Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features

David M. Dohan, DDS, MS,[a] Joseph Choukroun, MD,[b] Antoine Diss, DDS, MS,[c] Steve L. Dohan,[d] Anthony J. J. Dohan,[e] Jaafar Mouhyi, DDS, PhD,[f] and Bruno Gogly, DDS, MS, PhD,[g]
Nice and Paris, France, Los Angeles, Calif, and Göteborg, Sweden
NICE UNIVERSITY, UNIVERSITY OF PARIS V, UNIVERSITY OF PARIS VI, UNIVERSITY OF SOUTHERN CALIFORNIA, AND GÖTEBORG UNIVERSITY

Platelet-rich fibrin (PRF) belongs to a new generation of platelet concentrates, with simplified processing and without biochemical blood handling. In this second article, we investigate the platelet-associated features of this biomaterial. During PRF processing by centrifugation, platelets are activated and their massive degranulation implies a very significant cytokine release. Concentrated platelet-rich plasma platelet cytokines have already been quantified in many technologic configurations. To carry out a comparative study, we therefore undertook to quantify PDGF-BB, TGFβ-1, and IGF-I within PPP (platelet-poor plasma) supernatant and PRF clot exudate serum. These initial analyses revealed that slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycanic chains in the fibrin meshes. This result would imply that PRF, unlike the other platelet concentrates, would be able to progressively release cytokines during fibrin matrix remodeling; such a mechanism might explain the clinically observed healing properties of PRF. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:E45-50)**

Although platelet rich fibrin (PRF) looks like an autologous fibrin gel with cicatricial properties, it is actually a new platelet concentrate concept.[1-4] Its production protocol attempts to accumulate platelets and the released cytokines in a fibrin clot. These data are already documented for platelet concentrates of previous generations (ie, different kinds of concentrated platelet-rich plasmac (cPRP)).[5-7] However, to date they have never been evaluated for PRF technology.

The platelet cytokine quantification in the PRF therefore constitutes a significant step in the understanding of this biomaterial, because these soluble molecules are key inflammation and healing mediators.[8] However, in reality, much more than quantification, it is their mode of incorporation into the fibrin matrix that appears to be very important to determine.

PLATELETS, HEMOSTASIS, AND CICATRIZATION

Biologic mechanisms

Formed in bone marrow from megacaryocytes, platelets are discoidal and anuclear structures. Their lifespan is 8 to 10 days, and the cytoplasm contains many granules whose contents are secreted at the time of activation.

α-Granules contain many proteins, platelet specific (such as β-thromboglobulin) or nonplatelet specific (fibronectin, thrombospondin, fibrinogen, and other factors of coagulation, growth promoters, fibrinolysis inhibitors, immunoglobulins, etc.). The dense granules contain calcium, serotonin, etc. Moreover, the platelet membrane is a phospholipid double layer into which receptors for many molecules are inserted (collagen, thrombin, etc.).

Activation is fundamental to initiate and support hemostasis because of aggregation on the injured site and interactions with coagulation mechanisms. However, degranulation also implies the release of cytokines able to stimulate cell migration and proliferation within the fibrin matrix, launching the first stages of healing.

