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Review Article

A preferred choice for periodontists: Platelet concentrates

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ABSTRACT

This article provides a comprehensive overview of platelet concentrates, notably Platelet-Rich Fibrin (PRF). Due to its function in tissue repair and regeneration, PRF has gained importance in various fields like dermatology, endodontics, oral surgery, root coverage, osseous defect treatment, and implantology which are being discussed here. A-PRF+, Advanced PRF, Injectable PRF, Titanium PRF, PRF Lysate, Lyophilized-PRF, and Albumin PRF are a few examples of recent developments in PRF formulations that are emphasized. To effectively utilize the potential of platelet concentrates in regenerative procedures, further studies and standardization are required.

1. Introduction

Platelets are one among the components of the blood cells with 2μm in diameter with a lifespan of 7-10 days in the human body.[1] Around 100 years ago the role of platelets in hemostasis was established by Giulio Bizzozero, an Italian pathologist and since significant advent of light microscopy has illuminated the role of platelets in hemostasis and thrombosis in the era of 20^{th} century.[1]

Initially, platelet concentrates were used to prevent hemorrhage in severe thrombopenia. The property of adhesiveness in the fibrin matrix and the growth factors present in the platelets has made them the best choice in regenerative medicine.[2]

Platelet concentrates, in special favour of PRF has gained attention due to its special properties like potential for healing, biocompatibility and easy preparation. Ever since then, the structural and functional complexity of platelets has led to the introduction of various mechanical methods to quantify and qualify these blood elements; this in turn has led to the development of various forms of platelet concentrates that could be used for regeneration in various fields of medicine.[2–4]

2. Evolution

Wound healing, which is defined as the natural restorative response to tissue injury, involves a cascade of complex, orderly, and elaborate events involving many cell types guided by the release of soluble mediators and signals that are capable of influencing the homing of circulating cells to damaged tissues.[3] Stimulating the proliferation and activation of cells involved in wound healing, including fibroblasts, neutrophils, macrophages, and mesenchymal stem cells (MSCs) are brought about by the platelets along with the release of various growth factors, coagulation factors, adhesion molecules and chemokines.[4,5]

PRP is an example of an autologous blood product with high platelet concentrations that was created to take the role of fibrin sealants and enhance the healing process. PRP represents first generation of platelet concentrate and was introduced for the first time in 1998.(Table 1)

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Table 1: Evolution of platelet concentrates

Year	Nomenclature
1954	Kingsley first used the term PRP to thrombocyte concentrates. [6]
1970	"Fibrin glue" was introduced by Matras. [7]
1975-1978	Platelet-Fibrinogen-Thrombin Mixtures. [8]
1986	Platelet-Derived Wound Healing Factors (PDWHF) by Knighton. [9]
1997	Whitman et al coined the term "platelet gel". [10]
1999	Popular methods advertised on large scale to prepare pure platelet rich plasma commercialized as plasma rich in growth factors.
2000	Choukran et al developed the second generation platelet concentratre "PRF-Platelet Rich Fibrin". [11]
2006	Bielecki et al proposed Platelet Rich Gel. [12] Concept of Concentrated Growth Factors introduced by Sacco. [13]
2008	Everts et al introduced the concept of inactivated product "platelet-leukocyte rich plasma (P-LRP) and activated gel as platelet-leukocyte-gel" (PLG). [14]
2009	Classification of platelet concentrates proposed by Dohan Ehrenfest. [15]
2010	Sohn introduced the concept of sticky bone. [16]
2012	Mishra et al and DeLong et al introduce two new system of classifications. [17,18]
2014	Choukron et al introduced A-PRF. Tunali et al proposed T-PRF. [19]
2015	Mourao et al proposed detailed preparation methods for i-PRF. [20]

3. Biological Properties

The PRF is produced through centrifugation-induced progressive polymerization, unlike other fibrin adhesives. It then organizes into a three-dimensional structure that is more coherent than natural clots. The complicated fibrin mesh grows more complex as the polymerization process goes on, trapping both the intrinsic cytokines and the moving platelets. This guarantees continuous cytokine release and action during remodelling and healing. Osteoprotegerin (OPG) and phosphorylated extracellular signal-regulated protein kinase (p-ERK) are both produced more readily when PRF is present. [21]

Neutrophil migration, activation, and protease release are promoted by fibrin and fibrin breakdown products. At the location of the lesion, these neutrophils destroy contaminating bacteria by producing oxygen radicals and enzyme digestion. Additionally, the interaction of fibrin with monocytes and macrophages modifies phagocytosis, demonstrating the critical role played by macrophages in the transition from wound inflammation to wound repair. Because accurate cell-to-cell contact is necessary for tissue regeneration and is not achievable without leukocytes, platelets are not only responsible for tissue regeneration but also require leukocytes to function. [21]

Growth factors for periodontal regeneration [22]

1. **Transforming growth factor-β (TGF-β)**: Stimulates proliferation and chemotaxis of osteoblasts thereby enhancing the woven bone formation**.**
2. **Vascular endothelial growth factor (VEGF):** Initiates angiogenesis.
3. **Insulin growth factor-1 (IGF-1):** Acts in synergy with the TGF. Stimulates osteoblast proliferation. Stimulates proliferation and chemotaxis of osteoblasts thereby enhancing the woven bone formation.
4. **Fibroblast growth factor (FGF):** Enhances fibroblast proliferation, migration and differentiation.
5. **Epidermal growth factor (EGF):** Stimulation of cell proliferation and extracellular matrix.
6. **Platelet-derived growth factor (PDGF):** Stimulates migration and proliferation of mesenchymal lineage cells.

