Tissue response to platelet-rich fibrin: clinical evidences. Part 1: socket extractions

Joseph Choukroun and **Marco Del Corso** examine the use of platelet-rich fibrin in accelerating tissue healing

Summary

Platelet-rich fibrin (PRF) can be considered as an autologous healing biomaterial, incorporating leukocytes, platelets and the majority of the molecules that take part in the tissue healing processes within the autologous fibrin matrix.

Due to the presence and the slow release of its growth factors, PRF enhances healing phases; used as a membrane, it can be used on its own to fill extraction sites, or in combination with filler materials to protect the graft and to anticipate the wound margins healing. Several clinical applications are discussed in this article.

One of the goals in oral surgery is to obtain the best aesthetic result after the healing phases. Several techniques have been developed to reduce incisions and facilitate reconstructive procedures, while at the same time preserving the micro-circulation to the flap margins. The search for protocols promoting haemostasis and healing is thus a recurrent problem in all surgical disciplines.

Thanks to the work of Lynch (1987), there has been a large interest in growth factors in periodontology and implantology. Marx, in 1998, published the first studies on the use of platelet growth factors in oral surgery by fabricating platelet-rich plasma (PRP) (Marx et al 1998; Marx 2001). Since then, different platelet aggregates have been used in a large number of clinical situations.

In periodontal, implant and maxillofacial surgery, platelet aggregates play a role as biological bonding agents between the different components of a bone or gingival graft and act as a protective gel for the operation site, similar to the autologous fibrin glues used in the past (Clark 2001), where the fibrinogen was activated by the action of calcium and thrombin.

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Aims and objectives

The aim of this article is to demonstrate the applications for the autologous healing biomaterial platelet-rich fibrin in implant treatment.

The reader will:

- Gain a full understanding of the definition of platelet-rich fibrin
- Understand the tissue response to platelet-rich fibrin
- Be made aware of its indications for clinical treatment.

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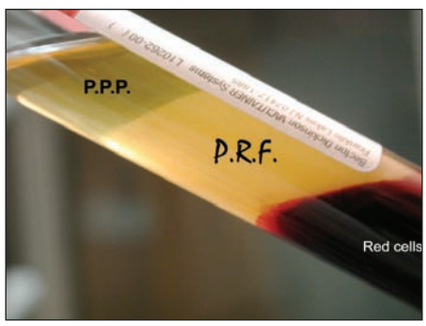
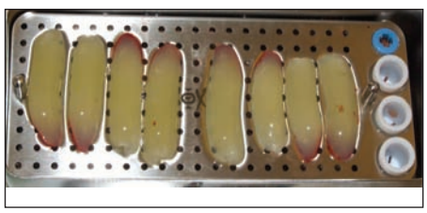




Figures 1a & 1b: The PRF clot in the vacutainer after centrifugation. Thanks to the specific Box, the clot is transformed in membrane and the serum exudate is recuperated

