Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution

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Platelet-rich fibrin (PRF) belongs to a new generation of platelet concentrates geared to simplified preparation without biochemical blood handling. In this initial article, we describe the conceptual and technical evolution from fibrin glues to platelet concentrates. This retrospective analysis is necessary for the understanding of fibrin technologies and the evaluation of the biochemical properties of 3 generations of surgical additives, respectively fibrin adhesives, concentrated platelet-rich plasma (cPRP) and PRF. Indeed, the 3-dimensional fibrin architecture is deeply dependent on artificial clinical polymerization processes, such as massive bovine thrombin addition. Currently, the slow polymerization during PRF preparation seems to generate a fibrin network very similar to the natural one. Such a network leads to a more efficient cell migration and proliferation and thus cicatrization. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:E37-44)**

Among the great challenges facing clinical research is the development of bioactive surgical additives regulating inflammation and increasing healing. Indeed, after each intervention, surgeons must face complex tissue remodeling phenomena and the consequences on healing and tissue survival. Although the use of fibrin adhesives in many field-related protocols is well documented from the past 30 years,[1,2] it remained controversial owing to the complexity of the production protocols (for autologous adhesives) or risk of cross-infection (for commercial adhesives). The development of platelet concentrate technologies offers simplified and optimized production protocols for a new kind of fibrin adhesive, concentrated platelet-rich plasma (cPRP). Because of legal restrictions on blood handling, a new family of platelet concentrate, which is neither a fibrin glue nor a classical platelet concentrate, appeared in France. This new biomaterial, called platelet-rich fibrin (PRF), looks like an autologous cicatricial matrix.

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WHAT IS FIBRIN?

Fibrin is the activated form of a plasmatic molecule called fibrinogen.[3] This soluble fibrillary molecule is massively present both in plasma and in the platelet α-granules and plays a determining role in platelet aggregation during hemostasis. It is transformed into a kind of biologic glue capable of consolidating the initial platelet cluster, thus constituting a protective wall along vascular breaches during coagulation. In fact, fibrinogen is the final substrate of all coagulation reactions. Being a soluble protein, fibrinogen is transformed into an insoluble fibrin by thrombin while the polymerized fibrin gel constitutes the first cicatricial matrix of the injured site.[4-6]

FIBRIN AND SURGICAL ADDITIVES

Despite advancements achieved in effective antihemorrhagic surgical techniques, finding hemostatic agents remains a persistent problem. There is a wide variety of hemostatic agents, such as collagen sponges, oxidized cellulose, and cyanoacrylate synthetic adhesives. Within our therapeutic arsenal, fibrin adhesives are well documented; they correspond to a natural biologic

mechanism (fibrin polymerization during hemostasis) amplified in an artificial way.[7]

However, over a long period of time, fibrin adhesives have been criticized owing to the fact that they are blood-derived products. Produced by pharmaceutic industries (eg, Tisseel from Baxter Healthcare), they constituted an infinitely small viral contamination risk and are currently being marketed in the US. More simplified tools inherent to the production of autologous fibrin adhesives have recently been developed with the evolution in similar technologies such as cPRP-type platelet concentrates.[8]

Methods

The operating mode of fibrin adhesives reproduces the last stages of the enzymatic cascades of coagulation during which the fibrinogen is converted into fibrin in the presence of thrombin, factor XIII, fibronectine, and calcium ions.[2]

The Tisseel kit from Baxter Healthcare is a perfect example. It consists of:

— a lyophylized fibrinogen concentrate, associated with fibronectin and factor XIII
— a bovine aprotinin solution (for protease inhibition), acting as an antifibrinolytic able to increase the lifespan of fibrin sealing
— a bovine thrombin concentrate
— a calcium chloride solution

Fibrinogen is first mixed with aprotinin to constitute solution A, which in turn is heated to 37°C. Solution B is obtained from mixing bovine thrombin with calcium chloride solution. Solutions A and B are blended just before use with a self-mixing syringe.

It is noteworthy that the speed of adhesive polymerization depends on the thrombin concentrations used to reconstitute solution B. Broadly speaking, hemostatic activity relies on the quick hardening of the adhesive and high thrombin rate. However, slow polymerization always remains an option, even if this is done to the detriment of the surgical interest of this additive.