Figure 1: The PRF

4. Classification

Based on the presence of a cell content (mostly leukocytes) and the fibrin architecture: [15]

Leukocyte-poor platelet-rich plasma (L-PRP) or Pure Platelet-Rich Plasma (P-PRP) products are preparations without leukocytes and with a low concentration of platelets, network of fibrin density following activation. The things of this family can be dissolved in liquids or utilized in a gel form of activation. Consequently, it is injectable. (For use in sports medicine) or utilized when forming a gel on a skin incision or suture (like the use of fibrin adhesives).

Leukocyte-and platelet-rich plasma (L-PRP) products are leukocyte- and fibrin-network-containing preparations that have been activated. This family's products are all available as activated gels or liquid solutions for application.

Leukocyte-poor platelet-rich fibrin (L-PRF), sometimes known as pure platelet-rich fibrin (P-PRF), refers to preparations having a high-density fibrin network but no leukocytes. These products are only available as strongly activated gels, by definition. Like conventional fibrin glues, this cannot be injected or otherwise applied. They may be

handled like a true solid material for other applications because to their robust fibrin matrix.

Leukocyte- and platelet-rich fibrin (L-PRF) products are preparations with leukocytes and with a high-density fibrin network. According to their description, these materials are only available as a strongly activated gel and cannot be injected or utilized like conventional fibrin glues. They may be handled like a true solid material for other applications thanks to their strong fibrin matrix.

Based on presence and activation of PRP:[17]

Type 1 PRP is a L-PRP solution

1. Type 2 PRP is a L-PRP gel
2. Type 3 PRP is PPRP solution
3. Type 4 PRP is a P-PRP gel.

This has been proposed for exclusive use in sports medicine.

To organize and analyze results in the literature, a different system known as PAW (Platelets, Activation, White Cells) was established. It places emphasis on the amount (absolute number), mechanism of platelet activation, and presence of white cells. This method, which is quite similar to Mishra et al.'s idea, has the drawback of just covering PRP.[18]

According to the Shirbate et al the very first generation are the concentrated platelet and growth factor count that has been validated. Development of PRP preparation devices were also considered. The second generation included modifications to previous preparation approaches, as well as the development of new platelet-rich plasma derivatives, such as platelet-rich fibrin (PRF). In the third generation, Platelet-rich plasma derivatives are compared in terms of their mechanical stability, strength, and biodegradability, as well as their ability to react, retain, and release numerous growth factors. Fourth generation studied the coupling of PCs (partner cells).[23]

5. Preparation

PRP is made using a sample of the patient's blood taken at the time of the procedure.30 cc venous blood draw will produce 3-5 cc of PRP. To prevent platelet activation before to usage, an anticoagulant, such as citrate dextrose A, is added to the blood drawn. The first spin is carried out(2500rpm for 15 min) the middle portion is collected and second spin(3500 rpm for 10 min)is done to obtain PRP. PRP is mixed with bovine thrombin and calcium chloride at the time of application. When blood is collected, it is immediately centrifuged at 3000 rpm for 10 min for PRF. Red blood cell-containing lower part and the middle fraction the fibrin clot is obtained. The middle portion, 2 mm below the lower dividing line is collected. The fibrin is formed in the center of the tube.[24]

6. Applications

1. **Non-surgical periodontal therapy**: Ozcan et al. analysed the effect of PRF application with scaling and root planning and the expression of TGF-β and Col-1 and found that there was significant improvement in the clinical parameters during the follow up.[25] Rakhewar et al. used I-PRF application as an adjunct to non-surgical therapy in chronic periodontitis and found that there was an improvement in every clinical parameters assessed.[26]
2. **Treatment of osseous defects:** Follow up studies have found that the results were superior when PRF was used along with bone grafts in treatment of intra bony defects.[27] Open flap debridement along with PRF produced satisfactory results rather than the former alone.
3. **Treatment of furcations:** Regenerative treatments for Grade II furcation defects have shown that PRF along with bone graft resulted in a better outcome when compared to bone grafts alone.[28]
4. **Root coverage Procedures:** PRF when used as a membrane along with laterally positioned flap has shown to give grater coverage as recorded in a study conducted by et al.[29] It can also be used at the donor site for better wound healing in free gingival grafting procedures.[30]
5. **Biotype enhancement**: PRF has proven to increase the thickness of the gingiva and provide promising results when used for biotype enhancement procedures.[31] It is preferably used as a membrane over the root surfaces with coronally advanced flap. I-PRF has been employed to enhance the thin biotype with and without microneedling and significant results were recorded in the literature.[32]
6. **Oral surgical procedures:** Satisfactory results were seen when PRF was used in post enucleation of large periapical lesions and surgical removal of impacted third molars canines. It has also been employed for ridge preservation following multiple extractions.[33]
7. **Endodontic procedures:** Regenerative endodontics, treatment of periapical lesions and open apex cases has showed better results and is a promising material for future procedures.[34]
8. **Implantology:** Platelet concentrates with special regards to PRF has been put in use for sinus lift procedures, as tissue scaffold in immediate implant procedures and periimplantitis management.[35]
9. **Dermatology:** Wide range of use has been reported in literature like in hair therapy, scar and acne treatment. It has been used in conjunction with other therapies like dermal fillers, laser, micro needling and autogenous grafting.[36]

