Guided bone regeneration procedure with platelet rich fibrin (PRF) membranes in the resolution of a severe maxillary bone defect: report of a case

Andrea Pacifici[1], Daniele Carbone [1]*, Giuseppe Soda[2], Antonella Polimeni[1], Luciano Pacifici[1]

[1] 1Department of Oral and Maxillofacial Sciences, Sapienza University of Rome, Italy
[2] 2Department of Molecular Medicine, Sapienza University of Rome, Italy

*Corresponding author Dr. Daniele Carbone, Department of Oral and Maxillo-Facial Sciences, "Sapienza" University of Rome, Via Caserta, 6 - 00161 Rome, Italy; Tel .+393281789264; e-mail: dr.danielecarbone@gmail.com

Article history
Received: June 21, 2015
Accepted: June 29, 2015
Published: June 30, 2015

Abstract

Platelet-Rich Fibrin (PRF) is a new generation autologous platelet concentrate, invented by Choukroun in 2001, consisting of fibrin matrix which releases growth factors and cytokines in a slow and substained way, promoting physiologic healing processes. Its preparation procedure provides a single centrifugation of a venous blood sample without any addition of anticoagulant substances and/or bovine thrombin (or other gelling agents), unlike other platelet concentrates. PRF is a very versatile material which can be used as an adjuvant tool for regenerative surgery of both hard and soft tissues in the form of membrane, cylinder, or fragments, according to occurrences. The aim of this work is to show an explanatory clinical case of the use of PRF membranes as adjuvant tool in a pre-implant guided bone regeneration (GBR) procedure of a horizontal bone defect in the premaxillary area.

Keywords: *Platelet rich fibrin, PRF, GBR, bone regeneration, platelet concentrate.*

Introduction

Pre-implant reconstructive oral surgery primarily aims to restore an adequate morphology of the alveolar ridge in terms of height and width, in order to allow the appropriately sized implant placement in the best position to ensure the success of prosthetic rehabilitation, according to the dictates of prosthetically driven implantology [1].

One of the new frontiers in the scientific research applied to regenerative medicine is the use of growth factors such adjuvants for bone graft techniques [2]; these polypeptide molecules act as biological messengers capable of determining different effects on target cell populations, with an overall action of promoting tissue healing and new bone formation.

Platelet concentrates represent one of the methodologies which allows to use the biological potential of the growth factors for regenerative purposes [3]. They are hemocomponents characterized by a high concentration of platelets obtained by centrifugation of a blood sample of the patient and applied at the site of intervention. Their effectiveness in accelerating the healing of hard and soft tissue is based on platelets' action: these are a source of autologous biological mediators such as PDGF, TGF-β, EGF, IGF-I and IGF-II and, once activated, massively release them outside.

Within this hemocomponents' family, Platelet Rich Fibrin (PRF) is certainly one the most interesting. It is born in France in 2001 by the work of Choukrun, and consists of a matrix of autologous fibrin which harnesses between its meshes platelets, leukocytes, and respective growth factors and cytokines [4].

The PRF protocol is extremely simple in comparison to other platelet concentrates; it provides a single centrifugation of the blood without any biochemical manipulation [4].

Venous blood is collected from the patient immediately before surgery, and is placed in 10 ml glass vials without anticoagulant; these are immediately

centrifuged at 3000 r.p.m. (with a centrifugal acceleration of approximately 400g) for 10 minutes in a PC-02 table centrifuge. Since no anticoagulant is present, the contact of the blood with the walls of the tube determines the trigger of the coagulation cascade and platelet activation in a few minutes. Once the centrifugation is complete, the content of the tube is arranged in 3 different layers: one composed of red corpuscles and lying on the bottom of the tube; an intermediate formed by the fibrin clot (PRF); a supernatant of Platelet poor plasma (PPP) or acellular plasma [4]. The supernatant liquid (PPP) is easily aspired: it is readily separable from the remaining part of the preparation, which has a higher consistency. At this point the remaining layers are removed from the tube and the fibrin clot (PRF)is detached from the corpuscular phase via scissors or tweezers (Fig. 1a).