Clinical applications

Despite the considerable differences existing among the protocols described in the literature, most studies show the efficiency of fibrin adhesives in controlling slow and diffuse bleeding as well as lymphatic exudates, serous collections, and all diffuse bleeding of the parenchyma. However, these adhesives do not guarantee hemostasis of severe vascular hemorrhages and will never be used in replacement of generally accepted surgical techniques.[2]

Fibrin adhesives are often used in cardiothoracic and vascular surgery. These adhesives are successfully used for the sealing of diffuse microvascular bleeding through spray application.

Fibrin adhesives are above all well known for their use in the sealing of wound borders and the facilitation of cutaneous reapplication in general and plastic surgery.[9] Surgeons therefore use the mechanical properties of the adhesive as well as the fibrin biologic properties to promote cicatrization.

These adhesives are also particularly well described in oral and maxillofacial surgery.[10-12] In addition to its capacity to accelerate healing, sealing with fibrin adhesive is conventionally known for reducing postoperative hematoma.[13]

Many other surgical disciplines have tried the application of these adhesives in several surgical areas in research on animals before human application. The results were sometimes controversial, namely in orthopedic surgery and neurosurgery. In fact, the sealing of dura mater or nerves in traumatic or tumoral reconstructive surgery remains less well documented.

In conclusion, these additives remain more than anything else an autologous fibrin glue whose main biologic activities are tissue adherence and biodegradability.[14,15]

CONCENTRATED PLATELET-RICH PLASMA: BIOLOGICAL ADHESIVE OR CELLULAR THERAPY?

Because of the risk of transmission of hepatitis, many marketed fibrin adhesives have been prohibited in the USA since 1978. Consequently, attempts at the development of autologous fibrin adhesives increased, but with mitigated success. Indeed, it is difficult to obtain using nonindustrial technique such high fibrinogen rates as in an industrial product similar to Tisseel. And when technology allowed the production of an acceptable autologous adhesive, practitioners encountered extremely long and complex protocols: when Tayapongsak et al. described their autologous fibrin adhesive in 1994,[16] which was useful to maintain bone graft fragments in a coherent mass (in order to avoid the postoperative osseous sequestrum), blood was harvested 1 to 3 weeks before the intervention and required 2 days of handling before being ready to use.

These efforts might have been in vain, but the development of a new therapeutic concept induces the sudden reawakening of these quiescent technologies: The use of platelet concentrates, based on the concept of cell therapy by growth factors,[17] reopens technologic research on the autologous fibrin adhesives.[8,18] But do these surgical additives remain simple fibrin glue?

Definitions

In a strict sense, PRP platelet concentrates are blood-derived products used for the prevention and the

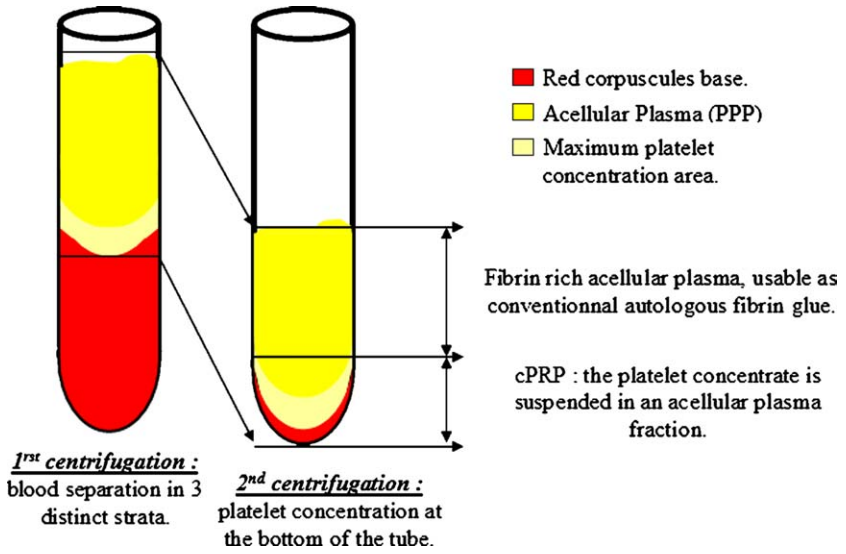

Fig. 1. Technologic concept of cPRP processing.

treatment of hemorrhages due to serious thrombopenia of central origin, such as medullary aplasia, acute leukaemia, etc. Thus they remain of very limited use.

