, a fibrin clot can be observed in the upper layer of tubes as depicted in Figure 2. This technology requires a centrifuge and a collection system present within the office, and since anticoagulants are not utilized, clotting forms rapidly. Therefore, centrifugation must take place within seconds after blood harvesting. The original PRF protocol was first established with a very simple technique: a blood sample was taken without anticoagulant in 10-mL tubes that was immediately centrifuged at 750 g for 12 minutes. The absence of anticoagulant implies the activation in a few minutes of most platelets of the blood sample in contact with the tube walls and the release of the coagulation cascades. Fibrinogen is initially concentrated in the upper layer of the tube, before the circulating thrombin transforms it into fibrin. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom of the tube and the acellular plasma at the top (platelet-poor plasma).

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Without anticoagulants, the blood samples start to coagulate almost immediately upon contact with the tube glass, and it takes a minimum of a few minutes of centrifugation to concentrate fibrinogen in the middle and upper part of the tube. Quick handling is the only way to obtain a clinically usable PRF matrix. If the duration required to collect blood and launch centrifugation is overly long, failure will occur. By driving out the fluids trapped in the fibrin matrix, practitioners will obtain very resistant autologous fibrin membranes.

Advanced Platelet Rich Fibrin (A-PRF)

The impact of WBCs on vascularization and bone formation led to modifications in the centrifugation speeds and times, due to the excessively high g-force. Due to high spin cycles, the protocols were modified accordingly to prevent cell loss within the PRF matrix. These new cycles, termed *A-PRF*, reduce the rpm to 1,300 for 8 minutes to maintain a higher amount of WBCs in the fibrin matrix and special glass tubes that are designed to induce a more rapid clotting. After the centrifugation, the tubes are removed and placed in their holders and left for 5 minutes to induce fibrin clot formation. This new fibrin clot is richer in WBCs, and recent research by the authors' group has shown that a higher release of growth factors from A-PRF also improves collagen matrix synthesis and recruitment of progenitor cells.[13]

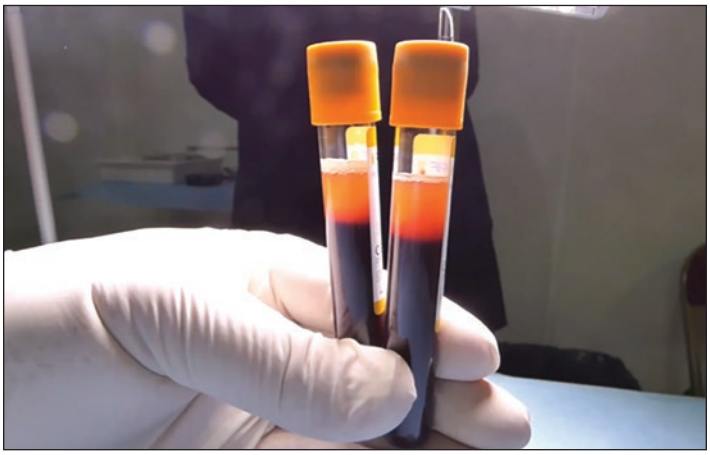

Figure 3. The newer formulation of injectable PRF (i-PRF) is a liquid formulation of PRF found in the top one-mL layer of centrifugation tubes following a 700-rpm spin for 3 minutes. This liquid can be collected in a syringe and reinjected into defect sites or mixed with biomaterials to improve their bioactive properties.

Injectable Platelet Rich Fibrin (i-PRF)

With the same concept of non-additive platelet derivatives, i-PRF was developed to fulfill the goal of acting as a regenerative agent that could be delivered in liquid formulation by drawing blood rapidly in a specific centrifugation tube at a very low speed of 700 rpm (60 g) for a short centrifugation time (3 minutes). The objective was

