Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing

Joseph Choukroun, MD,[a] Antoine Diss, DDS, MS,[b] Alain Simonpieri, DDS,[c]
Marie-Odile Girard, DDS,[c] Christian Schoeffler, DDS,[c] Steve L. Dohan,[d]
Anthony J. J. Dohan,[e] Jaafar Mouhyi, DDS, PhD,[f] and David M. Dohan, DDS, MS,[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 fourth article, investigation is made into the previously evaluated biology of PRF with the first established clinical results, to determine the potential fields of application for this biomaterial. The reasoning is structured around 4 fundamental events of cicatrization, namely, angiogenesis, immune control, circulating stem cells trapping, and wound-covering epithelialization. All of the known clinical applications of PRF highlight an accelerated tissue cicatrization due to the development of effective neovascularization, accelerated wound closing with fast cicatricial tissue remodelling, and nearly total absence of infectious events. This initial research therefore makes it possible to plan several future PRF applications, including plastic and bone surgery, provided that the real effects are evaluated both impartially and rigorously. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:E56-60)**

Platelet-rich fibrin (PRF) is an immune and platelet concentrate collecting on a single fibrin membrane all the constituents of a blood sample favorable to healing and immunity.[1-3] Though platelet and leukocyte cytokines play an important part in the biology of this biomaterial, the fibrin matrix supporting them certainly constitutes the determining element responsible for the real therapeutic potential of PRF.[4,5]

To understand the biologic effect of this fibrin matrix, it is important to divide clinical observations into 4 highly specific aspects of healing: angiogenesis, immune control, harnessing the circulating stem cells, and wound protection by epithelial cover.

ANGIOGENESIS, IMMUNITY, AND EPITHELIAL COVER

These are the 3 keys to healing and soft tissue maturation. The membranes of PRF are able to simultaneously support the development of these 3 phenomena.

Fibrin is the natural guide of angiogenesis

Angiogenesis consists of the formation of new blood vessels inside the wound. It requires an extracellular matrix to allow migration, division, and phenotype change of endothelial cells. It has been clearly demonstrated that fibrin matrix leads directly to angiogenesis.[6]

The angiogenesis property of fibrin matrix[7] is explained by the 3-dimensional structure of the fibrin gel and by the simultaneous action of cytokines trapped in the meshes. Furthermore, main angiogenesis soluble factors such as fibroblast growth factor–basic (FGFb), vascular endothelial growth factor (VEGF), angiopoïetin and platelet-derived growth factor (PDGF) are included in fibrin gel. Some studies[8,9] indicate that FGFb and PDGF can bind to fibrin with high affinity. Therefore, direct fibrin angiogenesis induction could be explained by fibrin binding of numerous different growth factors.

In vitro models developed by Nehl and Hermann[10] have shown that the structure and mechanical properties

of the fibrin clot are also important factors. The rigidity of the matrix considerably influences the capillary formation by endothelial cells in response to FGFb or VEGF stimulation. These differences in the fibrin matrix configuration are crucial for understanding the differences of biologic kinetics between fibrin glue, concentrated platelet-rich plasma (cPRP), and PRF.

Finally, an important phase of angiogenesis is αvβ3 integrin expression by endothelial cells, allowing the cells to bind to fibrin, fibronectin, and vitronectin. Important regulation of this integrin expression could be direct, brought on by the fibrin itself. In endothelial human cell culture, fibrin stimulates αvβ3 integrin expression. This is not the case with collagen.[8]

Fibrin constitutes a natural support to immunity

Fibrin and fibrinogen degradation products (FDP) stimulate the migration of neutrophil and increase the membrane's expression of CD11c/CD18 receptor. This receptor permits adhesion of the neutrophil to endothelium and fibrinogen as well as the transmigration of neutrophils.[11]

Moreover, the phagocytosis of neutrophils and the enzymatic degradation process are modulated by FDP.[12]

Monocytes arrive at the injury site later than neutrophils. It has been demonstrated that the wound colonization by macrophages is controlled by fibronectin via the chemical and physical properties of fibrin and by chemotactic agents trapped in its meshes.[13]

For example, FDP D-dimer added to the culture medium of human promonocytic cell lines increases the interleukin (IL)-1 and plasminogen activator (uPA) secretion.[14] This implies a positive feedback of fibrin in inflammatory events.

Fibrin and wound coverage

Fibrin matrix guides the coverage of injured tissues, affecting the metabolism of epithelial cells and fibroblasts.

Around the wound's margins, epithelial cells lose their basal and apical polarity and produce basal and lateral extensions toward the wound side. The cells subsequently migrate on the transitory matrix made by fibrinogen, fibronectin, tenascin, and vitronectin. This migration is more like a genuine matrix degradation than a simple translation.

Fibrin, fibronectin, PDGF, and transforming growth factors (TGF-β) are essential to modulate integrin expression, fibroblast proliferation, and their migration inside the wound.[15] These can be bound directly with fibrin by different integrins, of which αvβ3 integrin is primary. Thanks to the expression of 2 plasminogen activators, fibroblasts develop an important proteolytic activity to move within the fibrin clot. Furthermore, the in vitro migration of rat fibroblasts in fibrin gel is optimal when there is a maximum number of crossed connections between α-chains.[16] This fact represents one of the most important differences between swift polymerization of fibrin glues (and by extension, cPRP) and slow gelation of PRF.

