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PLATELET CONCENTRATES: A BOON IN PERIODONTAL REGENERATION

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Abstract

Periodontitis is a multifactorial disease of the supporting tissues of the teeth. Bone loss and attachment loss occur by the untreated periodontitis. Periodontal tissue regeneration has always been a dare for the periodontist because of its structural intricacy. However, with tissue engineering as a rising multidisciplinary branch, this aim has partially been fulfilled. Regeneration is reconstruction of both hard and soft tissues in structure and function. Various treatment modalities i.e. bone grafts and substitutes, guided tissue regeneration (GTR) membranes and growth factors (PGFs) are used for periodontal regeneration. Platelet concentrates are abundant source for growth factors. Platelet concentrates are prepared from the patient's own blood throughout that the activated platelets are become close among a fibrin matrix scaffold and release growth factors and cytokines played key role in tissue regeneration; including cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis. This review highlights various types of platelet concentrates, and there clinical applications within the treatment of periodontal diseases.

Index Terms - PRP, PRF, A-PRF, T-PRF, Sticky Bone, ALB- PRF.

I. INTRODUCTION

Primary goal of day-to-day ongoing researches is to optimize healing and the biggest challenges that the researchers are face are that the development of a regenerative biomaterial to regulate inflammation and accelerate wound healing. [10]

The knowledge of wound healing has been greatly improved by the identification and understanding of the role of blood clot and growth factors in the healing process; [30] During the initial phase of wound healing, platelets interact with the fibrin clot to form a haemostatic plug and to create a temporary scaffold to capture cytokines and support and stimulate cell migration and proliferation through the release of certain growth factors from platelet's α granules upon activation.[1]

Role of platelets in regeneration was confirmed way back in the 1970s,[7] because of the fact that it is a pool of growth factors that are responsible for neo vascularization, collagen synthesis, cell division, cell differentiation, induction, and migration of other cells to the injured site[8].

The key role of platelets in inflammation and wound healing is owing to the presence of several growth factors and cytokines [9].Furthermore, they contain fibrin, fibronectin, and vitronectin that contribute connective tissue, a matrix and create an efficient network for cell migration [1]. This has resulted in the idea of using platelets as therapeutic tools to improve tissue repair, especially in wound healing.

II. HISTORICAL BACKGROUND

The history of these preparations started in 1970 with the research works by Matras about the "Fibrin glues" used to improve skin wound healing in a rat model.[31] Later between (1975–1979) new strategies commanded in the role of platelets within the fibrin gel and upgraded the concept for the use of blood extracts, termed "Platelet fibrinogen-thrombin mixtures or platelets gels". [23, 32] The development of these techniques continued very slowly until the article of Marx et al., [24] which started the insane for these techniques. However, all these products were identified as PRP without conferring of their content or architecture, and this deficit of terminology continued for many years. Some commercial companies, in place of better visibility, started stamping their products with distinct commercial names. Platelet concentrates which was self-clogged and was developed by Choukroun J et al. in France in 2006 and termed "Platelet-Rich Fibrin (PRF)" due to the enduring fibrin gel polymerization of the preparation and was known as the (Second Generation). [3, 24] Later, the "Leukocyte Platelet-Rich Fibrin (L-PRF)" was often considered as an [25] and allowed to define new therapeutic principles: NTR (Natural Tissue Regeneration). [6] Later on, number of technologies and protocols were established to enhance the characterization of the preparations in terms of release duration and content of growth factors or cells and physical properties including "Advanced and Injectable PRF". [26-21] The "Titanium-prepared platelet-rich fibrin (T-PRF)",and "Advanced Fibrin Glue (AFG)", Autologous albumin gel and liquid platelet-rich fibrin (Alb-PRF) are further modified form of PRF prepared by modify centrifugation protocol or material of collection tubes [5,16 28,33]. (Fig. 1)

III. Classification of Platelet Concentrates:

The first classification was described by Dohan Ehrenfest et al., in 2009. This classification divided the products based on 2 key parameters; the presence of cell content (mostly leukocytes) and the fibrin architecture.[13]

1. **Pure Platelet-Rich Plasma:** These PRPs are without leukocytes and with a low-density fibrin network after activation so can be placed as injectable liquid solutions or in an activated gel form to be used or injected on wound or surgical site.

2. **Leukocyte-and Platelet-Rich Plasma:** These PRPs are with leukocytes associate with various degreed with a low-density protein network when activation thus is placed as injectable liquid solutions or in an activated gel kind to be used or injected on wound or surgical site.