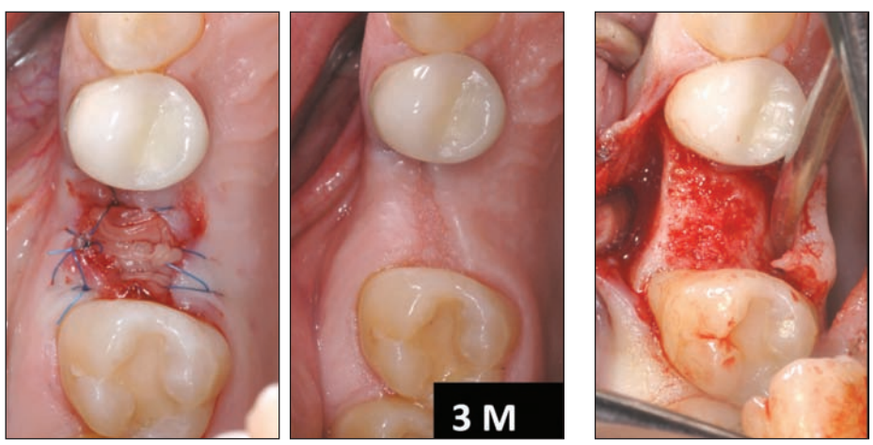


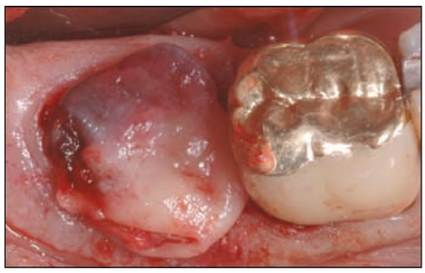
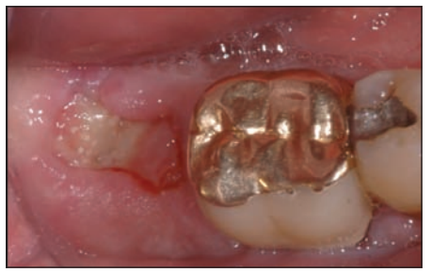
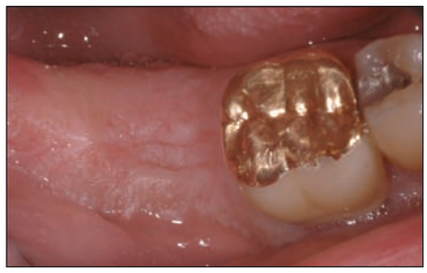
Figure 3: The membrane can be left exposed and sutured to the wound margins. This helps avoid discharging incisions and respects the vascularisation of the site. Healing at three months: good reconstitution of the defect with no evidence of biomaterial granules.

In 2001, a new protocol was suggested in France by Choukroun for concentrating growth factors as an alternative to PRP: the platelet-rich fibrin, or PRF (Choukroun et al 2001).

Definition of PRF

PRF can be considered as an autologous healing biomaterial incorporating, in a matrix of autologous fibrin, a high percentage of leucocytes, platelets and growth factors obtained from a simple blood sample drawn from the patient at the time of the surgical procedure.

PRF's fibrin contains growth factors as

Figures 4a, 4b, 4c: PRF membrane exposed in a post-extractive site: healing at 15 days and aspect at two months

PDGF (platelet-derived growth factor), TGFβ (transforming growth factor beta), IGF (insulin-like growth factor), and VEGF (vascular endothelial growth factor), and provides an accelerated healing of superficial and bone tissues, developing a good neo-angiogenesis and faster wound healing (Dohan et al 2006; Dohan et al 2006; Dohan Ehrenfest et al 2010).

Methodology of PRF

The blood sample is treated with a single centrifugation, in a specific centrifuge, with no blood manipulation, no anti-coagulant, no bovine thrombin, no sodium nitrate or calcium chloride. At the end of the centrifugation process, three distinct fractions are found (Dohan et al 2006; Dohan et al 2006)

- A deeper level part containing the red cells
- A superficial part containing platelet-poor plasma
- An intermediate fraction including the platelet-rich fibrin (PRF) clot, which is then clinically used in the form of membrane. The PRF's fibrin membrane is more elastic and consistent than the platelet concentrated clot traditionally obtained in some PRP protocols (Mosesson 2005) and can be employed in several clinical situations (Figures 1a & 1b).

