

From Platelet-Rich Plasma to Advanced Platelet-Rich Fibrin: Biological Achievements and Clinical Advances in Modern Surgery

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Abstract

In the past 20 years, the platelet concentrates have evolved from first-generation products, i.e., platelet-rich plasma (PRP) and plasma rich in growth factors to the second-generation products such as leukocyte-platelet-rich fibrin (L-PRF) and advanced platelet-rich fibrin (A-PRF). These autologous products with a higher leukocyte inclusion and flexible fibrin mesh act as a scaffold to increase cellular migration in the angiogenic, osteogenic, and antimicrobial potential of these biomaterials in tissue regeneration. In the second-generation platelet concentrates, the protocols are easier, cheaper, and faster with an entire physiological fibrin matrix, resulting in a tridimensional mesh, not as rigid as one of the first generations. This allows the slow release of molecules over a longer period of time and triggers the healing and regenerative process at the site of injury. The potential of A-PRF to mimic the physiology and immunology of wound healing is also due to the high concentration of growth factors released as follows: vascular endothelial growth factor, platelet-derived growth factor, transforming growth factor- β , and anti-inflammatory cytokines that stimulate tissue cicatrization, vessels formation, and bone cell proliferation and differentiation. Furthermore, the number of neutrophils and monocytes/macrophages is higher releasing important chemotactic molecules such as chemokine ligand-5 and eotaxin. Thus, L-PRF and A-PRF have been used, especially in implantology, periodontology, and maxillofacial surgery. Future clinical applications include tissue regeneration/grafts, ulcers/skin necrosis in the diabetic patient and others, plastic surgery, and even musculoskeletal lesions.

Keywords

- ▶ advanced platelet-rich fibrin
- ▶ fibrin
- ▶ plasma rich in growth factors
- ▶ platelet concentrates
- ▶ platelet growth factors
- ▶ platelet-rich fibrin
- ▶ platelet-rich plasma
- ▶ tissue regeneration

Introduction

Platelet concentrates are products that result from the centrifugation of a blood sample. They concentrate platelets, fibrin, and leukocytes (depending on the used protocol) converting them into a clinical and useful form.

With the knowledge of the biological features of the concentrates, the initial protocols evolved from the platelet concentrates of first generation that include platelet-rich plasma (PRP) and plasma rich in growth factors (PGRF) to

the second-generation concentrates. These ones include protocols such as leukocyte-platelet-rich fibrin (L-PRF) and advanced platelet-rich fibrin (A-PRF). The substantial evolution between the first- and second-generation platelet concentrates is based on the principle that in the second-generation ones, there is no blood manipulation with additives and they remove the anticoagulants from the centrifugation protocol.¹

The biological and clinical properties of these second-generation platelet concentrates make them highly attractive to

use in regenerative medicine. In the past decades, they were used mainly in the dentistry field to speed revascularization of damaged tissues and bone regeneration before implant placement. Other oral applications include periodontology, maxillofacial surgery such as gingival recession, intrabony defects, alveolar filling postextraction, and sinus lifting.² Nowadays, the autologous platelet concentrates are used not only in dentistry but also for ulcers/skin necrosis, plastic and reconstructive surgery, and even musculoskeletal lesions.³

The success of the platelet concentrates depends on the platelet concentration, the number/type of leukocytes entrapped in the fibrin membrane, and also of the release of bioactive molecules at the sites of injury that will trigger the regenerative process.⁴ Growth factors released include vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor- β (TGF- β), epidermal growth factor (EGF), insulin-like growth factor (IGF-I), hepatocyte growth factor (HGF), cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-4 (IL-4), and interleukin-10 (IL-10), and chemotactic molecules such as chemokine ligand-5 (CCL-5) and eotaxin. Thus, platelet concentrates such as A-PRF have all the biological features to induce an optimal healing.⁵

Platelet Concentrates

Characteristics of Platelet-Rich Plasma, Plasma rich in Growth Factors, Platelet-Rich Fibrin, and Advanced Platelet-Rich Fibrin

In modern tissue regeneration, the evolution of the platelet concentrates was directed toward less time, better quality, and less invasiveness using autologous products and reducing the risk of rejection from the patient. The fact that the second-generation concentrates respect the European Directive 2004/03/CE can also account for another characteristic that compares the effectiveness of first- and second-generation platelets, there is no manipulation of the patients' blood, so the protocol is also simple and cheaper.^{6,7}