Platelet concentrates for topical surgical, such as the standard platelet concentrates of transfusion hematology, use were thus arbitrarily called PRP. Moreover, the described protocols generally use a double centrifugation to increase the collected platelets concentration. To correct this misuse of language, many names were suggested: cPRP,[19] plasma rich growth factors,[20] etc. It seems however, that cPRP is the simpler and more adequate term.

These protocols are founded on a simple idea: The blood collection is made just before the intervention, and the sample is immediately transformed into platelet concentrate using a cell separator from the hematology laboratory (in the first years) and subsequently increasingly specific simple and automated machines (the most impressive example is Harvest SmartPReP).[21] The platelet concentrate is then mixed with thrombin and calcium chloride in order to induce massive activation of concentrated platelets and preparation gelling.

It is at this stage that platelet cytokines are normally released. The cicatricial properties of these soluble molecules are already well documented. The idea of cell therapy by autologous growth factors addition generated an increasing passion of clinical workers for this kind of biotechnologic approach to healing enhancement.[22-25]

Techniques

Many different protocols can be applied to the cPRP concept. But we can schematically divide them into 2 families: complex techniques using hematology cell separators, and simplified techniques with ready-to-use commercially available kits and 2-step centrifugation to concentrate platelets. These commercial systems are being increasingly automated to simplify clinical use.

Therefore, we will describe a general concept rather than any one particular system:

a) Venous blood is taken with anticoagulant to avoid platelet activation and degranulation.
b) The first centrifugation ("soft spin") allows the blood separation in 3 distinct layers (Fig. 1):

   At the bottom of the tube, the red blood corpuscles constitute 55% of total volume.
   At the top of the tube, the acellular plasma layer is mainly made up of circulating plasmatic molecules (in particular, fibrinogen) and low in platelets. It is designated platelet-poor plasma (PPP) and constitutes 40% of total volume.
   Between the 2, an intermediate layer is where platelets concentrations are largely increased. It constitutes only 5% of total volume and presents a characteristic buffy aspect that led to it being called "buffy coat." It will compose the major part of the future cPRP, but at this stage, there is still no easy scientific process allowing its separation from the other layers.

c) Using a sterile syringe, the practitioner aspirates PPP, PRP, and some red blood corpuscles (which are systematically attracted during the operation). Then the material is transferred to another tube, without anticoagulant.
d) This second tube will then undergo another centrifugation, purported to be longer and faster than the

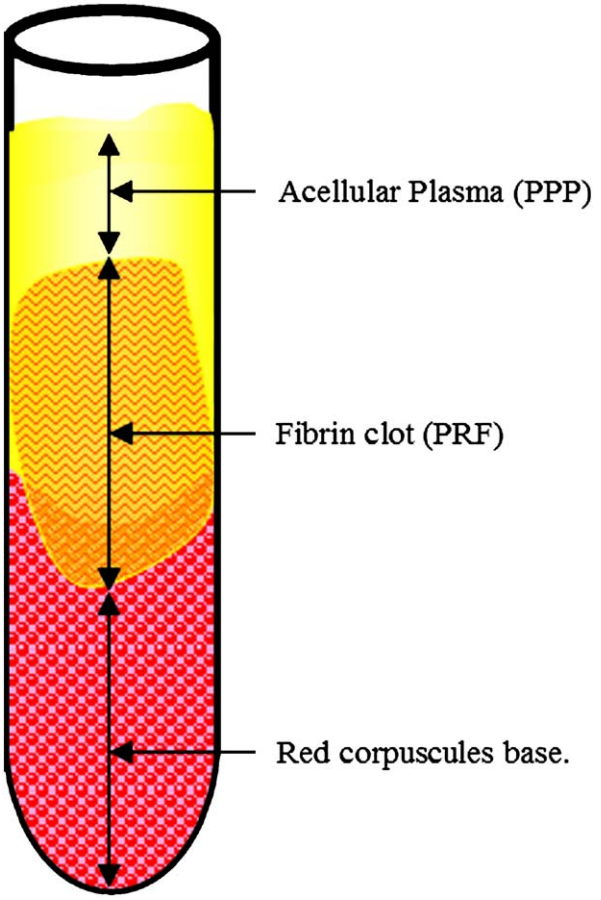

Fig. 2. Blood centrifugation immediately after collection allows the composition of a structured and resistant fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top.