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[a]Assistant Professor, Biophysics Laboratory, Faculty of Dental Surgery, University of Paris V; Department of Oral Surgery, Odontology Service, Hopital Albert Chenevier, Paris.
[b]Private Practice, Pain Clinic Center, Nice, France.
[c]Assistant Professor, Laboratory of Surface and Interface in Odontology, Odontology Faculty, Nice University; Department of Periodontology, Odontology Service, Hopital St Roch, Nice, France.
[d]Student, Biophysics Laboratory, Faculty of Dental Surgery, University of Paris V; Odontology Service, Hopital Albert Chenevier, Paris.
[e]Student, Saint-Antoine Faculty of Medicine, University of Paris VI.
[f]Private practice, Casablanca, Morocco; Assistant Professor, Advanced Periodontology, University of Southern California; Researcher, Department of Biomaterials/Handicap Research, Institute for Surgical Sciences, Sahlgrenska Academy at Göteborg University.
[g]Professor, Faculty of Dental Surgery, University of Paris V; Chief, Odontology Service, Hopital Albert Chenevier, Paris.
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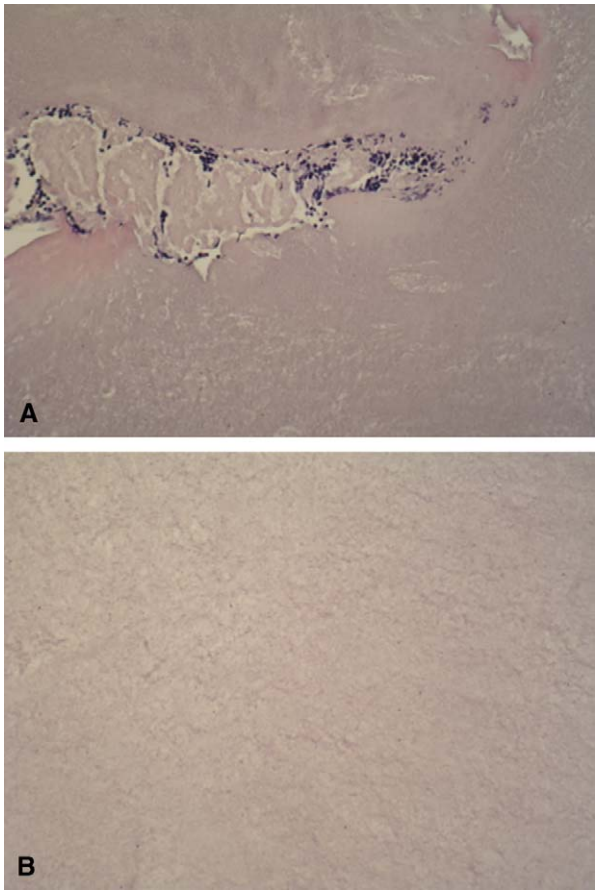

Fig. 1. The lower part of the PRF fibrin matrix is occupied by whitish streaks looking like cell fragment aggregates on histological sections. These are the platelet accumulations and constitute a "buffy coat" (**A**). But there is no platelet or any other cellular body in the upper part of the PRF fibrin clot (**B**). Hemalun-eosin staining, 52×.

Platelet cytokines

TGFβ-1: Fibrosis agent. Transforming growth factor β (TGFβ) is a vast superfamily of more than 30 members. The reference molecule in speaking about "the" TGFβ will actually be TGFβ-1. It is the most massively produced isoform, not only in the platelet α-granules, but also in general during intercellular dialog.[9]

In vitro, its effects are extremely varied according to the amount applied, the matrix environment and cell type. For example, it has been shown that it could stimulate the proliferation of osteoblasts just as easily as it could cause their inhibition.[10]

Although its effects in terms of proliferation are highly variable, for the great majority of cell types, it constitutes the most powerful fibrosis agent among all cytokines.[11] In other words, it will induce a massive synthesis of matrix molecules such as collagen I and fibronectin, whether by osteoblasts or fibroblasts.

Thus, although its regulation mechanisms are particularly complex, TGFβ-1 can be considered as an inflammation regulator through its capacity to induce fibrous cicatrization.

PDGFs: Stimulant of mesenchymatous lineages. PDGFs (platelet-derived growth factors) are essential regulators for the migration, proliferation, and survival of mesenchymatous cell lineages.[12,13] According to the distribution of their specific receptors, they are able to induce stimulation as easily as inhibition of the development of these cells.[14]

This position of regulation node plays a fundamental role during the embryonic development and all tissue remodeling mechanisms. For this reason, PDGFs play a critical role in the mechanisms of physiologic cicatrization and the pathogenesis of atherosclerosis and many other fibroproliferative diseases (eg, neoplasia and pulmonary and renal fibrosis).[15]

The IGF axis: Cell-protective agent. Insulin-like growth factors (IGFs) I and II are positive regulators of proliferation and differentiation for most cell types, which unfortunately include tumor cells (which use the IGF system to increase their survival potential).[16] Although these cytokines are cell multiplication mediators, in the main they constitute the major axis of programmed cell death (apoptosis) regulation, by inducing survival signals protecting cells from many matricial apoptotic stimuli.[17] Moreover, even though IGFs are released during platelet degranulation, they are initially massively present in blood circulation.