Platelet-Rich Plasma

Inside the PRP family, a clarification is necessary to allow comparing with other recent protocols. The protocol developed by Jo et al⁸ uses the same amount of blood as the others of first- and second generations—9 mL. It is considered a first-generation concentrate using calcium glutamate/bovine thrombin to activate coagulation and an anticoagulant solution of citrate phosphate dextrose adenine (CPDA). There is a first centrifugation (900 rpm/5 minutes) to separate the red cells from the white cells, and three layers are obtained—a superior one rich in platelet and white cells, a medium one of buffy coat, and an inferior layer with the red cells. After collecting the two superior layers, a second centrifugation at 1500 rpm/15 minutes excludes the red cells. Both centrifugations use plastic tubes and the final result is 2 mL of gel containing the $\times 4.2$ increase in the initial number of platelets.^{8,9}

Plasma Rich in Growth Factors

Furthermore, the first-generation concentrate with anti-coagulants (sodium citrate) and coagulation activators

(calcium chloride) and the final product is obtained after an 1850 rpm/8 minutes centrifugation also in plastic tubes. After that, three layers of blood include a superior one divided into platelet-poor plasma (PPP) and PRP, a medium one with buffy coat, and the inferior one with the red cells. Depending on the clinical use, we can have different forms of presentation and the most used in regenerative medicine is the fibrin mesh induced by PPP that forms after 15 to 20 minutes.¹⁰

Leukocyte-Platelet-Rich fibrin

This is a second-generation platelet concentrate, in which the final product is obtained by a total physiologic process. For Inchingolo et al,¹¹ the lack of “additives” allows the natural activation of the platelets and coagulation when in contact with the tube. This last one is made from glass, not plastic because according to Jianpeampoolpol et al,¹² the silica from the glass is a natural coagulation inducer. The fibrinogen initially concentrated in the superior layer of the tube when in contact with the thrombin is converted into fibrin that will imprison the platelets: a quick process between the harvest and blood centrifugation. According to Marrelli and Tatullo,¹³ PRF has a mesh of fibrin that allows cellular migration and a slow release of growth factors essential for tissue regeneration. The final product is originated after centrifugation at 2700 rpm/12 minutes with the fibrin mesh in the middle of the tube between the noncellular and cellular plasma (with platelets, leukocytes, and fibrin).

Advanced Leukocyte-Platelet-Rich Fibrin

In this second-generation platelet concentrate, the fibrin mesh is obtained using lower G forces (1500 rpm/14 minutes) that clinically translate in an increased concentration of growth factors and neoangiogenic potential.^{14,15} They also use glass tubes for centrifugation, and according to Dohan Ehrenfest et al,¹⁴ the changes in the speed and time in the centrifugation protocol result in a shorter membrane, lighter, and narrower with the increased capacity of cell imprisonment. Changing the centrifugation protocol to Choukroun's PRF translated in the change of the leukocyte pattern in the fibrin-rich clot: the number of neutrophils increased.¹⁶

The main characteristics of all the platelet concentrates are summarized in **Table 1**.

Platelet Growth Factors

The biological properties of the platelet concentrate explore the platelet's function in organism homeostasis, namely, the fibrin coagulation and tissue regeneration. Such potential is due to growth factors such as VEGF, PDGF, TGF- β , EGF, IGF-I, and HGF. After centrifugation, all these factors contribute to soft- and hard-tissue regeneration and wound healing after tissue injury^{17,18} (**Table 2**).

Leukocytes and the Inflammatory Process

As mentioned before, the first goal in tissue regeneration was to separate the plasmatic components to obtain a rich-platelet concentrate. Afterward, it was observed that besides platelets the inclusion of other components such as leukocytes could improve the healing process. Thus, leukocytes release

Table 1 Biological comparison between the first- and second-generation platelet concentrates

Platelet concentrate	PRP ⁸	PRGF	L-PRF	A-PRF
Generation	1	1	2	2
Anticoagulants use	Yes (CPDA)	Yes (sodium citrate)	No	No
Coagulation activation	Calcium glutamate/bovine thrombin	Calcium Chloride	No	No
Protocol (rpm/min) centrifuge tubes	900/5+1500/15 (plastic tubes)	1850/8 (plastic tubes)	2700/12 (glass tubes)	1500/14 (glass tubes)
Protocol cost	High	High	Low	Low
Fibrin membrane	–	Yes	Yes	Yes
Fibrin membrane formation	–	Induced	Physiological	Physiological
Leukocytes	Nondetermined	0%	50–65%	50–65% (↑↑↑neutrophils)
Growth factors	√	√√	√√√√	√√√√√
Bone regeneration	√	√√	√√√	√√√
Presentation form	Gel	Liquid Clot Supernatant Fibrin membrane	Plugs (for alveolar filling) Exudate Fibrin membrane	Plugs (for alveolar filling) Exudate Fibrin membrane

