

Use of PRF in Bony Regeneration Surgery

M. del Corso, J. Choukroun & D. Dohan, France

Platelet-rich fibrin (PRF) can be regarded 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. In this article, the clinical and

biological aspects of the action of PRF on bone regeneration in the various fields of application in oral surgery are analyzed.

The search for protocols promoting hemostasis and healing is a recurrent problem in all surgical disciplines. Thanks to the

work of Lynch¹, there has been interest in growth factors in peri-implantology, and Marx in 1998 published the first studies on the use of platelet growth factors in the form of platelet-rich plasma.^{2,3} Since then, platelet aggregates of biological quality high in cytokines have been used

in a large number of clinical situations.

In periodontal, peri-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 past⁴⁻¹² where the fibrinogen was activated by the action of calcium and thrombin.

In 2004, a new protocol was suggested in France for concentrating growth factors as an alternative to PRP: platelet-rich fibrin or PRF.¹³

Definition of PRF

PRF is a matrix of autologous fibrin high in platelets and growth factors obtained from a simple blood sample drawn from the patient at the time of the surgical

procedure. PRF can also be regarded as a platelet concentrate of high immunological value incorporating, in the form of an autologous fibrin membrane, all the elements involved in healing and the immune response contained in a 10 ml blood sample.¹⁴

The blood sample is treated with a single centrifugation in a suitable machine; at the end of the process, three distinct fractions are found¹⁵:

- a low part containing the red cells;
- a superficial part containing platelet-poor plasma (PPP);
- an intermediate fraction comprising the platelet-rich fibrin clot, which has various clinical uses.

This simple protocol allows a platelet concentrate that is held inside the autologous fibrin mesh to be obtained by a physiological process and without manipulation of the sample or addition of excipients. The fibrin clot is then solidified in the form of a membrane that is more elastic and consistent than that obtained in some PRP protocols.^{2,3,16-9} The presence of the leukocytes and the growth factors that these contain (PDGF and VEGF)¹⁴, further differentiates PRF from other more complex platelet concentration protocols.^{14,21,23}

Biology of PRF

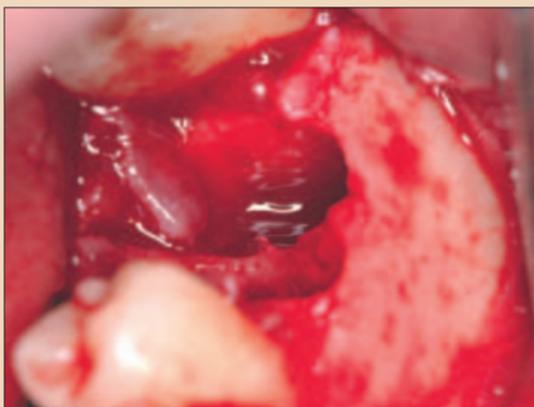
Following tissue injury, under normal conditions, the patient's fibrin is colonized rapidly by inflammatory cells, fibroblasts and endothelial cells, which remodel it into granulation tissue and subsequently to mature connective tissue.²⁵

It has already been demonstrated in the past that a matrix of fibrin or fibronectin could modulate the response of the fibroblasts to certain cytokines, acting on the expression of the endothelial cells.²⁴ Other studies confirm that the fibrin matrix allows recruitment, migration, adhesion and differentiation of the different cell types necessary for tissue repair.²⁵⁻²⁸

Two studies^{29,30} have demonstrated the presence of all the platelets and their growth factors in the fibrin clot of fibrin obtained with the PRF technique. The platelet cytokines and especially PDGF, RGFb-1 and IGF are released gradually during physiological reabsorption of the fibrin matrix, and this allows the healing process to be achieved protected from external attack.