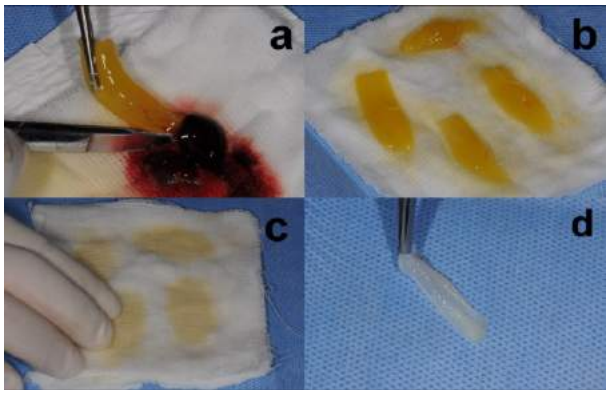

Figure 1. a) Removal of the corpuscular phase from the fibrin clot; b) Fibrin clots; c) Fibrin clots' compression; d) PRF membrane appearance after fibrin clot compression.

The obtained PRF, in this stage, has a yellowish color, a glossy surface and an almost gelatinous consistency (Fig. 1b). It can be compressed resulting in condensation and compaction of the fibrin strands, so that a portion of fluid trapped in the matrix comes out and the product acquires a greater consistency.

Compression can be carried out, in order to obtain a dense PRF membrane, by placing the clot between two sterile gauzes (Fig. 1c, 1d) or in a device specifically designed for the purpose, the PRF Box [5]. This consists of a metal box which presents, at the base, a grid on which the fibrin clot is laid, and a cover that exerts a constant pressure on it. The PRF Box presents, moreover, devices of cylindrical shape, equipped with a piston, in which the clots may be compressed into dense PRF cylinders [5]. The fluid which is issued with the compression of the PRF is a serous transudate, rich in proteins such as vitronectin and fibronectin, and it may be recovered and used for hydrating the graft material or rinsing the surgical site.

The Platelet-rich Fibrin is a very versatile material which can be used in the form of membrane, cylinder, and fragments, depending on the occurrences [6]. Its clinical field of application in dentistry includes both hard tissues and soft tissue regenerative surgery. Several authors has studied its use in GBR and sinus lift procedures [6-13], socket preservation technique [5,6,14], peri-implant defects' management [15,16], periodontal infra-bony defects regeneration [17,18], reparation of large maxillary sinus perforations [19], muco-gingival surgery [6,20].

PRF membranes have recently been evaluated as hypothetical scaffold for tissue engineering application: in vitro studies have shown its effectiveness in stimulating the proliferation and differentiation of animal [21] and human [22-24] osteoblasts, and on other human cells, such as gingival fibroblasts, skin pre-keratinocytes and pre-adipocytes [25], involved in the healing process of soft tissue; PRF has also proved to be capable of inducing in vitro proliferation and differentiation of mesenchymal stem cells from human bone [26,27], periodontal ligament [28,17] and dental pulp [29].

The reported case shows the use of PRF membranes as graft material coverage in order to enhance tissue healing in a pre-implant guided bone regeneration (GBR) procedure of a horizontal bone defect in the premaxillary area.