Abbreviations:↑↑↑, increased concentration; √, small amount; √√, medium amount; √√√, good amount; √√√√, very good amount; √√√√√, excellent amount; A-PRF, advanced platelet-rich fibrin; CPDA, citrate phosphate dextrose adenine; L-PRF, leukocyte-platelet-rich fibrin; PRGF, plasma rich in growth factor; PRP, platelet-rich plasma.

VEGF and TGF that, again, improve chemotaxis and angiogenesis.^{29–31} Nowadays, there are in the market first-generation protocols with or without leukocytes and second-generation ones that always include white cells.

Literature results about the impact of leukocytes in the first-generation platelet concentrates revealed heterogeneous results. On the one hand, there is lack of clinical evidence of the positive impact of leukocytes, and on the other hand, some authors claim that the leukocyte exclusion improves the healing process, reducing the inflammatory response modulated by the secretion of metalloproteinases, hydrolases, and proteases.^{32,33}

The second-generation platelet concentrates, namely, L-PRF and A-PRF emerged in the market to overcome the limitations found in the first-generation concentrates. A complete autologous concentrate with a higher leukocyte inclusion in a fibrin mesh (to act as a scaffold) should increase its osteogenic and antimicrobial potential. Following this research new developed protocols also seem to substantiate the positive biological impact of the fibrin in the cells support and migration, inducing rapid tissue regeneration. Besides, there is also an increase in VEGF, TGF production, and anti-inflammatory cytokines that will mediate the inflammatory process.³⁴

IL-4 drives Th cell differentiation in a Th2 pattern, inducing activation and proliferation of B-cells. During the inflammatory process, it negatively regulates IL-1 and tumor necrosis factor- α , inducing IL-10, another Th2 cytokine. It induces the antagonist of IL-1 receptor contributing to the control of postsurgery pain.¹ IL-6, according to Kumar and Shubhashini,³⁵ is a pleiotropic cytokine produced by cells such as fibroblasts, endothelial cells, monocytes, and macrophages, also

inducing B- and T cell differentiation. It is involved in the acute-phase response not only sharing some properties with IL-1 but also inducing IL-1 receptor and a negative feedback in the inflammatory cascade.^{36,37}

However, there is a lack of clinical studies validating the role of the leukocytes and cytokines in the inflammatory process beginning with inflammation, then proliferation and finally regeneration. There are not enough *in vivo* studies to support that, when using the platelet concentrates, we are also using M2 (alternatively activated macrophages [anti-inflammatory molecule producer])-polarized macrophages instead of M1 (classically activated macrophages [pro-inflammatory molecules producer]).^{38,39}

Tissue repairing requires a strategic collaboration of all leukocytes, epithelial and endothelial cells, fibroblasts, and platelets. In Choukron's A-PRF, the number of leukocytes includes more neutrophils that also can contribute to a pro- or anti-inflammatory state of the macrophages modulating tissue regeneration.¹⁶

The Inclusion of Fibrin as a Scaffold

In platelet concentrates PRGF, L-PRF, and A-PRF, the fibrin mesh represents a big innovation in regenerative tissue processing. The fibrin and the final products from fibrinogen degradation stimulate neutrophil migration and increase chemotaxis, by overexpression of the membrane receptors CD11c/CD18.⁴⁰

In dental tissues regeneration, such as periodontal tissue, the fibrin scaffold supports and acts as a carrier for cells. It is a natural polymer that interacts directly with the cells/tissue, inducing an, almost natural, tissue regeneration. This is one of the foundations of tissue engineering applications.⁴¹