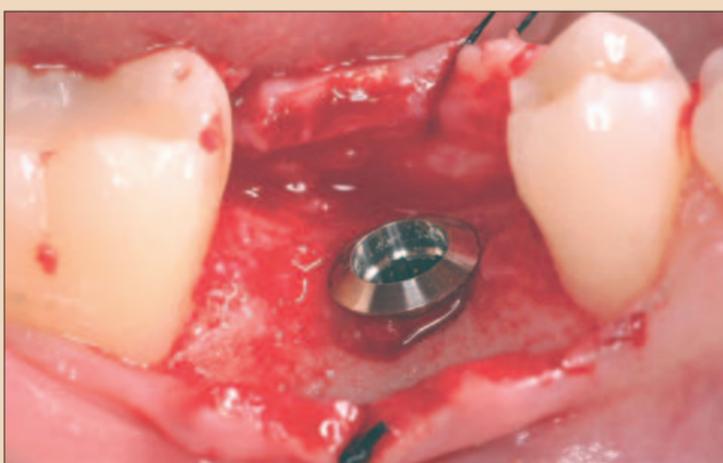
Figs. 1, 2: Clinical Case 1: tooth 3.6 is extracted following endodontic failure. Note the chronic lesion at the tip of the distal root.



Figs. 3-5: After careful curettage, the extraction socket is filled with PRF without adding any other graft material. The suture does not fully approximate the wound margins, which will heal by second intention in a faster period.



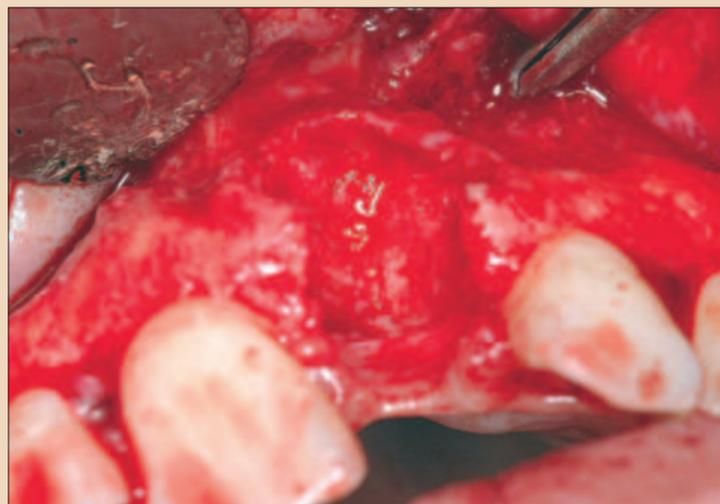
Figs. 6-8: After 4 months, the site treated with PRF alone has the clinical appearance of excellent mucosa and, upon reopening, all the bone appears to have re-formed. The clinical impression is of type D1 compact bone.



Figs. 9, 10: It is then possible to place an Intra-Lock implant (Boca Raton FL, USA) with a diameter of 4.75 mm.



Figs. 11, 12: **Clinical Case 2:** a young patient was referred to us because of a persistent mucosal lesion despite endodontic treatment performed after trauma to the frontal group.



Figs. 13, 14: The frontal incisor is extracted together with an apical lesion involving the vestibular bone margin.

Fig. 15: Detail of the residual infraosseous lesion: absence of an intact vestibular bony roof is a major detriment for the future dental esthetic and necessitates bone regeneration treatment.

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According to some studies^{31,32} the fibrin matrix appears to possess osteoinductive properties. It has also been shown that osteoblastic cells manifest marked sensitivity to PRF, which is shown by an increase in proliferation and differentiation (J. Choukroun, International Conference on Immediate Loading, Naples, Italy, 25-27 May, 2006).

As regards the difference between PRF and PRP, according to studies by Zhu and Choi³³, it appears that the addition of fibrin to the PRP protocols provides better histological results in bone tissue engineering.

The presence of both components (high concentration of cytokines and fibrin with a physiological architecture) thus suggests the use of PRF in bone with the aim of providing a stimulus to cellular differentiation in osteogenesis. The gradual release of the cytokines appears finally to play a regulatory part in the inflammatory phenomena within the graft.

Use in Post-extraction Sites

Management of the extraction site is complex both for esthetic reasons (for example, in the incisor region) and to avoid bone resorption following extraction. For this reason, it is often necessary to insert a filler material inside the extraction site to preserve the residual bone volume.

The elasticity and consistency of PRF have suggested use of this biomaterial as a guided tissue regeneration (GTR) membrane to cover and protect the bone graft material and the operative site in general.

In cases where the size of the socket does not allow the margins of the wound to be approximated perfectly, the fibrin matrix of the PRF promotes re-epithelialization of the site, accelerating fusion of the margins of the muco-gingival incision.²⁵ Epithelial and connective tissue healing is a consequence of the density of the fibrin matrix which, at more superficial and well vascularised levels, is rapidly degraded by the circulating thrombin.