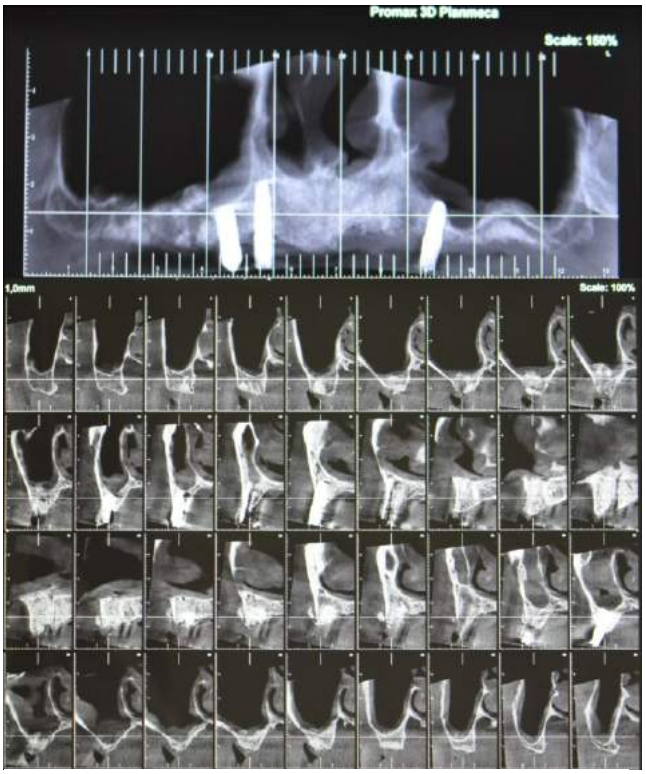

Figure 2. Pre-operatory CBCT; panorex and sagittal views.

Case Report

A 63 years old apparently healthy male subject presented to our observation requiring a total fixed prosthetic rehabilitation on implants of the upper arch. The clinical examination (Fig 3a) and the Cone Beam

Computed Tomography (CBCT) (Fig. 2) showed the presence of 3 implants with retentive attacks supporting a provisory total upper denture and revealed the presence of a severe vertical bone defect in the posterior maxillary regions on both sides and horizontal bone deficiency in the left premaxillary area. The subject was completely edentulous in the upper arch.

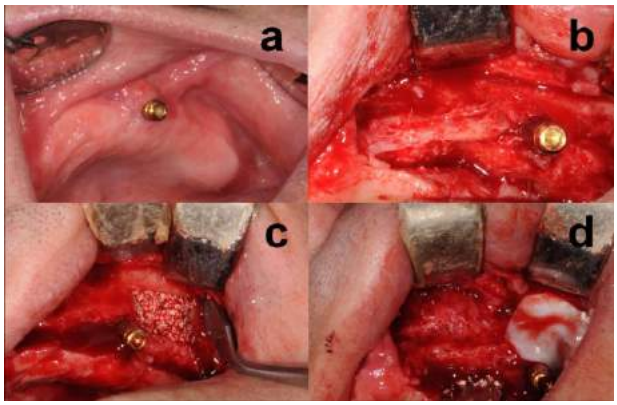

Figure 3. a) Pre-operatory clinical appearance of the upper left maxilla; b) Upper maxillary bone skeletonization; c) Maxillary sinus filling; d) Collagen membrane positioned above the antrostomy and perforations of the pre-maxillary alveolar bone.

Preliminarily to the insertion of other fixtures, we decided to operate on the crestal bone insufficiency in order to ensure the retention of a upper fixed prosthesis. On the right side a sinus lift surgery with lateral antrostomy was planned. In this article we report the left upper arch pre-prosthetic surgery: contextually to the maxillary sinus elevation, a horizontal guided bone regeneration (GBR) procedure was performed in the premaxillary area through the use of PRF membranes.

The surgery was carried out under local anesthesia. Four nerve blocks were performed at the posterior superior alveolar nerve, greater palatine nerve, infraorbital nerve and nasopalatine nerve; furthermore an infiltrative anesthesia at upper vestibular fornix and at the palatal mucosa were performed by 3% mepivacaine added of epinephrine (1:100.000).

A trapezoidal flap was incised by Bard-Parker n°15 scalpel, with the horizontal incision drawn along the alveolar ridge and extended from the left maxillary tuberosity region to the midline and the two oblique releasing incisions extended to the buccal vestibule, 5 mm beyond the mucogingival line.The flap was properly raised and tilted in order to allow the maxillary bone exposure (Fig. 3b).

The antrostomy and the Schneiderian membrane detachment were performed by piezoelectric and manual instrumentation. The maxillary sinus was filled with hydroxyapatite of bovine origin, previously mixed with the corpuscular fraction of the patient's blood obtained by centrifugation of the blood sample performed for PRF membranes obtainment (Fig. 3c). A collagen membrane was placed to protect the antrostomy (Fig. 3d).