Table 2 Platelet growth factors properties

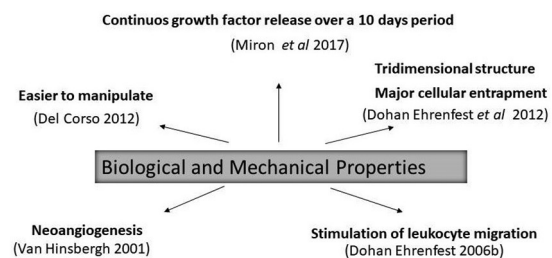
Growth factors	Biological action
VEGF	The VEGF was first mentioned by Ferrara and Gerber ¹⁹ as a signaling protein, angiogenic and with specificity for the vascular endothelial cells, stimulating chemotaxis and endothelial cell proliferation. Nör et al ²⁰ demonstrate that VEGF also regulates vascular permeability, the main process for the beginning of angiogenesis and also induces bone tissue regeneration. Thus, Di Alberti et al ²¹ wrote that it is the main growth factor for tissue regeneration in dentistry implants application
PDGF	This growth factor concentrates mainly in the platelets α - granules, liberated during the coagulation cascade. Its effect is dependent on other growth factors presence, inducing fibroblast, macrophages, and other leukocytes chemotaxis. ²² According to Caplan and Correa, ²³ the target of PDGF is mainly the bone tissue inducing osteoblast chemotaxis, vascular regeneration, and fracture repairing. It is the first growth factor to be found in a wound, responsible for collagen synthesis in the connective tissue
TGF- β	As referred by Miyazono, ²⁴ this factor as a role in the growth, proliferation, adhesion, and apoptosis of several cell types being a key player in the inflammatory process. It induces chemotaxis and mitogenesis of undifferentiated cells to the place of repair activating fibroblasts, osteoblasts, and chondroblasts proliferation. It induces the first step of repairing and extracellular matrix healing ²⁵
EGF	The EGF promotes chemotaxis and mitogenesis of epithelial, mesenchymal cells and fibroblasts also inducing tissue regeneration. It stimulates epithelial proliferation in peri-implantar tissues inducing the formation of the peri-implant junctional epithelium. Thus, its presence together with EGF from saliva increases in oral surgery ²⁶
IGF-I	As referred by Kurten et al, ²⁷ IGF-I is synthesized by almost every tissue with increased concentrations in bone. It mediates growth, differentiation, and cellular transforming and stimulates osteoblasts. It is also involved in keratinocytes migration and wound healing ²⁸
HGF	This platelet growth factor regulates the migration and cellular morphogenesis having an important role in wound repair through its interaction with the mesenchymal epithelium ¹⁰

Abbreviations: EGF, epidermal growth factor; HGF, hepatocyte growth factor; IGF-I, insulin-like growth factor; PDGF, platelet-derived growth factor; TGF- β , transforming growth factor- β ; VEGF, vascular endothelial growth factor.

Aggour and Abd El-Hady⁴² observed in vitro that fibrin-based membranes are better scaffolds for the proliferation of bone cells when compared with collagen membranes. This strong mechanical and polymerization characteristic makes it a more suitable support for regeneration, increasing fibrinogen concentrations, and inducing the formation of a stronger matrix. In PRP, the fibrin mesh is quite “immature” with small diameter fibrils. This mesh supports platelets during surgery but rapidly dissolves like glue, not having the same angiogenic and chemotactic potential of a membrane.⁴⁰

In PRGF, the use of an anticoagulant and a gel agent influences the velocity and polymerization of the membrane, and as a result, it forms a tetra-molecular mesh condensate with an increased quantity of fibrin. Thus, in PGRF, where the use of coagulation activator modifies the thrombin concentrations, the thickening of the fibrin polymers results in the formation of a rigid mesh that favors the sealing of the biological tissues but impairs cellular migration and cytokine release.⁷

In PRF (L-PRF and A-PRF), there is no patient blood manipulation, and the fibrin matrix formation is completely physiological creating a tridimensional mesh. With no manipulation, the thrombin concentrations are lower favoring the cellular imprisonment and molecules deliverance during 10 days.⁴³ These cells and the VEGF are crucial for vascular angiogenesis and pathogen elimination avoiding possible contaminations. The spatial confirmation of the fibrin mesh also supports the platelets and stem cells ideal for concentration at the injury/surgery site⁴⁴ (► **Fig. 1**).^{37,40,45-47}

**Fig. 1** Fibrin: an autologous matrix.

Leukocyte-Platelet-Rich Fibrin and Advanced Platelet-Rich Fibrin: Recent Applications

All the studies until now reveal that the biological effects achieved depend on the platelet concentration and the number/type of leukocytes entrapped in the fibrin membrane. Thus, the release of bioactive molecules at the sites of injury will trigger the healing and regenerative process.⁴