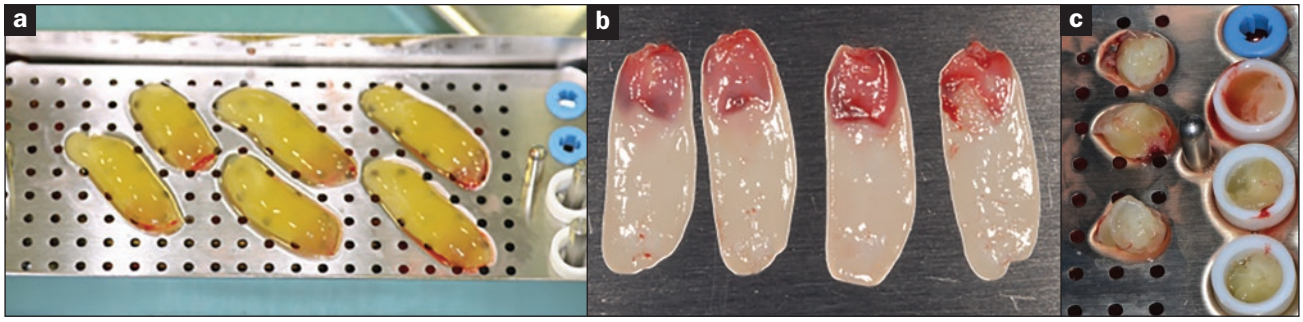

Figure 4. PRF clots formed to either make membranes or PRF plugs.

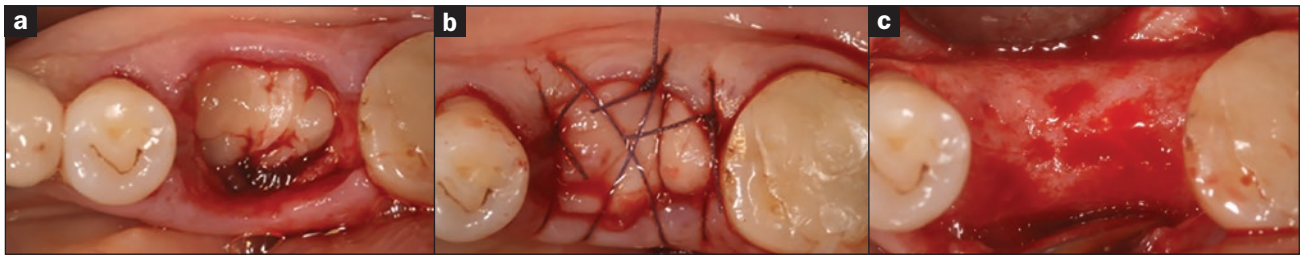

Figure 5. One of the main uses of PRF has been for the management of extraction socket healing. This is a demonstration of an extraction socket filled with multiple PRF plugs utilized alone. After 3 months of healing, substantial bone regeneration is observed without use of a bone grafting material.

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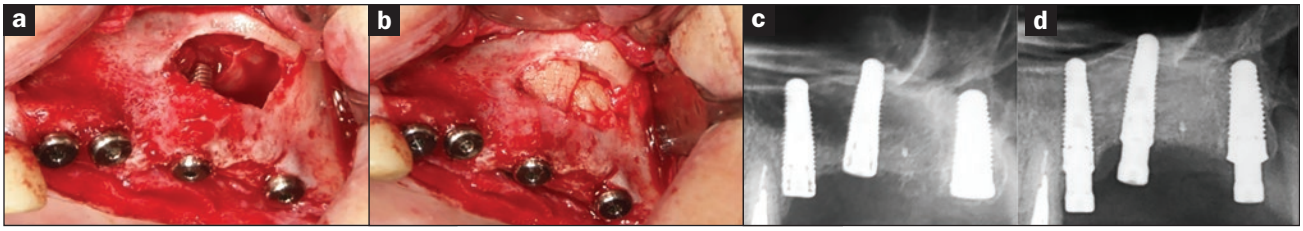

Figure 6. (a to c) Lateral window sinus augmentation procedures performed by Dr. Alain Simonpieri (Marseille, France) utilizing PRF alone. **(d)** Notice the bone gain occurring after a 6-month healing period utilizing PRF.

to centrifuge without anticoagulants or additives (Figure 3). This new formulation can be utilized for a variety of procedures including mixing with bone grafts to form a stable fibrin bone graft for improved handling after a short period of time (one to 2 minutes), which improves graft stability. This takes full advantage of the low-speed centrifugation concept and additionally improves wound healing when combined with bone biomaterials.

Clinical Use of PRF and Indications

In terms of soft-tissue management and maturation, PRF is able to support the development of angiogenesis, immunity, and epithelial coverage. Fibrin has been shown to act as the natural scaffold guiding angiogenesis, which consists of the formation of new blood vessels inside the wound.

Regarding the clinical use of PRF in daily dental practice, PRF may be utilized as a tissue matrix/scaffold (provisional extracellular matrix) with the ability to simultaneously release growth factors during a 10-day period. The clots are prepared in a PRF metallic box, which allows the slight compression of the clots into membranes or plugs to be later utilized as depicted in Figure 4. These can be utilized for a variety of clinical procedures including acting as autologous barrier membranes, extraction socket healing (Figure 5), sinus lifting procedures (Figure 6), implant dentistry, and for the treatment of intrabony defects and gingival recessions (Figure 7), amongst others. Research is continuously ongoing in this field, and patient satisfaction has been considered high due to the use of regenerative materials from autologous sources (patient's own blood).