PLATELETS AND PRF

Platelet distribution in PRF

Preliminary hematologic studies revealed that platelet in the acellular supernatant (platelet-poor plasma (PPP)) or in the red blood corpuscles base, did not remain. A few histologic analyses were sufficient enough to determine the platelet distribution within the various layers of the centrifuged collection tube: They accumulate in the lower part of the fibrin clot, mainly at the junction between the red corpuscles (red thrombus) and the PRF clot itself (Fig. 1). This last observation underscores the idea that the PRF red extremity would be of interest for clinical use and even more effective than the higher part of the fibrin clot (Fig. 2).

Lastly, it is of high interest to note that the PRF matrix enmeshes glycosaminoglycans (heparin, hyaluronic acid) from blood and platelets. Their histologic aspect after alcian blue staining (Fig. 3) follows the fibrillary architecture of fibrin, meaning that these glycanic links are incorporated within fibrin polymers. Glycosaminoglycans have a strong affinity with small circulating peptides (such as platelet cytokines) and a great capacity to support cell migrations and healing processes.[18]

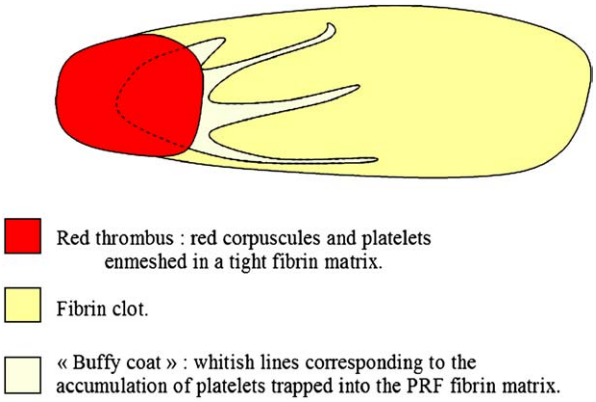

Fig. 2. The PRF fibrin clot obtained according to the Process protocol is divided into 3 parts: a red thrombus in contact with the red blood corpuscle base, an acellular fibrin gel, and a network of buffy columns corresponding to platelet accumulation.

Questions to be studied

The 3 main platelet cytokines play a fundamental role in initial healing mechanisms owing to their capacity to stimulate cell migration and proliferation (particularly by PDGFs) and induce fibrin matrix remodelling as well as secretion of a cicatricial collagen matrix (particularly by TGFβ). Their quantification must first determine where these molecules accumulate: Are they concentrated in the acellular supernatant or trapped in the PRF clot? And, more significantly, are they in solution in the fibrin clot or incorporated in the fibrin polymer network?

We therefore studied the secretion profile of 3 isoforms of these cytokines (PDGF-BB, TGFβ-1, and IGF-I) within the different parts of the PRF collection tube and compared our values with those obtained according to a whole range of cPRP protocols.

This comparative analysis should clarify the relations between fibrin matrix and platelet cytokines released during centrifugation and the main biologic architecture of PRF.

MATERIAL AND METHODS

Blood collection was carried out on 15 healthy volunteers, nonsmoker males from 20 to 28 years of age. In accordance with French law no. 88-1138 of December 20, 1988, dealing with the protection of people participating in biomedical research, and the World Medical Association Helsinki Declaration, volunteers received clear and honest information about the nature and the objectives of our study before testing.