3. **Pure Platelet-Rich Fibrin:** These PRFs without leukocytes and with a high-density fibrin network and only exist in a strongly activated gel form, so cannot be injected or placed like traditional fibrin glues. However, they can be manipulated like fibrin membranes or solid materials for other applications.

4. **Leukocyte and Platelet-Rich Fibrin:** These PRFs with leukocytes and with a high-density fibrin network and only available in a strongly activated gel form and cannot be injected.

IV. Protocols and Characterizations for Different Preparations:

1) Platelet Rich Plasma—First Generation Platelet Concentrates (PRP)

The use of autologous preparation with high platelet concentrations like Platelet rich plasma (PRP), Platelet concentrates (PC) and platelet gels grove to combine the fibrin sealant properties with growth factor effects of platelets—providing a flawless growth factor delivery system at the site of injury. The meticulous rationale behind the use of these preparations lies in the fact that growth factors (GFs) are known to play a vital role in hard and soft tissue repair mechanisms. [2,24] These GFs manifests chemotactic and mitogenic properties that enhance and manipulate cellular functions involved in tissue healing, regeneration and cell proliferation.[3]

Preparation

Generally, PRP is developed via a two-step centrifugation preparation of anticoagulated blood sample. In the first step (Soft Spin) of centrifugation (300g for 5 min at 12°C or 240g for 8 min at 16°C), three layers are distinguished: platelet poor plasma (PPP) plasma on top, buffy coat' (BC) middle layer that contains platelets and leukocyte and red blood cells (RBCs) on the bottom. For production of Pure PRP (P-PRP), PPP and superficial BC are transferred to another tube, then centrifuged for a second time (Hard Spin) to make sure proper plasma separation (700g for 17 min at 12°C), most of the PPP layer is throw away. The final P-PRP concentrate comprised of an undetermined section of BC (containing a large number of platelets) put up in some fibrin-rich plasma.

For production of Leukocyte-rich PRP (L-PRP), PPP, the whole BC layer and some residual RBCs are shifted to another tube. After hard spin centrifugation, the PPP is threw away33. The final L-PRP made up of the entire BC, which comprises most of the platelets and leukocytes, and residual RBCs put up in some fibrin-rich plasma (Figure 2).[1]

2) Platelet Rich Fibrin (PRF)—Second Generation Platelet Concentrate

Choukroun's Pure Platelet-rich fibrin (P-PRF) designed to a new generation of platelet concentrates, with simplified technique and without biochemical blood handling. The research on PRF was guided by Dr. Joseph Choukroun. [3, 12]

A standard protocol for PRF preparation ought to be followed to get correct amount and quality of the fibrin matrix, platelets, and growth factors; needle is employed for ten mil blood assortment in sterile glass coated plastic tubes while not anticoagulant that are directly centrifuged at 3000 revolutions per minute for ten min. throughout the process, once the blood gets in contact with the test tube wall the platelet gets activated and continuing to the initiation of action cascade. When action, the resultant product contains of 3 layers. The highest most layers consisting of one-celled PPP (platelet poor plasma), RBCs at the lower, and PRF clot are in between of the test tube. The fibrin clot obtained when action is off from the tube and therefore the hooked up red blood cells scraped off from it and threw away. PRF can even be created within the type of a membrane by compression and removing the fluids present within the fibrin clot. [11, 14]

Leukocyte Platelet-Rich Fibrin (L-PRF):

The L-PRF clot or membrane are changed PRF to comprise most of the platelets and leukocytes present within the initial blood collect and the platelet growth factors and stem cells that are contained inside the fibrin network with intense strength.[8] It's ready by modification of initial technique of (P-PRF); Blood ought to be accumulated quickly in 9ml glass-coated plastic tubes (less than twenty seconds per tube) and quickly centrifuged in Intra-Spin centrifuge at room temperature (2700 revolutions per minute for twelve minutes) to develop L-PRF clots. The clots are often harvested fastidiously into a sterile adapted surgical box and squeezed into membranes, stay as fibrin plug or mixed with particulate bone for preparation of sticky bone. [9]

Advanced Platelet rich fibrin (A- PRF):

The centrifugation concept is 1500 rpm 14 mins. Later on, it was diminished to 1300 rpm 14 mins. It is based on the lower centrifugation protocol. Besides Platelets, Macrophages also develop growth factors. Accordingly, this might be able to affect the differentiation of host macrophages and macrophages within the clot after implantation. Thus, they advocate this would influence bone and soft tissue regeneration, especially through the survival of monocytes/macrophages and their growth factors. [1] with the lower centrifugation concept, it was conformed that presence of macrophages in advanced platelet rich fibrin. PRF clots developed with A-PRF centrifugation arrangement showed a loose structure with more confine inter fibrous space, and more cells in distal part of fibrin clot.[8]