first ("hard spin"). This makes it possible to concentrate platelets at the bottom of the tube and subsequently to obtain once again 3 distinct layers (Fig. 1):

some residual red blood corpuscles trapped at the bottom of the tube
acellular plasma (PPP) for 80% of total volume
between the 2, a buffy layer, or PRP.

e) At this stage, it becomes easy to collect the PRP. With a syringe, the practitioner can discard the major part of the PPP, leaving just enough serum to place the concentrated platelets in suspension. The unit is then gently shaken to obtain a ready-to-use cPRP.

Note that the red blood corpuscles trapped at the bottom of the tube are also suspended by this last operation, which explains the rosy aspect of the final cPRP.

f) cPRP is then mixed with bovine thrombin and calcium chloride at the time of application, with the help of a mixing syringe. Gelling of platelet concentrate will then quickly occur: Fibrinogen is also concentrated during the cPRP preparation, and its polymerization will constitute a fibrin matrix with particularly interesting hemostatic and adhesive properties.

Moreover, cPRP application can be accomplished in gel or spray form (according to the syringe nozzle used). In both cases, fibrin polymerization is completed in a few minutes. Note that to obtain a denser gel, or even a cPRP membrane, it is possible to add Tisseel to the mixture.[26]

Clinical results indissociable from fibrin activity

The cPRP platelet concentrates constitute very recent but already well documented technologies.[27-46] Unfortunately, the first results indicate that their clinical effects are very near those observed with conventional fibrin adhesives.[47-63] Indeed, the potential effect of the platelet cytokines, massively released during platelet activation and fibrin gelling, looks to be extremely limited in time.[64-69] Although fibrin gel should be a perfect support for cytokine action, these small soluble molecules are released too quickly to be closely built in inside the fibrin matrix during polymerization. This last theory could explain the mitigated effects of these preparations; however, much research remains to be done to validate this concept.

PLATELET-RICH FIBRIN—A NATURAL FIBRIN MATRIX

Technique

PRF was first developed in France by Choukroun et al.[70] for specific use in oral and maxillofacial surgery. This technique requires neither anticoagulant nor bovine thrombin (nor any other gelling agent). It is nothing more than centrifuged blood without any addition, which makes it possible to avoid all the restrictions of the French law related to blood-derived product reimplantation. This technology requires a PC-02 table centrifuge and a collection kit from Process (Nice, France).

The PRF protocol is very simple: A blood sample is taken without anticoagulant in 10-mL tubes which are immediately centrifuged at 3000 rpm (approximately 400*g* according to our calculations) for 10 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 high part 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 and acellular plasma at the top (Figs. 2 and 3).