Blood samples were treated according to the PRF protocol with a PC-02 table centrifuge and collection kits provided by Process (Nice, France).[19] They were thus taken without anticoagulant in 10-mL glass-coated plastic tubes immediately centrifuged at 3000 rpm (approximately 400*g*) for 10 minutes. After PRF processing, 2 distinct samples were collected (Fig. 4):

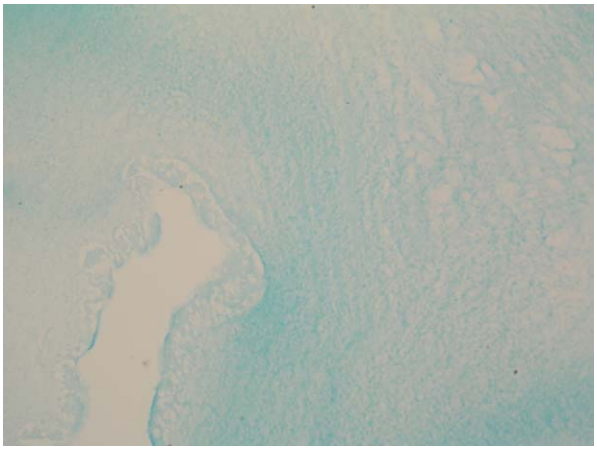

Fig. 3. Glycanic chain distribution within PRF fibrin clot (pH 1 alcian blue staining, 52×).

— The supernatant represented acellular plasma or PPP.
— The exudate resulting from PRF clot corresponded to the solution trapped in the fibrin meshes. For collection, it was necessary to leave our PRF clots in a sterile metal cup for approximately 10 minutes to let them slowly release the serum contained therein.

The 2 sample types were stored in Eppendorf's 2 mL collection tubes at −80°C. The rates of PDGF-BB, IGF-1, and TGFβ-1 ere quantified in these samples by ELISA (Quantikine; R&D Systems, Minneapolis, Minn). Average values were deferred on histograms and analyzed statistically.

These values were then compared to those obtained on total blood according to 2 protocols:

— The blood sample was taken in a dry tube and kept still for 30 minutes, to leave it enough time to coagulate thoroughly. Then the tube underwent a 15-minute centrifugation at 1000*g*, making it possible to recover a representative serum for cytokine rates from completely activated blood.
— The blood sample was taken with anticoagulant (EDTA, citrate, or heparin) and immediately centrifuged for 15 minutes at 1000*g*. Plasma analysis then gave the free circulating cytokine rates in nonactivated blood.

The experimental results were correlated using a Student unilateral test, with 5% significance threshold. These tests were carried out in 2 different ways:

— between the PPP supernatants and the PRF clot exudates;

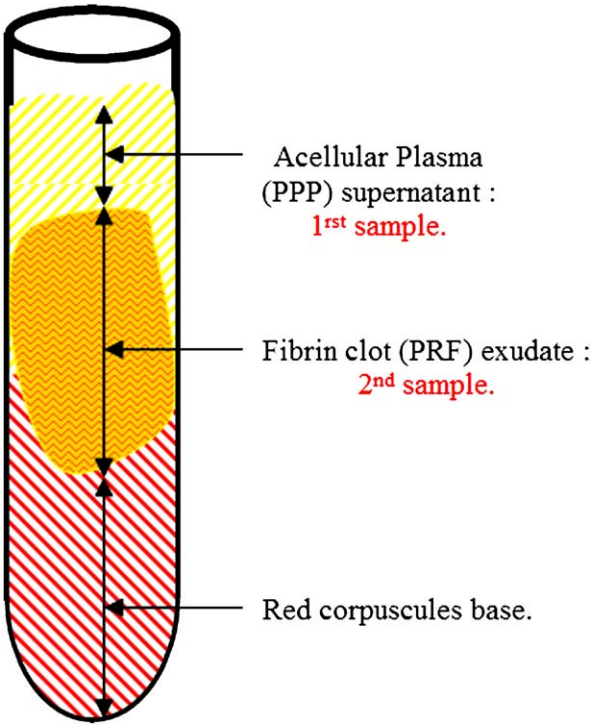

Fig. 4. Schematic representation of the 3 centrifugation strata obtained after PRF processing according to Process official protocol.