Advanced Platelet rich fibrin (A- PRF) +:

The centrifugation concept is 1300 rpm 8 mins. It is also based on lower centrifugation protocol. However, more research is needed to and the effect of APRF+ on Regeneration.[9]

Injectable-PRF(I- PRF):

The production of this injectable formulation of PRF was with the goal of delivering to clinicians a simple to use platelet concentrates in liquid form which can be either employed alone or combined easily with many biomaterials. Taking advantage of slower and shorter centrifugation speeds, an intense presence of regenerative cells with elevated concentrations of growth factors can be seen when compared to other formulations of PRF. The i-PRF is designed by: 10 ml of whole blood accumulated in plain vacuum tubes with no use of anticoagulant was quickly centrifuged at 700 rpm for 3 min. The 1 ml top most upper plasma layer was then collected by needle and designated as i-PRF.[27] Addition of i-PRF to particulate bone will activate the polymerization in 15 min to develop red colored sticky bone.

Titanium Platelet-Rich Fibrin (T-PRF):

Based on the theory, that titanium may be a well structured platelet activator than silica, for designing L-PRF. Tunali et al in 2014, introduced a new product called (Titanium- prepared PRF) in which the 9ml of blood was immediately collected in grade IV titanium tubes, and the tubes were rapidly centrifuged at 2800 rpm for 12 minutes and found well arranged, thicker and longer fibrin network.[33]

Autologous Fibrin Glue (AFG) and Sticky Bone:

A concept of fabricating growth factors - enriched bone graft matrix (also referred to as “sticky bone”) exploitation Autologous protein glue (AFG) has been incontestable since 2010; to get Autologous protein glue, 20- 60CC of blood in non-coated tubes is centrifuged at 2400-2700 rev for two min. Out of the 2 layers obtained, the deeper layer is RBC’s and also the superficial layer is AFG. This AFG is then extracted employing a syringe and mixed with particulate bone powder and allowed to rest for 5-10 min for polymerisation, which ends up in a very yellow coloured mass known as sticky bone.

V. NEWER CONCEPTS:

5.1 Albumin Gel-platelet-rich Fibrin Mixture (Alb-PRF)

The biological properties of Alb-PRF, which can show a potential enhancement in PRF-based clinical applications. Alb-PRF release of seven key growth factors found in blood up to a 10-day period, comprising PDGF-AA, PDGF-AB, PDGF-BB, TGF-β1, VEGF, epidermal growth factor (EGF) and insulin growth factor 1 (IGF-1). Then, it improves the cell biocompatibility, migration potential, proliferation assay and expression of TGF-β1 and collagen 1 production.

Blood samples were taken. Nine ml of blood in plastic tubes was centrifuged at 700 g for 8min. The upper most layer (platelet-poor plasma layer) was collected in two-ml syringes and heated at 75°C for 10 minutes to produce denatured albumin (albumin gel). By following heating, the albumin gel was allowed to cool down at room temperature for ten minutes. Then, liquid PRF as well as residual cells and growth factor found inside the buffy coat layer was thereafter mixed along with the cooled albumin gel to form Alb-PRF by employing a female-female luer lock connector. This amalgamation allowed that both the lower-resorption properties of the albumin gel along with the higher cell content and growth factor content of the liquid PRF layer to be remixed. The injectable AlbPRF gels were then shifted into 6-well cell culture plates forming a gelated membrane.[19]

5.2 BIO-PRF (Horizontal Centrifugation Protocol)

Miron et al (2019) found that horizontal centrifugation concept. Horizontal action created a big increase in each the quantity and concentration of platelets and leukocytes.[29]

Preparation of BIO-PRF

Blood samples were collected. the subsequent 3 action devices were used during this study as well as the IntraSpin associated an Eppendorf horizontal centrifuge. 2 separate protocols were tested on every machine as well as the manufacturer's recommendation to supply each liquid- and solid-PRF. On the Intraspin device, leukocyte and platelet rich fibrin (L-PRF) protocol were used for the solid-PRF clot whereas a ~700 RCF-max for three min was used to develop liquid-PRF. On the method for PRF device, the advanced platelet rich fibrin (A-PRF) protocol (~200g RCF-max for 8 min) was used to designed solid-PRF and a liquid-PRF was created using the i-PRF protocol of ~60g RCF-max for 3 min. A horizontal centrifuge used attributable to its advantage in separating layers based on density. Two protocols were used during this study as well as a solid-PRF protocol of 700g for eight min and a liquid-PRF protocol of 200g for eight min.