This fact implies faster remodeling by the gingival fibroblasts, which migrate on this matrix. The acceleration of the healing processes makes the treated site less sensitive to outside attack (mechanical, bacterial and chemical) and crucially influences the esthetic result and the patient's postoperative comfort. At deeper levels, there is a potential for influencing the long-term remodeling of more complex bony tissues.

Naturally, it is possible to combine the PRF membrane with different filling materials such as autologous bone (chin, ascending ramus of the mandible, maxillary tuberosity, etc), allogenic bone (inorganic human bank bone) or xenogenic bone (inorganic bovine, porcine, equine, etc).

In circumferential defects with more walls, PRF alone leads to rapid reossification combined with excellent healing of the wound, even when this does not provide perfect approximation of the margins (Figs. 1-20).

In vitro studies have shown that even in the absence of mo-

lecular signals (growth factors, cytokines), collagen is capable of stimulating differentiation of undifferentiated human mesenchymal cells (hMSC) into osteoblasts.³⁴ Clinical studies accompanied by histological analysis have demonstrated that preservation of the natural collagen present in some heterologous biomaterials provides a valid substrate capable of stimulating and accelerating the physiological process of

osseous regeneration, at the same time guaranteeing perfect biocompatibility and the absence of inflammatory reactions at the graft site.³⁵⁻³⁷

Based on this scientific evidence, in the last three years we have used a collagenated heterologous biomaterial (Gen-os porcine cortico-cancellous bone, OsteoBiol, Turin, Italy) as a filling material with excellent results.

In the case of infraosseous defects in particular, PRF was used in the form of a membrane and mixed with the graft material according to a specific protocol. The presence of the fibrin and collagenated material makes it easy for the surgeon to use, increasing the cohesion between the particles and facilitating persistence at the graft site (Figs. 11-19).

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Figs. 16, 17: The defect is filled with a mix of PRF and granular cortico-cancellous bone (Gen-Os, OsteoBiol, Turin) and covered with a PRF membrane.



Fig. 18: After 5 months, an Intra-Lock implant 13 mm in length (Boca Raton FL, USA) is placed.

Fig. 19: Upon reopening, performed after 4 months, resonance frequency analysis (RFA) gives a value of ISQ = 71 measured with the Ostell method (Integration Diagnostics, Savedalen, Sweden) (38-42). Tissue conditioning is performed with a resin temporary (in the photo).

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Use in Sinus Lift

The dense and resistant filling (from a mechanical and chemical aspect) of PRF can help to structure the bone graft, especially in protected sites such as the maxillary sinus. Mixed with sinus filling materials (autologous bone, heterologous bone, xenografts functioning as scaffolding), PRF functions both as a bonding agent between the bone fragments and as a matrix promoting neo-angiogenesis and migration of bone progenitor cells to the inside of the graft.^{44, 45}

The tissue-specific power of the cytokines contained in it allows good histological results to be obtained after only three months even when the sinus elevation is considerable. In these cases of considerable elevation, it is appropriate to use a topical antiseptic such as metronidazole 0.5%⁴⁶⁻⁴⁸ in order to avoid the formation of gas vacuoles produced by anaerobes.

In the event of perforation⁴⁹, the PRF membrane can also be placed in contact with the Schneiderian membrane with the aim of reconstructing and reinforcing its integrity before in-

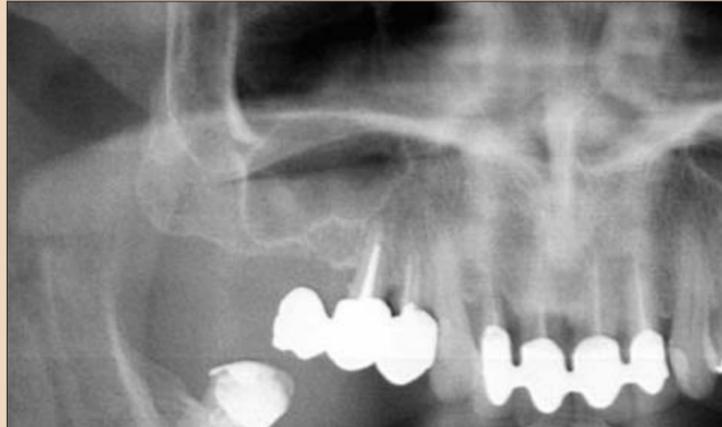


Fig. 20: Clinical Case 3: In a 65-year old patient, elimination of the extension element on 1.6 is planned, followed by prosthetic restoration of the missing molars with implants. The height of the basal bone in this area does not exceed 2 mm.