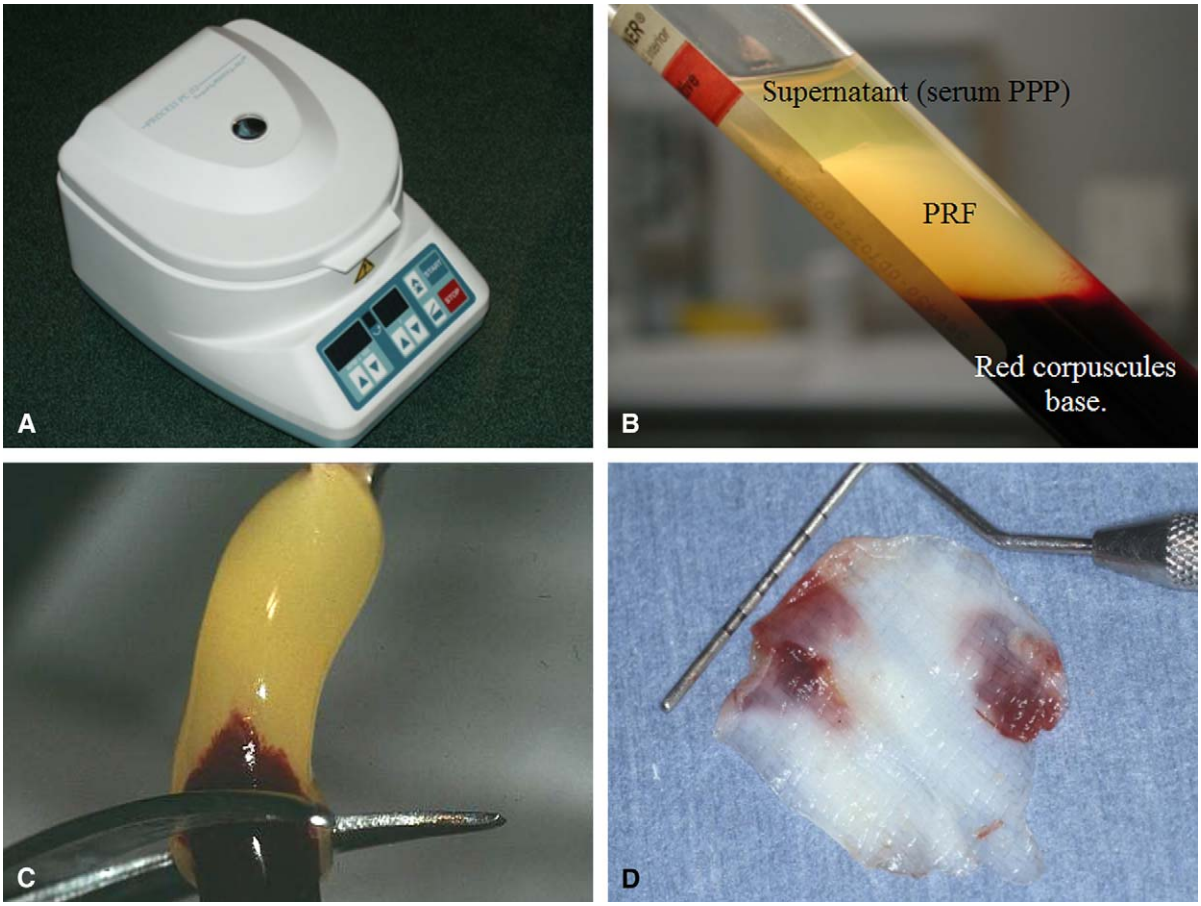

Fig. 3. Blood processing with a PC-O2 centrifuge for PRF (**A**; Process, Nice, France) allows the composition of a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top (**B**). After collection of the PRF itself (**C**), resistant autologous fibrin membranes are easily obtained by driving out the serum from the clot (**D**).

Platelets are theoretically trapped massively in the fibrin meshes.

The success of this technique entirely depends on the speed of blood collection and transfer to the centrifuge. Indeed, without anticoagulant, 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 clot. If the duration required to collect blood and launch centrifugation is overly long, failure will occur: The fibrin will polymerize in a diffuse way in the tube and only a small blood clot without consistency will be obtained.

In conclusion, the PRF protocol makes it possible to collect a fibrin clot charged with serum and platelets. By driving out the fluids trapped in the fibrin matrix, practitioners will obtain very resistant autologous fibrin membranes.

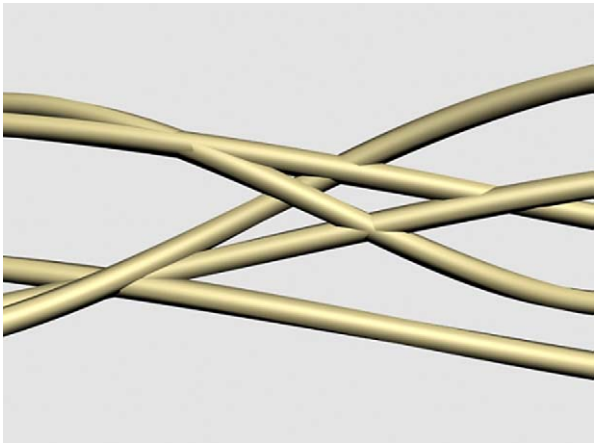

Fig. 4. Theoretical computer modelling of condensed tetramolecular or bilateral fibrin branch junctions. Note the rigidity of this architecture (D-TEP v1.3).