Miron et al (2019) found that horizontal action created each solid and liquid formulation with each higher concentrations and numbers of platelets and leukocytes in comparison to fixed-angle centrifuges. It conjointly disclosed that presently used manufacturer's suggested protocols need more optimization.[29]

VI. CONCLUSION

PRF, a brand new generation of platelet concentrate, could be a novel step in regenerative periodontal treatment with simplified process and while not biochemical modification. With the exception of its application in dental specialty, PRF is additionally been utilized in varied medical fields: orthopedics and cosmetic surgery. Though deserves and demerits of PRF are confirmed by varied systematic reviews and meta-analysis, varied prospective studies have nevertheless to be explored. Clinical studies in cooperating PRF for varied treatments are quite encouraging; but, more studies are necessary to support its common use in routine practice with high clinical effectuality and semi permanent stability.

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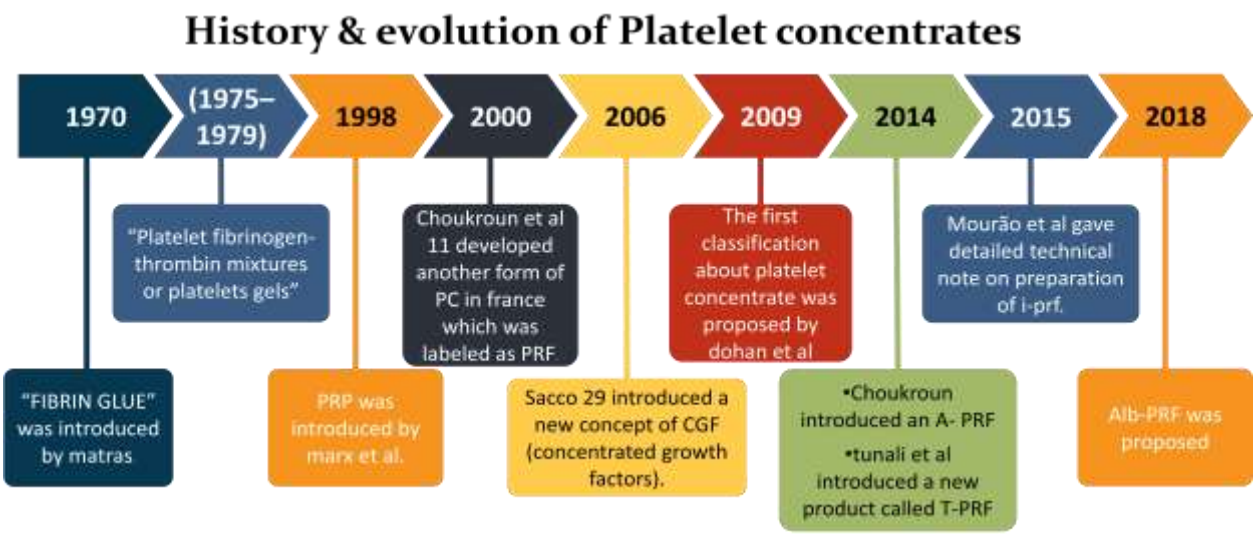

(Fig. 1) A chronological table illustrating recent advances in the development of PRP and its derivatives

Clinical implications	Advantages	Limitations
• intrabony defects • Sinus lift procedures • Augmentation techniques • Peri-implant defects • Ridge preservation • Root Coverage • preserve extraction socket	• Nontoxic to tissues. • Easily available and minimal invasive • Increased endothelial, epithelial and epidermal regeneration • Stimulates angiogenesis and enhance collagen synthesis • Promotes soft and hard tissue wound healing.	• Presence of bovine thrombin which cause allergic reaction • Lack of uniformity in PRP preparation

Table I: Clinical implications, advantages, limitations and contraindications of PRP

Clinical implications	Advantages	Limitations
• Extraction Socket preservations • Intrabony defects with or without bone grafts • Furcation defects • PRF membrane has been used for gingival recession coverage with coronally advanced or lateral pedicle flap for multiple and single recession respectively • Endo perio lesions • Sinus lifting procedure	• It is totally safe. • Standard concept for preparation.	• As it is produced in limited quantities, which limits the usage in general surgery • PRF membranes are totally specific to the donor and cannot Containing an allogenic graft tissue.

Table II: Clinical implications, advantages, limitations and contraindications of PRF