After migration and degradation of fibrin, fibroblasts start the collagen synthesis as described in the in vitro healing model.[17]

Clinical implications

With these fundamental considerations, PRF can be considered as a natural fibrin-based biomaterial favorable to the development of a microvascularization and able to guide epithelial cell migration to its surface. The interest of such a membrane is evident, namely, to protect open wounds and accelerate healing. Furthermore, this matrix contains leukocytes and promotes their migration. Its utilization seems to be of high interest in the case of infected wounds.

A current clinical example deals with the filling of a tooth socket by PRF. Quickly, a neovascularization forms through the PRF clot and the epithelial covering developed. Finally, in spite of the infectious and inflammatory statement of such sockets, rapid healing of the wound is observed without pain, dryness, or purulent complications (Fig. 1).

ANGIOGENESIS AND HARNESSING OF STEM CELLS

During any phenomenon of hemostasis and healing, the fibrin clot traps the circulating stem cells brought to the injured site thanks to initial neovascularization. Set in fibrin matrix, these cells converge on a secretory phenotype, allowing the vascular and tissue restoration.[18,19]

PRF, as a physiologic fibrin matrix, serves as a net to stem cells, especially when an accelerated angiogenesis develops in the fibrin membrane.[7] This aspect is of particular interest in the case of wide osseous defects. Indeed, such healing requires accumulation of medullar stem cells and their conversion toward the osteoblast phenotype.

Fibrin and mesenchymal stem cells

Mesenchymal stem cells from bone marrow contribute to regeneration of whole-type bone cells and many other tissues.

These undifferentiated cells are recruited from blood to injured tissues,[20,21] where they are able to differentiate themselves into several different cell types. This initial differentiation occurs necessarily in a transitory scar matrix formed by fibrin and fibronectin. That is why fibrin is preferentially used as support matrix for

Fig. 1. Tooth extraction and osseous filling in a case of terminal periodontitis of wide sites (**A** and **B**) are delicate interventions because of the difficulty in obtaining soft tissue coverage on the surface of the osseous injury. Sockets are filled with Phoenix allogenic bone (TBF, France), (**C**). The use of PRF as cover membranes (**D** and **E**) permits a rapid epithelialization of the surface of the site, neutralizing the infectious phenomena. Forty-eight hours postoperative, wound is totally closed and sutures are removed (**F**).

the transplantation of these cells. Several authors have demonstrated that a fibrin matrix is an optimal support to transplanted mesenchymal stem cells for obtaining osseous defect regeneration.[22-24]

An experimental fibrin matrix made of 18 mg/mL fibrinogen and activated by 100 IU/mL thrombin appears optimal, in vitro, for stem cell proliferation and migration.[22] Such an artificial matrix seems very similar to a natural fibrin clot such as PRF.