CONCLUSION

The use of PRF in regenerative medicine has now seen a huge increase in its use across many fields of medicine due to its ease of use and low costs while providing a completely autologous source of growth factor delivery. Recent modifications to the centrifugation speeds and times (A-PRF) further enhance its regenerative potential and bring to clinical practice a liquid formulation that is injectable during use (i-PRF). Future strategies are continuously being developed to further improve the clinical outcomes following regenerative procedures utilizing platelet concentrates.✦

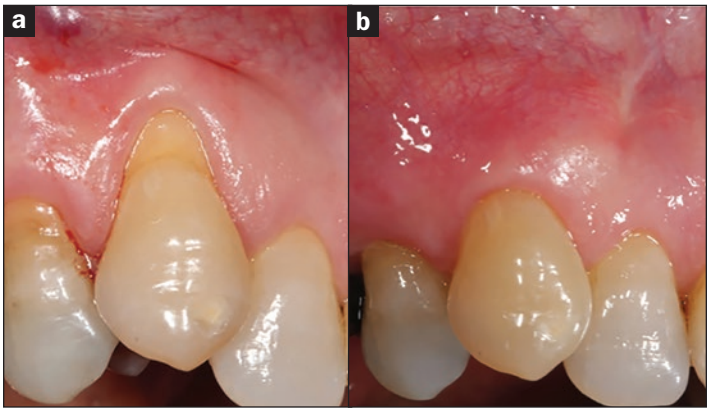

Figure 7. Use of PRF alone for the treatment of gingival recessions. Notice the soft-tissue wound healing observed. Due to the use of the blood-derived collection of growth factors, PRF alone can be utilized for the regeneration of simple gingival recessions.

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POST EXAMINATION QUESTIONS

1. How many times greater a concentration of growth factors do platelet concentrates (platelet-rich plasma [PRP] and PRF) have in comparison to normal blood?
a. 2x to 3x.
b. 4x to 6x.
c. 6x to 8x.
d. 10x to 15x.

2. What is the difference between PRF and leukocyte-PRF (L-PRF)?
a. L-PRF contains more leukocytes.
b. L-PRF is the newer formulation with better biological properties.
c. L-PRF has more optimal centrifugation speeds.
d. Nothing; they are exactly the same.

3. Which of the following procedures can PRF be utilized for?
a. Socket healing.
b. Sinus lifting procedures.
c. Gingival recession.
d. All of the above.

4. What is the main difference between PRP and PRF?
a. PRP is more natural and therefore produces better results.
b. PRF does not contain anticoagulants, whereas PRP does.
c. PRP is a liquid, whereas PRF is the equivalent fibrin solution.
d. Nothing; they are exactly the same.

5. What are the main actions of PRF on human tissues?
a. Supplies angiogenic factors capable of improving blood flow to defect sites.
b. Contains a fibrin clot that acts as a 3-D provisional matrix.
c. Contains a number of defense-fighting immune cells.
d. All of the above.

6. What are the advantages of the low-speed centrifugation concept?
a. It reduces the number of leukocytes in the final PRF clot, thereby improving wound healing.
b. It improves bone graft stability by acting as a sticky matrix.
c. It allows a greater collection of leukocytes in the upper layer of PRF by reducing centrifugation g-force.
d. It is a new technique to prepare PRP from changes in the centrifugation protocols.

7. How long has PRF been shown to release growth factors to the surrounding microenvironment?
a. In the order of minutes.
b. In the order of hours.
c. One to 3 days.
d. 10 to 14 days.

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8. What are the advantages of advanced-PRF (A-PRF) when compared to L-PRF?

a. Higher amount of growth factor release.
b. Higher amount of collected leukocytes.
c. Higher production of collagen from cells.
d. All of the above.

9. What is the centrifugation protocol to produce a liquid version of PRF (injectable PRF)?

a. 700 rpm for 3 minutes.
b. 700 rpm for 8 minutes.
c. 1,300 rpm for 3 minutes
d. 1,300 rpm for 8 minutes.

10. What is/are the primary role(s) of macrophages and leukocytes contained within PRF?

a. Secretion of a wide range of growth factors.
b. Tissue wound healing.
c. New blood vessel formation.
d. All of the above.

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