In order to ensure the vascularization of the graft, perforations on the cortical vestibular bone were performed in the premaxillary area (Fig. 3d); a homologous thermoplastic graft material was adequately modeled to the bone defect (Fig. 4a) and PRF membranes were placed on the site as graft coverage (Fig. 4b, 4c).

Periosteal releasing incisions were carried out on the flap in order to ensure adequate closure by primary intention of the soft tissue above the graft without any tension. The flap was correctly repositioned and sutured with 4.0 non absorbable monofilament suture (Fig. 4d).

After surgery antibiotic and anti-inflammatory systemic therapy and digluconate chlorhexidine (0,20 %) spray were prescribed to the patient. The patient was subjected to antibiotic, pain-relieving and anti-edema therapy for 7 days.

Clinical examination at 10, 16 and 21 days after surgery showed no inflammation and good progression of the soft tissues' healing process in the area involved by the surgery.

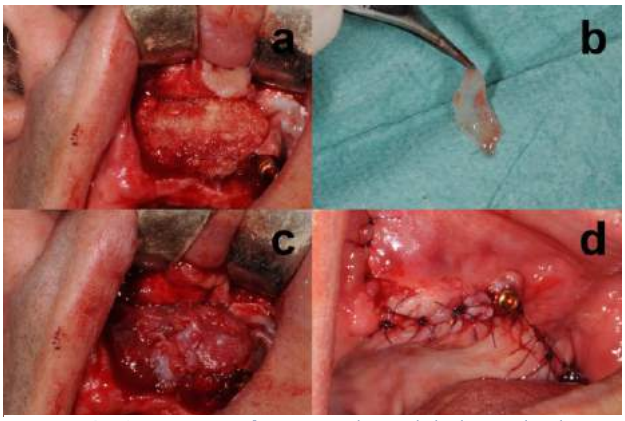

Figure 4. a) Bone graft material modeled on the bone defect; b) PRF membrane; c) PRF membranes positioned above the bone graft; d) Suture.

After 8 months, the case was radiographically re-evaluated through a Computed Tomography with Dentascan scan program and radiological template. The exam showed good healing of bone tissues, graft integration and the absence of inflammatory complications, except for the loss of the left implant not attributable to the described intervention (Fig. 5).

Subsequently 8 dental implants were inserted into the upper arch and a bone specimen was concurrently taken from the regenerated site in the premaxillary area using a 2 mm inner diameter steel trephine bur and submitted to histopathological examination. Dental implants were all successfully osseointegrated as detectable on the orthopantomography performed at 6 months after their placement (Fig. 6). The patient is currently rehabilitated

with a temporary fixed prosthesis and is awaiting prosthetic finalization.

Histological examination by optical microscopy of the hard tissue sample (Fig. 7), with hematoxylin and eosin staining, attested the success of the healing process, showing: lamellar bone being formed, deposited in the vicinity of young trabeculae; presence of osteoclasts and osteoblasts highlighting the active process of bone remodeling; visible resorption signs on the graft material particles.