— between the PRF clots exudates and respectively the plasmatic and serologic average rates established by the laboratory.

Finally, a comparative study was to be carried out between cytokine quantifications in the PRF clot exudates and established cPRP values originating from 5 different protocols. These latter values were collected in the literature:

— cPRP by cell separator: values of Weibrich et al.[20]
— cPRP by 3I PCCS protocol: values of Weibrich et al.[21]
— cPRP by Curasan protocol: values of Weibrich et al.[21] for IGF-I and TGFβ-1 and of Zimmermann et al.[22] for PDGF-BB
— cPRP by Friadent-Schütze and Harvest SmartPrep protocols: values of Weibrich et al.[23]

RESULTS

Two major items of data were highlighted by statistical analysis of the results.

First, there are no significant differences ($P < 5\%$) between the cytokine concentrations measured in PPP supernatant and those in the actual PRF clot.

Second, PDGF-BB and TGFβ-1 rates measured in supernatants and PRF clots exudates are all quite

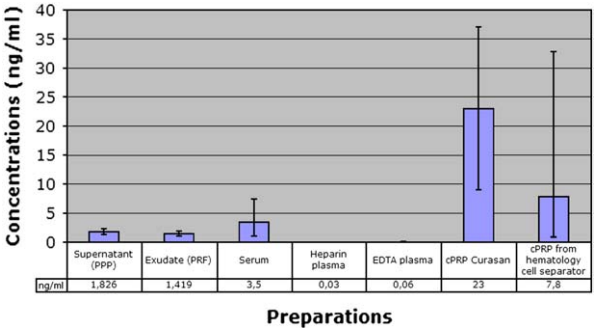

Fig. 5. PDGF-BB ELISA quantifications.

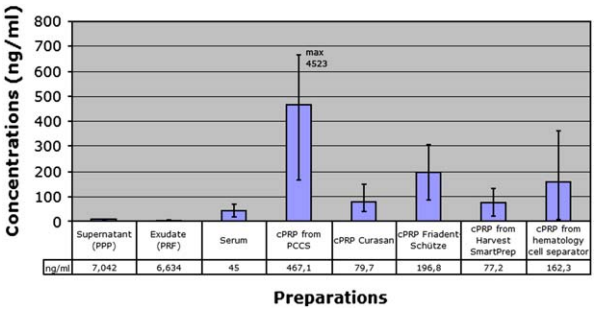

Fig. 6. TGFβ-1 ELISA quantifications.

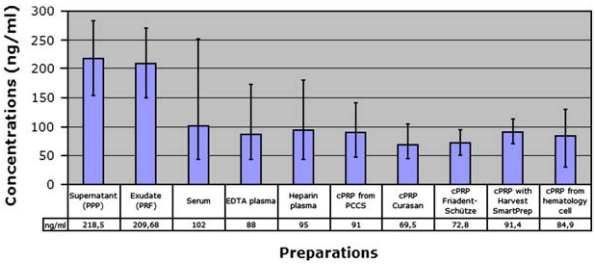

Fig. 7. IGF-1 ELISA quantifications.

significantly lower than those obtained with the several cPRP protocols (Figs. 5 and 6). On the other hand, for IGF-I the supernatant and exudate rates are both significantly higher than for the different kinds of cPRP (Fig. 7).