limiting bone resorption, the implant placement reduces the risk of vestibular perforation or dehiscence.⁵⁰⁻⁵²

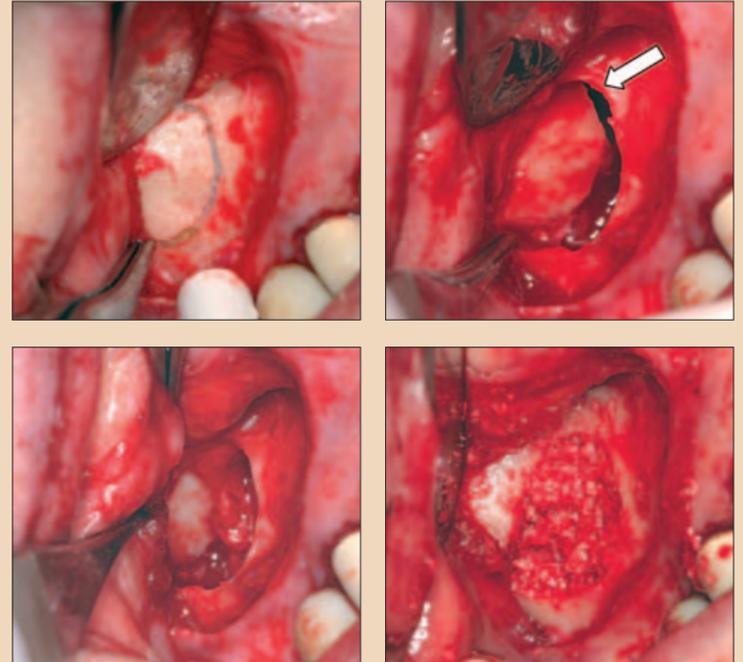
However, in these situations, the poor fit between the coronal part of the implant and the alveolar walls can be significant, especially in the case of replacement of an upper canine where the diameter of the implant often does not correspond to the oval shape of the root of the lost tooth. To fill the space between the implant and the thin bony vestibular wall, autologous or allogenic filling material and a covering membrane are used.

of the layer of vestibular bone in these cases.

If the edges of the wound are not perfectly approximated, closure of the site can be ensured by the membranes, which are held in place with sutures. Epithelialization of the surgical wound is complete in 12-15 days.

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



Figs. 21-23: Opercularization of the maxillary sinus shows laceration of the Schneiderian membrane mesially. Reconstruction of the anatomy of the floor is achieved by interposing a layer of PRF membrane.

Fig. 24 A particulate cortico-cancellous bone graft (Gen-Os, OsteoBiol, Turin) is placed mixed with PRF according to the specific protocol. A PRF membrane is positioned on the opening to protect the surgery site.

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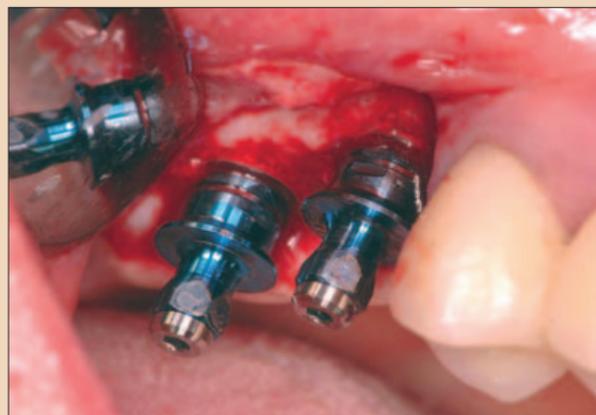
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Fig. 25-27: After 3 months, two 3i Osseotite implants (Implant Innovations, Palm Beach FL, USA) of 11.5 mm with XP platform are placed.



serting the graft material in the submucous space (Figs. 20-28). Finally, positioning the PRF membrane over the fenestration of the sinus graft achieves faster healing of the surgical margins with the aim of protecting the graft from external attack.