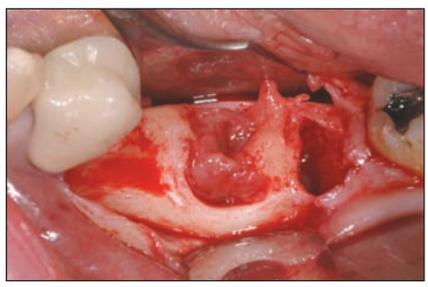
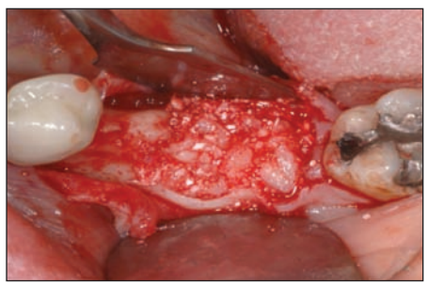
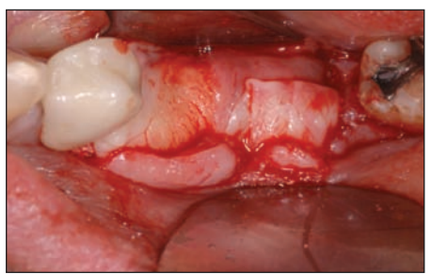
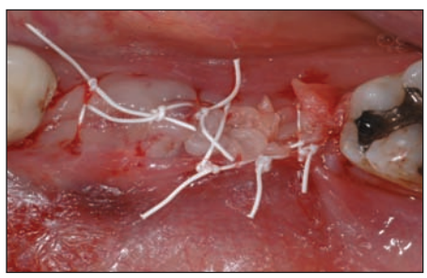
The presence of leukocytes (containing PDGF and VEGF) (Dohan et al 2006; Dohan et al 2006) and the absence of manipulation differentiate PRF from other, more complex platelet concentration protocols (Weibrich, Kleis and Hafner 2002).

The fibrin clots are treated immediately in the PRF Box, a tool that guarantees the adequate preparation of fibrin membranes or plugs destined to be applied in the socket extractions. The serum exudate is collected in the second level of the box and can be used for a longer conservation of the membranes, and is ready to be mixed with a bone biomaterial for grafting.

Tissue healing response to PRF

It has already been demonstrated in the past that a matrix of fibrin or fibronectin could modulate – in tissue healing phases – the response of the fibroblasts to certain cytokines, acting on the expression of the endothelial cells (Clark 2001). Other studies confirm that the fibrin matrix allows recruitment, migration, adhesion and differentiation of the different cell types necessary for tissue repair (Lanir et al 1988, Dohan et al 2003, Dohan et al 2004). It is inside fibrin that neo-vessels grow; following tissue injury, under normal conditions the patient's fibrin is colonised rapidly by inflammatory cells, fibroblasts and endothelial cells, which remodel it into granulation tissue and subsequently to mature connective tissue (Clark 2001).

Two studies (Dohan et al 2003; Dohan et al 2004) have demonstrated the presence of all the platelets and their growth factors in the fibrin clot obtained with the PRF technique. The platelet cytokines and especially PDGF, RGFb-1 and IGF are gradually released during fibrin matrix physiological resorption, and this allows the healing process to be protected from external injuries. Indeed, the gradual release of cytokines appears to play a regulatory role in the inflammatory phenomena within the graft (Gurevich et al 2002, Le Guehennec 2005).

Figures 5a, 5b, 5c, and 5d: Complex infra-bony defect following multiple extractions. An important graft of mixed biomaterial and PRF is performed. The graft is covered with a PRF membrane and sutured: some portions of the membrane are exposed

Use in post-extraction sites

The role of PRF is to bring autologous fibrin, leucocytes, platelets and their growth factors into the surgical site.

Fibrin is a tissue healing matrix in which neo-vessels grow-up, thanks to VEGF derived from leucocytes (Mosesson 2005; Laurens, Koolwijk and De Maat 2006). Clinical evidence demonstrates accelerated tissue reconstruction in the presence of PRF compared to physiological healing times.

Management of the extraction site is complex thanks to both aesthetic reasons (for example, in the incisor region) and the need to avoid bone resorption following extraction.

For this reason, it is often necessary to insert a filler material inside the extraction site to maintain the residual bone volume.