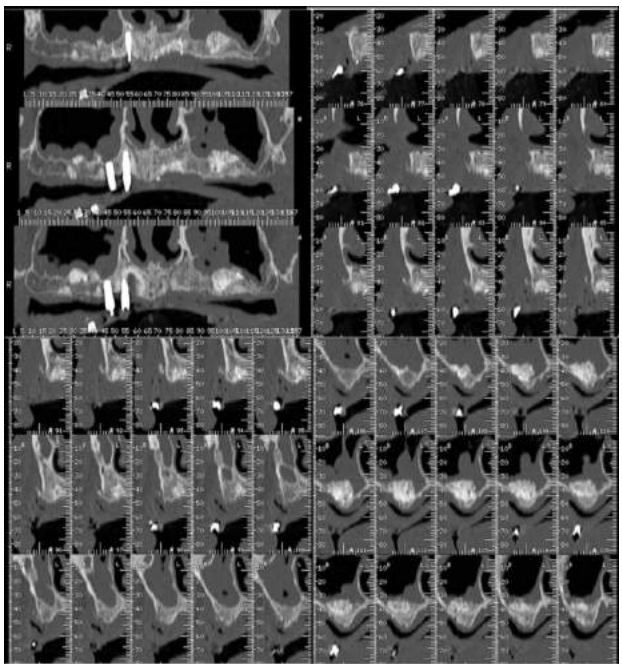

Figure 5. CT-Dentascan at 8 months from surgery; panorex and sagittal views.

Discussion

The PRF protocol provides a biomaterial with constant characteristics in terms of biological properties, composition and structure, assumed that its steps are carried out properly [30]. The contact between the wall of the tube and the blood without any anticoagulant results in rapid initiation of the coagulation cascade and platelet activation. Conversely to red corpuscles, that settle on bottom of the tube going to constitute the corpuscular phase, platelets and leukocytes get trapped between the meshes of fibrin; more exactly, in a PRF clot, it is concentrated 97% platelets and 50% of the leukocytes present in the blood sample used [30]. The distribution of cells trapped in the fibrin meshes is not uniform: the density of platelets and leukocytes is greater in the first millimeter of clot located at the interface with the corpuscular phase, and gradually decreases in proportion to the distance from that zone [30,31].

PRF can be considered as a natural cicatrizant autologous biomaterial capable of accelerating all the mucosa and bone physiological healing phenomena; it moreover acts as a modulator of local inflammatory response at the surgical site [4,31,32,33].

The set of biological features of PRF depends on the peculiar production protocol that allows the formation of a fibrin matrix very similar to the natural one, in which are harnessed activated platelets and leukocytes with their respective cytokines and growth factors. Each of the mentioned components contributes importantly to the characteristics of the biomaterial.

Platelet concentrates described in literature are all based on fibrin, even if the gelling mode through which these are obtained differs between "old generation" products and PRF. The gelling mode is essential in determining the three-dimensional organization of fibrin network [4,31]. The production of PRP and fibrin adhesives requires the use of bovine thrombin and calcium chloride to start the last phase of coagulation and fibrin polymerization. The speed of the reaction is proportional to the amount of enzyme used, greatly influencing the mechanical and biological properties of the final product [4]: high thrombin levels lead to the development of a rigid network not very favorable to cytokines embedding and cell migration, but with high mechanical resistance [4,31].

The fibrin matrix that constitutes PRF has the peculiarity of polymerizing slowly and naturally during centrifugation. The concentrations of autologous thrombin that act on fibrinogen are similar to the physiological ones, since there is no addition of enzyme of bovine origin (unlike other platelet concentrates such as PRP), causing the formation of a fine and flexible fibrin network, able to support the harnessing of cytokines (both those circulating in plasma and those produced by the cells embedded in the matrix itself), and growth factors; these are incorporated in a widespread and uniform way within the biomaterial (intrinsic cytokines) which acts as a slow resorption carrier, able to release significant amounts of signal molecules during the first 7 days from its production, inducing a longer lasting and effective stimulation on the regenerative process than other platelet concentrates [21,31,34].

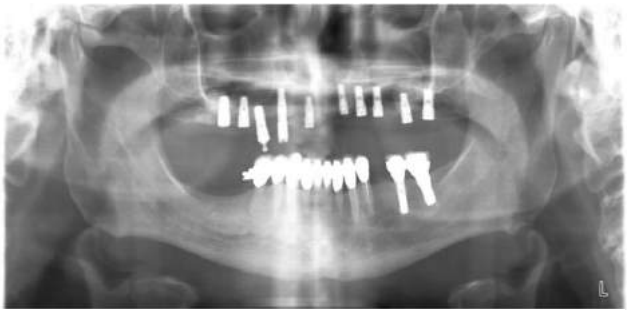

Figure 6. Orthopantomography at 6 months from implants' insertion.