7. Recent Advances

1. **Advanced platelet-rich fibrin:** The antecubital vein in the person's hand was used to draw a 10 mL venous sample, which was then immediately transferred to glass tubes. There was no anticoagulant in the tubes to initiate fibrin polymerization or platelet activation. The tubes in the A-PRF group were centrifuged at 1500 rpm for 14 minutes. The overall levels of secreted growth factors in A-PRF are significantly higher for transforming growth factor (TGF-1), vascular endothelial growth factor (VEGF), PDGF, epidermal growth factor (EGF), and insulin-like growth factor (IGF1).[37]
2. **Advanced platelet-rich fibrin plus:** According to Pavlovic et al.'s study, Fujioka Kobayashi et al. developed the A-PRF+ preparation procedure by reducing the centrifuge speed to 1,300 rpm at 200 g with a centrifugation time of eight minutes. Compared to the A-PRF and leukocyte PRF, the A-PRF+ contains significantly more of the secreted growth factors TGF-1, VEGF, PDGF, EGF, and IGF-1. A-PRF+ increased the migration and proliferation of human gingival cells.[38]
3. **Injectable platelet-rich fibrin:** The goal of the newly developed injectable-PRF, also known as i-PRF, is to offer clinicians a liquid formulation of conveniently accessible platelet concentration that may be utilized either alone as a regenerative agent or in conjunction with a range of biomaterials for regeneration. Due to the slower and shorter centrifugation speed in i-PRF, (3300rpm for 2 min) regenerative cells with higher concentrations of the growth factors are more common and may be observed. The expression of the growth factors PDGF, TGF, and collagen1 as well as the release of more of a number of growth factors can all be accelerated by the use of i-PRF.[20]
4. **Titanium platelet-rich fibrin:** A 10 mL blood sample is taken from the patient's arm's antecubital veins, and the blood is then transferred to a grade IV titanium tube. The titanium tubes were then immediately centrifuged for 10 minutes at room temperature at a designated table at a centrifugal force of 3000 rpm. Using sterile tweezers and sterile scissors, the T-PRF clot was removed from the tubes, split from the RBC's base, and centrifuged before being deposited on sterile woven gauze.[19]
5. **PRF lysate (PRF-Ly):** After the PRF preparation, the exudate (PRF lysate) is collected and incubated at 37°C in a humid environment. It is made up of 95% air and 5% CO2. All growth factors, including PDGF, TGF, VEGF, and EGF, are thought to be advantageous. Additionally, it has been utilized to greatly boost collagen production, migration, and proliferation rates to levels comparable to those of regular fibroblasts.[39]
6. **Lyophilized-PRF (Ly-PRF):** PRF is extracted from the routine protocol freeze dries and lyophilised and stored in granules for further use. Use of lyophilization as a fresh technique for PRF preservation, especially for preserving the physical microarchitecture of PRF and autologous PDGF-AB growth factor release. The Ly-PRF produced showed essential characteristics that suggested its potential application as a bioactive scaffold and bone regeneration stimulator.[39]
7. **Albumin PRF (Alb-PRF)**: After centrifugation, it is created by two steps that involve heating and incorporation (heating of the serum, low platelet plasma, and integration of cells) i.e For 8 minutes, entire blood was centrifuged at 700 g. Next, by tempering the albumin, the denatured albumin was obtained (10 minutes at 75°C) from the platelet-poor plasma layer. The buffy coat (liquid PRF) were once again combined with the cooled albumin gel. It is entirely made of autologous blood, in which the growth factor/GF and PRF cytokines have been liquidized and separated from the RBCs' leukocyte zone.[40,41]

8. Conclusion

The identification of the platelet concentrates (PC) that retain the growth factors entangled in the fibrin network and gradually release them speeds up the healing of wounds. It is one of the most interesting recent developments in the area of regenerative dental techniques. After an injury, platelets begin the coagulation cascade, form a solid clot, and release growth factors that aid in tissue growth and repair. Remarkable evolution from solid to injectable form have elevated the quality of usage of platelet concentrates. Recent advances and research have led to different specifications in terms of centrifugal speed, time that ultimately improves the growth factors required. The PRP preparation procedure is a drawback because the growth factors are only released for a brief time and have varying storage times. Further standardisation and quality maintenance protocols would lead to a better efficient method of utilisation of the promising regenerative potential of the platelet concentrates.

9. Source of Funding & Conflict of Interest

None.

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