DISCUSSION

Interpretation of results

The analysis of our results makes it possible to establish significant working hypotheses concerning biologic PRF features. After comparison of our values with those obtained by other authors and vast range of cPRP protocols,[20-23] it is possible to consider that on the whole, PRF platelet cytokines remain trapped in the fibrin meshes, and probably even in the fibrin polymers.[24]

Indeed, during PRF processing, the absence of anticoagulant in the collection tube necessarily induced

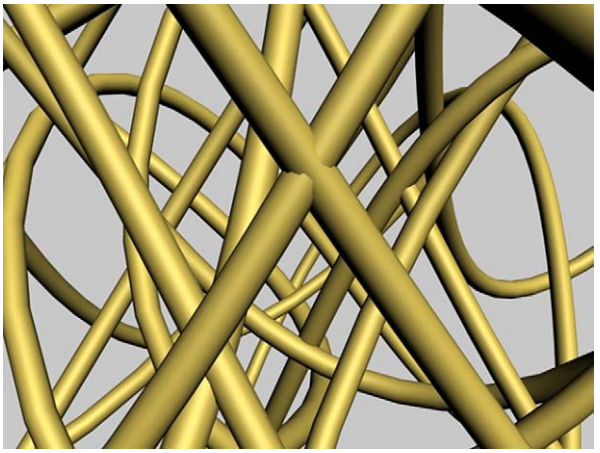

Fig. 8. Theoretical computer modeling of a fibrin network resulting from fibrin glue polymerization. Note that in adhesives such as Tisseel, fibronectin is trapped in the matricial meshes (not represented here) (D-TEP v1.3).

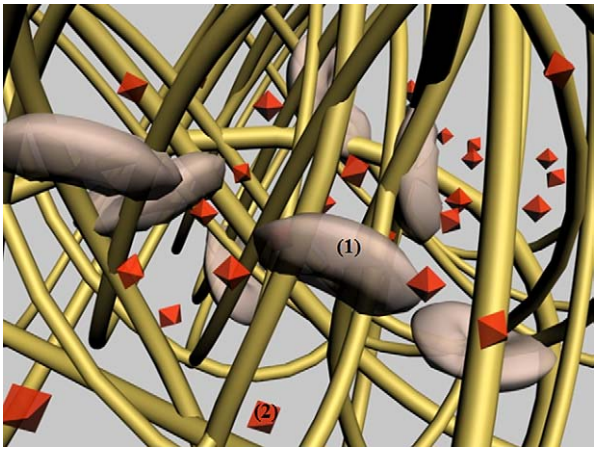

Fig. 9. Theoretical computer modeling of a fibrin network resulting from a cPRP polymerization. The activated platelets are trapped in the fibrin meshes and release a significant quantity of cytokines extrinsically retained in the fibrin architecture (D-TEP v1.3; scales not respected). (*1*) Platelet trapped in the fibrin gel. (*2*) Platelet cytokine in solution (extrinsic).

massive platelet activation, bolstered by the presence of a mineral phase on the tube walls (residual glass particles). These cytokines are small soluble molecules which centrifugation could naturally concentrate in the upper part of the tube, in other words, in the supernatant. However, this is clearly not the case. In fact, the majority of the said cytokines are found neither in the supernatant nor in the exudate. They thus remained trapped in the PRF fibrin matrix, even after serum exudation, which necessarily implies an intimate incorporation of these molecules in the fibrin polymer molecular architecture.

IGF-I is no exception. However, IGF-I is principally a circulating molecule: In the first stages of centrifugation, this cytokine is initially concentrated on the upper part of the tube, thereby explaining the high concentrations measured on that spot. On the other hand, IGF-I resulting from the platelet degranulation will certainly undergo the same matricial incorporation process as TGFβ-1 and PDGF-BB. Note that the IGF-I rates resulting from cPRP technologies are necessarily low, because published quantifications are performed solely on the cPRP platelet concentrate, but, the greater part of the circulating IGF-I is in the PPP (supernatant) which is conventionally discarded during the initial cPRP production steps.