Fibrin and osseous tissue

Direct interactions between fibrin and osseous cells during healing are insufficiently documented. On the other hand, numerous animal studies deal with the fibrin effect on osseous healing. The results are contradictory; osseous healing is either improved or remains unchanged.[25] These divergences may be caused by differences between the models used: animal type, osseous defect, and fibrin gel.
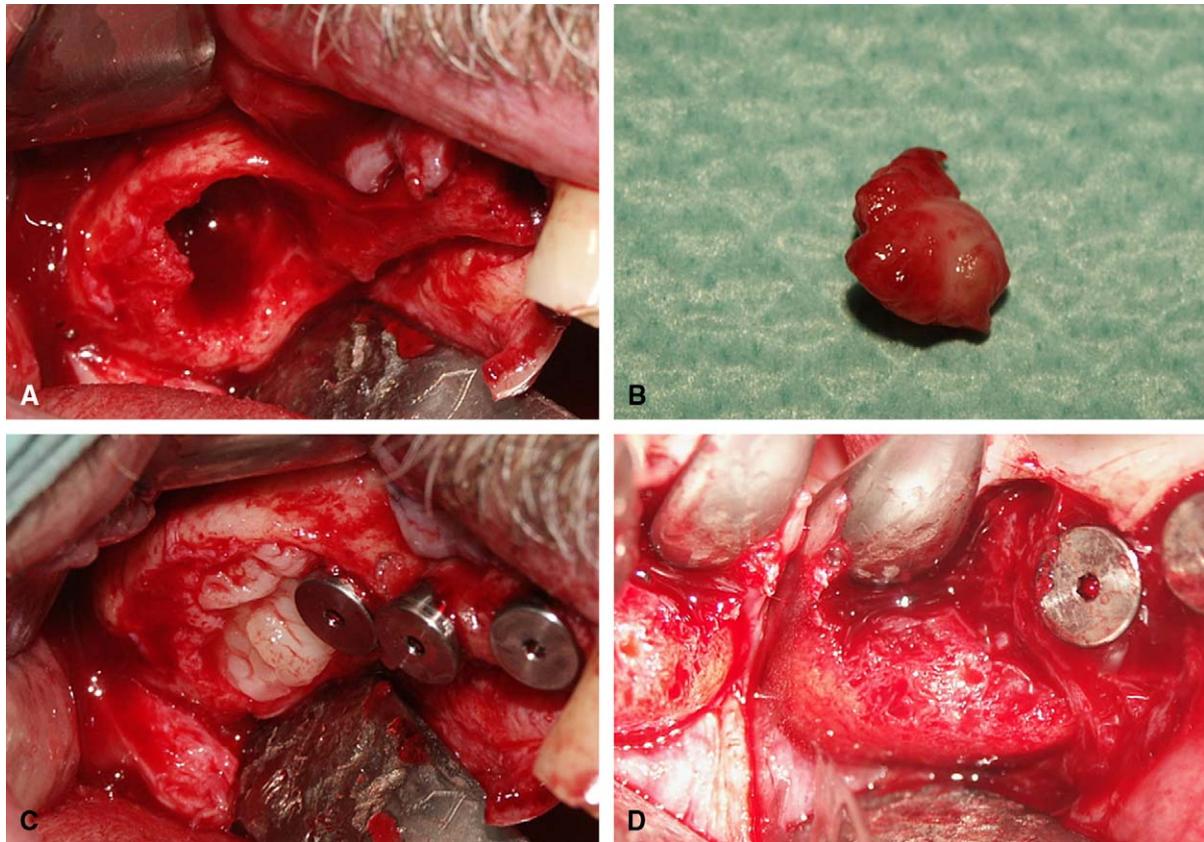
Fig. 2. During massive cystic ablation of the maxillary (**A** and **B**), residual cavity is filled with PRF (**C**). Two and a half months later, the osseous defect is replaced by a dense and cortical bone (**D**) instead of the average 10 months naturally. The use of PRF allows acceleration of the physiologic phenomena.

Nevertheless fibrin is a recognized support matrix for bone morphogenetic protein (BMP) transplants. Therefore, the fibrin matrix associated with BMPs has angiotrophic, hemostatic, and osseous conductive properties.[26] BMPs enmeshed in the fibrin matrix are progressively released, and when transplanted intramuscularly they are able to induce bone. This progressive release of cytokines is a common feature of *in vivo* natural fibrin clot and likely of PRF.

Clinical implications

These fundamental elements are illustrated during maxillary cystic ablation. After complete cystic ablation, the cavity fills quickly with blood. This blood clot is nothing more than a "light" version (physiologic version) of PRF. The fibrin clot matrix is a trap for the circulating stem cells. Thus, physiologic healing time of this cystic cavity lies between 6 months and 1 year.

When the cystic cavity is filled with PRF, this physiologic healing phenomenon is accelerated. Because the PRF fibrin matrix is better organized, it is able to more efficiently direct stem cell harnessing and the healing program.

A cystic cavity filled with PRF will be totally healed in 2 months instead of the 6 to 12 months required for physiologic healing (Fig. 2).

DISCUSSION: WHICH FIELDS OF APPLICATION FOR PRF?

PRF has to be considered as a fibrin biomaterial. Its molecular structure with low thrombin concentration is an optimal matrix for migration of endothelial cells and fibroblasts. It permits a rapid angiogenesis and an easier remodeling of fibrin in a more resistant connective tissue. Therefore, these PRF membranes can be used for all types of superficial cutaneous and mucous healing.

But PRF is not only a simple fibrin membrane. It is also a matrix containing all the molecular and cellular elements permitting optimal healing. The matrix carries all the favorable constituents present in a blood sample. That is why this biomaterial can be considered a physiologic concentrate. It is obtained without any addition or manipulation.

Numerous extraoral applications might be imagined. In plastic surgery, the esthetic result of cutaneous wound

healing constitutes a recurrent problem. In this respect, fibrin glues are still used in this discipline for their capacities to prevent formation of keloid scars. The use of PRF in this type of surgery has to be tested.

Nevertheless, only a limited volume of PRF can be used. Because it is obtained from an autologous blood sample, the quantities produced are low. This fact limits the systematic utilization of PRF for general surgery. PRF tissue banks are unfeasible. The fibrin matrix contains all the circulating immune cells and all the highly antigenic plasmatic molecules. That is why PRF membranes are totally specific to the donor and cannot constitute an allogenic graft tissue.

CONCLUSIONS

The clinical experience confirms that PRF can be considered as a healing biomaterial. It features all the necessary parameters permitting optimal healing. These consist of a fibrin matrix polymerized in a tetramolecular structure, the incorporation of platelets, leukocyte, and cytokines, and the presence of circulating stem cells.

Despite the fact that cytokines trapped in PRF are gradually released and able to accelerate the cellular phenomenon, the structure of the fibrin network is the key element of all improved PRF healing processes.

Finally, from a clinical standpoint, this biomaterial appears to accelerate physiologic healing and the numerous perspectives of PRF have still to be clinically tested.

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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