Isolated Use in Peri-implant Regeneration

Immediate implant placement in a post-extraction site combines post-extraction healing with the phase of implant osseointegration. In addition,

Naturally, it is possible to insert a mix of PRF and filling material to act as scaffolding, which will maintain the vestibular emergence convexity of the crown. When the height of the vestibular bone crest and the distance between this and the implant surface allow⁵⁵, it is possible to use PRF alone as a filler and protective barrier for the implant and peri-implant space thus treated (Figs. 29-35). Review four months later shows the perfect mucosal healing of the site and good preservation

processes of tissue healing together with its function as a potent stimulus to neo-angiogenesis, which is a characteristic of the biomaterial.

Used in the form of a membrane, PRF allows the surgical site to be protected from external attack and constitutes a matrix for faster healing of the wound edges. It generally provides a perceptible reduction in healing times in the superficial tissues, and the patients often complain less of postoperative pain.

Mixed with graft materials, fragments of PRF attract mesenchymal cells and new blood vessels. This may explain the rapidity of ossification of the graft. The high concentration of plasma 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 action of this versatile healing biomaterial. □



Fig. 28: The radiograph after one year shows good preservation of the deep tissues subject to biomechanical load.

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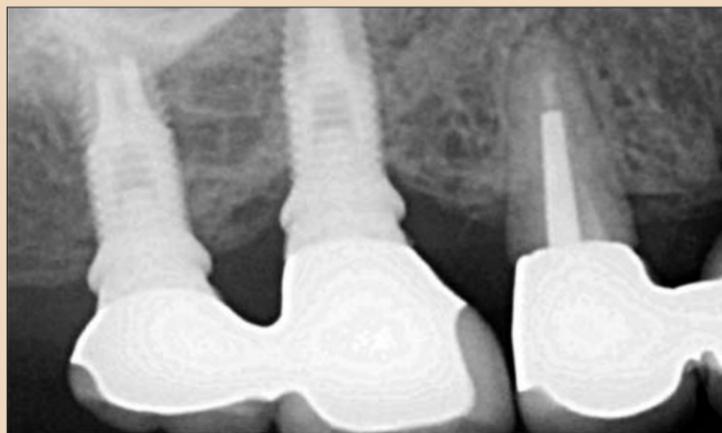


Fig. 29: Clinical case 4: Infiltration of the crown of 2.3 requires avulsion and implant prosthetic restoration.

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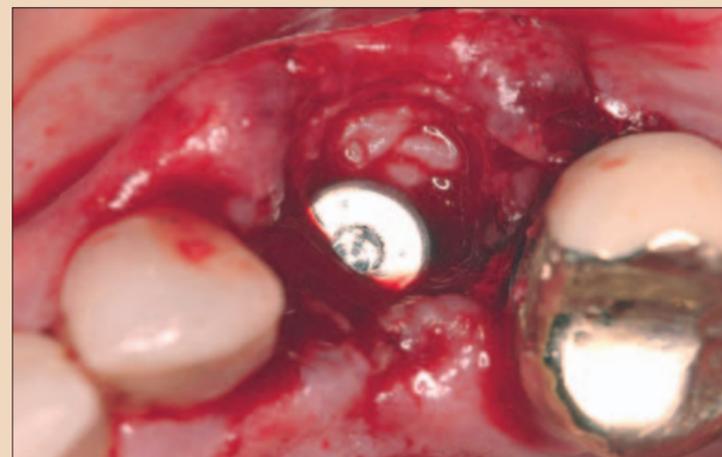
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Author Info

M. del Corso (DDS, DIL) is in private practice and is a contracted professor at the University of Turin, University of Lyon. Contact via e-mail at, mdelc@fastwebnet.it.

J. Choukroun (DMS, DU) is an anesthetist at Private Pain Center in Nice, France.

D. Dohan (DDS, MS, PhD) is an assistant in oral surgery at the University of Paris, France.



Figs. 30-32: The bone situation allows in the context of avulsion, placement of an Intra-Lock implant (Boca Baton FL, USA) with a 4.3 mm prosthetic platform. The space between the implant and the thin vestibular wall is filled with PRF only.



Fig. 33: The surgery site is covered and protected by another PRF membrane.



Figs. 34, 35: Upon reopening, the vestibular tissue has been preserved and is mature. The temporary can condition esthetic emergence.

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