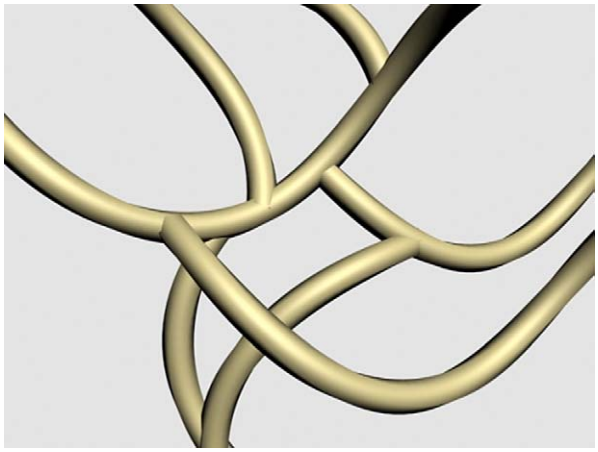

Fig. 5. Theoretical computer modelling of trimolecular or equilateral fibrin branch junctions. Note the flexibility of this net architecture (D-TEP v1.3).

Still unknown biology

The major characteristic of this method is derived from the absence of anticoagulant: Blood handling will thus launch the massive activation of collected platelets and release of the many cytokines they contain. If these soluble molecules are most likely partially trapped in the fibrin meshes of the PRF, there is still no comparative quantification in support of this theory.

The objective of this series of articles will be thus to look further into PRF-associated biologic mechanisms, to correlate them with clinical results,[71] and foresee new prospects for the use of this promising biomaterial.

DISCUSSION: DIFFERENT POLYMERIZATIONS, DIFFERENT BIOLOGIES

One of the main differences between fibrin adhesives cPRP and PRF is attributable from the gelling mode.[72-74] Fibrin adhesives and cPRP use a bovine thrombin and calcium chloride association to commence the last stages of coagulation and sudden fibrin polymerization. The speed of this reaction is dictated by the use of these surgical additives, and their hemostatic function implies a quasi-immediate setting and therefore significant quantities of thrombin. This mode of polymerization will considerably influence the mechanical and biologic properties of the final fibrin matrix.[3]

PRF has the characteristic of polymerizing naturally and slowly during centrifugation. And the thrombin concentrations acting on the collected autologous fibrinogen are almost physiologic because there is no bovine thrombin addition.

This aspect is crucial to determine the 3-dimensional organization of a fibrin network. Indeed, during gelling, the fibrin fibrillae can be assembled between them in 2 different biochemical architectures: condensed tetramolecular or bilateral junctions and connected trimolecular or equilateral junctions.[3] Bilateral junctions are constituted with strong thrombin concentrations and allow the thickening of fibrin polymers; this leads to the constitution of a rigid network, not very favorable to cytokine enmeshment and cellular migration (Fig. 4). However, the great resistance of such a gel is completely appropriate to firmly seal biologic tissues: Therefore, there will be a fibrin adhesive and, by extension, a cPRP.

In contrast, weak thrombin concentrations imply a very significant percentage of equilateral junctions. These connected junctions allow the establishment of a fine and flexible fibrin network able to support cytokines enmeshment and cellular migration (Fig. 5). Moreover, this 3-dimensional organization will give great elasticity to the fibrin matrix: It is what we observe in a flexible, elastic, and very strong PRF membrane.

These 3 fibrin biotechnologies therefore use different polymerization modes which imply very different biologic integration mechanisms.

CONCLUSION

Although PRF belongs to a new generation of platelet concentrates, it is in the first place a fibrin technology. Indeed, the biologic activity of the fibrin molecule is enough in itself to account for the significant cicatricial capacity of the PRF. And the slow polymerization mode confers to the PRF membrane a particularly favorable physiologic architecture to support the healing process.

However, it is now necessary to look further into platelet and inflammatory features of this biomaterial. Only a perfect understanding of its components and their significance will enable us to comprehend the clinical results obtained and subsequently extend the fields of therapeutic application of this protocol.

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