By using allografts, depending upon the volume of post-extractive defects, the revascularisation of the site could be delayed. Indeed, the management of soft tissue over the graft could request flap traction and discharging incisions that reduce micro-vascularisation at the margins.

While it is possible to combine PRF with different filling materials such as autologous bone (chin, ascending ramus of the mandible, maxillary tuberosity, etc), allogenic bone (inorganic human bank bone) or xeno-bone (inorganic bovine, porcine, equine, etc), the fibrin itself and the cytochines help cellular migration and revascularisation of the grafted site.

The consistency of PRF has suggested the use of this biomaterial as a guided tissue regeneration membrane, to cover and protect the bone graft material and the operative site in general. In particular, the elasticity of the PRF fibrin allows it to be included in the suture (Figures 2 and 3).

In cases where the size of the extraction socket does not allow the margins of the wound to be sutured perfectly, PRF's fibrin matrix promotes re-epithelialisation of the site, accelerating the fusion of the muco-gingival incision margins (Figures 4a, 4b,and 4c) (Lanir et al 1988).

Epithelial and connective tissue healing is a consequence of the density of the fibrin matrix, which is remodelled faster by gingival fibroblasts migrating on this matrix.

The acceleration of these healing processes makes the treated site less sensitive to outside attacks (mechanical, bacterial and chemical) and crucially, influences the aesthetic result and the patient's postoperative comfort.

At deeper levels, PRF increases the cohesion between the grafted biomaterial particles and facilitates the growth factors releasing locally at the graft site (Laurens, Koolwijk and De Maat 2006; Salasznyk 2004).

Infra-bony defects treated with PRF

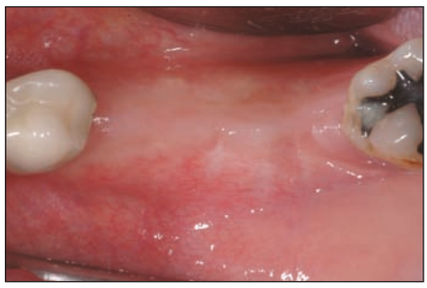
The treatment of infra-bony defects could be positively influenced by using PRF's membrane. In circumferential defects with entire walls, PRF alone leads to rapid re-ossification combined with excellent healing of the wound, even when this does not provide perfect approximation of the margins.

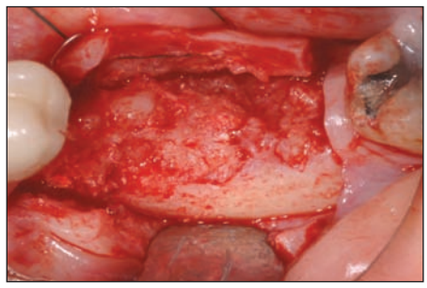
If a single wall is missing, the use of filling material in combination with PRF is indicated (Figures 5 and 6)

Conclusions

Clinical interest in the use of PRF lies not only in the simplicity of the protocol and morphological versatility of the fibrin membrane, but also in its potential for accelerating the processes of tissue healing. The powerful stimulus for neo-angiogenesis is a characteristic of this biomaterial.

Used as a membrane, which can also be sutured, the PRF allows the surgical site to be protected from external injuries and constitutes a matrix for faster healing of the wound edges. It generally provides a perceptible reduction in superficial tissue healing times, and patients often report reduced postoperative pain.





Figures 6a and 6b: Aspect of the mucosa at four months; aspect of the crest at four months

Mixed with graft materials, PRF attracts mesenchymal cells and new blood vessels: this may explain the rapidity of healing times. The high concentration of plasmatic cytokines and fibrin exert an osteogenic effect on bone progenitor cells and the concentration of leukocytes contained in the PRF appears to guarantee an immune action that facilitates the success of large grafts.

Other basic and clinical studies should be conducted to provide a better understanding of the mechanisms of this versatile healing biomaterial. I

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