A further interesting aspect of the PRF matrix is its

ability to harness glycosaminoglycans, such as heparin and hyaluronic acid, and glycoproteins such as fibronectin [31]. These components are arranged so as to closely follow the fibrillar architecture of the fibrin meshes, and present considerable affinity for cytokines and a great ability to support cell migration and tissue healing. Finally this three-dimensional organization makes the fibrin matrix very elastic by a mechanical standpoint.

Some authors have revealed a higher concentration of platelet cytokines and growth factors in PRF compared to PRP, presumably due to the different gelling modes of the two products from which derive different structures [4,21,31].

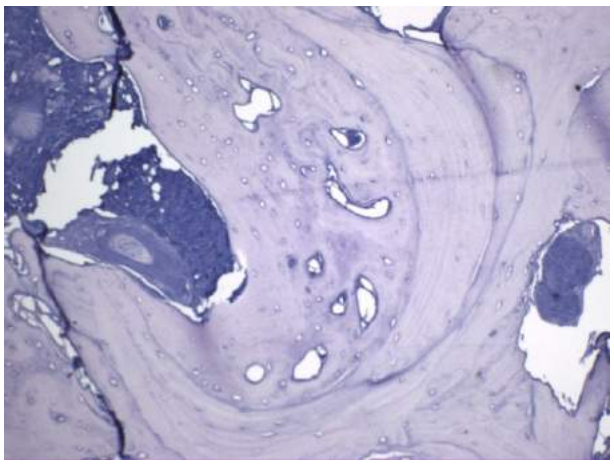

Figure 7. Appearance of the sample at the optical microscopy. Hematoxylin-eosin 200x.

Approximately 50% of the leukocytes present in the centrifuged blood sample is incorporated within the PRF matrix [30]. The presence of this cell population within the biomaterial is essential in the determination of some of its biological features. The degranulation of leukocytes is triggered by the process of centrifugation.

Therefore the secretion of certain molecules such as TNF-α, IL-Iβ, IL-6 and IL-4 is increased in the PRF; these cytokines remain included into the meshes of fibrin in the same way of growth factors, and their gradual release could modulate the inflammatory process in the surgical site. The PRF can therefore be considered as a leukocyte regulating node with the ability of controlling inflammation [33]. Furthermore, during the first 7 days after the biomaterial's production, leukocytes secrete various growth factors such as VEGF and TGFβ1 thereby contributing to the tissue regenerative process [21,34]. The Platelet-rich Fibrin is a highly versatile material that can be used as membrane, cylinder and fragments, according to eventualities [6]. In regenerative procedures with bone grafts, PRF membrane plays a important mechanic role since it stabilizes the graft material and protects it. The fibrin matrix, then, functions as carrier of platelet growth factors with progressive and slow release over time of the intrinsic cytokines, which positively guide the healing process for an extended time period and the reshuffle of hard and soft tissues, with a physiological enhancement of angiogenesis and bone growth [21,31,34,35] . The promotion of graft covering soft tissue's cicatrization also enhances bone healing since the regeneration site is better protected from the external environment with reduced risk of wound dehiscence and exposure [6,7,35].

Conclusions

The Platelet Rich Fibrin is a new generation autologous biomaterial and represents a new opportunity for pre-implant bone regeneration. Several studies have demonstrated its validity and effectiveness as a tool for regenerative purposes. The presented case showed a way of using PRF in a GBR procedure.

PRF It is an autologous product and therefore its use does not expose the patient to any risk of immune reactions or infections; furthermore the method of preparation of PRF is simpler and more economical than other platelet concentrates and does not involve any chemical manipulation of the patient's blood. Its interesting biological and mechanical characteristics make it a very versatile biomaterial, which can be used both for hard and soft tissues regenerative procedures.

The scientific research focused on this platelet concentrate is bringing to light a growing number of notions concerning its biological and clinical characteristics, and makes expected a further expansion of its possible fields of application in the therapeutic field and in tissue engineering.

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