Recent in vitro studies show that L-PRF ability to potentiate angiogenesis and vasculogenesis at the injury site is mediated not only by the liberation of bioactive molecules but also by the presence of hematopoietic stem cells and endothelial progenitor cells.³⁹

The second-generation concentrates L-PRF and A-PRF have a similar level of platelets and cytokines. However, A-PRF releases a significantly higher quantity of growth factors TGF- β , PDGF, VEGF, and chemotactic molecules CCL-5 and eotaxin, when compared with traditional PRF.⁴³ Furthermore, A-PRF

has an increased number of neutrophils. These inflammatory cells contribute to monocyte/macrophage differentiation, despite the similar total amount of leukocytes.¹⁶ Thus, A-PRF is an excellent candidate for tissue repair and vessel formation.⁵

The main clinical applications of L-PRF and A-PRF include tissue regeneration in oral and maxillofacial surgery (alone or with bone grafts). In regenerative medicine and dentistry, several clinical studies showed better outcomes with PRF than open-flap debridement, in intrabony periodontal defects. Furthermore, its use together with bone substitutes such as nanohydroxyapatite had a therapeutic effect compared with the substitutes alone.⁴⁸ Osteonecrosis of the jaws can also benefit from L-PRF application: necrotic bone replacement by L-PRF was used as a physical barrier against microorganisms, preventing secondary infections.⁴⁹ PRF can also act in the treatment of ulcers/skin necrosis, plastic surgery,³ and even musculoskeletal lesions.⁵⁰

In A-PRF, the release of TGF- β -1, PDGF, and VEGF and the presence of monocytes/macrophages facilitate wound healing and tissue regeneration.⁵¹ All of these biological factors were previously associated with a high success in several dentistry areas as follows: periodontal reconstructive surgery,⁵² sinus lift, and implants.⁵³ Furthermore, A-PRF can be used with freeze-dried bone allograft improving bone osteogenesis and alveolar stability in the use of implants.⁵⁴

Current clinical trials are evaluating the use of A-PRF in periodontal angular defects. Thus, there are studies focusing the treatment of intrabony defects with PRF, it lacks evidence of the benefit of A-PRF in the same patients.⁵⁵

In orthopaedic surgery, the use of L-PRF with bone cell proliferation/differentiation inducers such as bone morphogenetic proteins-2 also seems to be a benefit for tissue regeneration.⁵⁶ Furthermore, Crisci et al study the use of L-PRF in ulcers in a diabetic foot and found that treatment improved wound healing with no evidence of infection.⁵⁷ In other types of chronic ulcers such as pressure and venous leg ulcers, the use of L-PRF has also improved healing and cicatrization.³

Conclusion

When analyzing the efficacy in clinical applications has shown in the literature, second-generation platelet concentrates result in more natural protocols, lower costs, and faster time for obtaining a valid clinical product when compared with the first-generation protocols.

When comparing the biological properties, proliferation *versus* differentiation, L-PRF and A-PRF use a high number of leukocytes, and platelets and growth factors promoting both proliferation and differentiation. L-PRF and A-PRF mimic the physiological process, opposite to the first-generation concentrates that use anticoagulants and coagulation activators that can reduce the positive leukocyte impact in wound healing. The TGF- β , PDGF, VEGF, eotaxin, and CCL-5 released by the leukocytes promote local vascularization and tissue repairing, mainly due to the control of the inflammatory process by anti-inflammatory cytokines IL-4, IL-6, and IL-10 also having an antimicrobial potential.

Furthermore, the fibrin scaffold has different physical and biological properties, capturing a higher number of cells, and including stem cells that can differentiate in other autologous cells. In A-PRF, there is an increase not only in the number of neutrophils but also the time of growth factors liberation increases in 10 days. This results in better cellular conduction, migration, angiogenesis, and natural healing of the tissues patient. For these reasons, the potential benefits of leukocyte (mainly inflammatory neutrophils) and fibrin are reciprocal, and they are mutual stimulation “actors” in the healing process.

Nowadays, the L-PRF and A-PRF are used not only in dentistry has adjuvants in tissue regeneration before implant rehabilitation but also for chronic ulcers, musculoskeletal lesions, plastic surgery, and even orthopaedic surgery. In the future, there is a wide range of clinical applications due to the low costs and rapid healing properties of these concentrates, but still, there is a vast research to be done translating the laboratory results to the clinical practice.

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Conflict of Interest

None declared.

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