PRF and cPRP: Different polymerizations and biologies

Unlike simple fibrin adhesives (Fig. 8), or cPRP platelet concentrates (Fig. 9), the PRF results from a natural and progressive polymerization occurring during centrifugation. The fibrin network thus formed presents a particularly homogeneous 3-dimensional organization, even more highly coherent than natural fibrin clots. This is confirmed by alcian blue staining: Glycanic

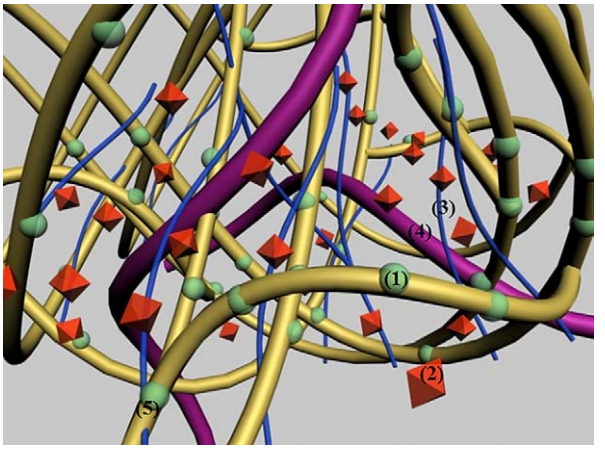

Fig. 10. Theoretical computer modeling of a PRF clot. Note the presence of structural glycoproteins (fibronectin) and extrinsic cytokines (in solution) enmeshed in the fibrin matrix. The PRF slow polymerization process would also allow the intrinsic retaining of glycanic chains and cytokines within fibrin polymers. PRF would be thus very close to a natural fibrin thrombus (D-TEP v1.3; scales not respected). (*1*) Cytokine intrinsically retained within fibrin fibrillae. (*2*) Platelet cytokine in solution (extrinsically associated with fibrin polymers). (*3*) Fibrin-associated glycanic chains. (*4*) Circulating glycoproteins (fibronectin). (*5*) Fibrin fibrilla associated with glycanic chains and intrinsic cytokines.

chains scrupulously follow the fibrillary architecture of the fibrin meshes and these results are observable with key structural glycoproteins such as fibronectin (Fig. 10).

Moreover, a progressive polymerization mode signifies increased incorporation of the circulating cytokines in the fibrin meshes (intrinsic cytokines). Such a

configuration implies an increased lifespan for these cytokines, because they will be released and used only at the time of initial cicatricial matrix remodeling (long-term effect). The cytokines are thus maintained available in situ for a convenient period, when the cells start cicatricial matrix remodeling, ie, when they have to be stimulated to launch injured site reconstruction.

Lastly, slow polymerization with physiologic thrombin concentrations implies a very elastic matricial architecture (equilateral junctions between fibrin fibrillae particularly favorable to cell migration and soluble molecule retention. In contrast, the brutal polymerization mode of cPRP (and fibrin adhesives) makes intimate incorporation of the cytokines in the fibrin matrix difficult. Because of the high thrombin rates necessary for rapid setting of the adhesive, this fibrin will polymerize rigidly (bilateral condensed junctions). The released platelet cytokines will thus be extrinsic, ie, trapped in the colloidal suspension between the fibrin network meshes during gelling (Fig. 8 and 9). Their physiologic elimination will therefore be necessarily fast and a great share of the theoretical cytokines/fibrin synergies from PRF will then be lost (Fig. 10).

All these comparative parameters make it possible to consider the PRF as a healing biomaterial more than a new kind of fibrin biological adhesive.[25,26]

CONCLUSION

This first biochemical analysis of the PRF composition indicates that this biomaterial consists of an intimate assembly of cytokines, glycanic chains, and structural glycoproteins enmeshed within a slowly polymerized fibrin network. These biochemical components have well known synergetic effects on healing processes. As an example, fibronectin, as cell proliferation and migration guide, potentiates the stimulative effects from PDGF-BB. These preliminary data therefore imply that PRF would not only be a new generation of platelet gel, but a completely usable healing concentrate.

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Reprint requests:

David M. Dohan, DDS, MS
Faculty of Dental Surgery
Biophysics Laboratory
1 Rue Maurice Arnoux
92120 Montrouge
France
drdohand@hotmail.com