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Various platelet-rich fibrin products and its applications: A review

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Abstract

Platelet-rich fibrin (PRF) belongs to a new generation of platelet concentrates geared to simplified preparation without biochemical blood handling. Its role in improving healing is something that is very exciting to the field of dentistry. It is the aim of this review to acquaint the reader with PRF and its various applications.

Keywords: PRF, platelet, healing, growth factors

Introduction

Improving healing is a constant issue in all surgical disciplines, and the development of advanced biomaterials and pharmaceutical preparations has always influenced the history of surgery [1].

Although the use of fibrin adhesives in many field-related protocols is well documented from the past 30 years, it remained controversial owing to the complexity of the production protocols (for autologous adhesives) or risk of cross-infection (for commercial adhesives) [1], progressively leading to the evolution from fibrin glues to platelet concentrates (PC) [2].

Platelet-rich fibrin (PRF) belongs to a new generation of platelet concentrates geared to simplified preparation without biochemical blood handling [2].

Important Milestones in History of Platelet Concentrates

Year	Development
1970	Matras introduced "Fibrin glue", which improved healing of skin wounds in rat models [1, 3, 4, 5]
1974	Platelets regenerative potentiality was introduced [5]
1986	Knighton <i>et al.</i> first demonstrated that PC successfully promote healing and named it as "platelet-derived wound healing factors (PDWHF)" [3, 6].
1997	Whitman <i>et al.</i> First to introduce the use of platelet-rich plasma in oral surgical procedures; reported great advantages because it enhanced osteoprogenitor cells in the host bone and bone graft [7, 3].
1998	In the first articles about platelet concentrates, various nomenclatures were proposed, but the term PRP (Platelet-Rich Plasma) became the reference after the founding article of Marx <i>et al.</i> [1, 3, 8].
1999	Plasma rich in growth factors (PRGF) or also called as preparation rich in growth factors introduced [3].
2001	Choukroun <i>et al.</i> developed PRF, "second-generation" platelet concentrate [2, 3, 5, 7, 9].
2006	Sacco developed CGF (concentrated growth factors) also called as Sacco's PRF [10, 11].
2009	Dohan Ehrenfest <i>et al.</i> proposed first classification about platelet concentrate [3, 12].
2013	Tunali <i>et al.</i> introduced T-PRF (Titanium-prepared PRF) [3, 13].
2014	Advanced PRF called A- PRF (claimed to contain more monocytes) by Ghanaati <i>et al.</i> introduced [3, 14].
2015	Technical note on preparation of i-PRF (injectable) by Mourão <i>et al.</i> [3, 15].

Classification of Platelet Concentrates

Dohan Ehrenfest *et al.* proposed first classification about platelet concentrate. This classification defined 4 main families based on separation of the products using 2 key parameters: The cellular content (primarily leukocytes) and the fibrin architecture:

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- 1) Pure platelet-rich plasma (P-PRP)-or leukocyte-poor platelet-rich plasma (LP-PRP).
- 2) Leukocyte-and platelet-rich plasma (L-PRP).
- 3) Pure PRF (P-PRF) - or leukocyte-poor PRF; and
- 4) Leukocyte- and platelet-rich fibrin (L-PRF) (called Choukroun PRF) [3, 12].

The classification described previously is the only system covering all forms of platelet concentrates for surgical use.

Currently used centrifugation protocols for platelet rich fibrin products

Original Choukroun PRF protocol (standard protocol)	3000 rpm/10 minutes [2, 5, 17]
Dohan Ehrenfest's Group - Leukocyte- and Platelet-Rich Fibrin (L-PRF)	2700 rpm/12 minutes [12, 14, 17]
Choukroun advanced PRF (A-PRF), enriched with leukocytes (A-PRF+)	1300 rpm/8 minutes [14, 17, 18]
Choukroun i-PRF (solution/gel)	700 rpm/3 minutes [15, 17]
Choukroun advanced PRF (A-PRF)	1300 rpm/14 minutes [14, 18]
T-PRF	2800 rpm/12 min [13]
CGF (Third generation platelet concentrate)	One step centrifugation protocol: 30sec -acceleration, 2min - 2700 rpm, 4min - 2400 rpm, 4min - 2700 rpm, 3min - 3000 rpm, 36sec - deceleration and stop [10, 11, 19].
Modified T-PRF (MT-PRF)	Ten-minute periods clockwise and counter-clockwise at 2700 rpm for a Total of 20 minutes [20].

Why so many PRF products?

Platelet-rich fibrin was first developed as an autologous leukocyte- and platelet-rich fibrin (L-PRF) biomaterial in France since 2001. Unlike other platelet-rich products, this technique required neither anticoagulant nor bovine thrombin (nor any other gelling agent). Thus, this platelet-rich fibrin is considered a second generation platelet concentrate. Without an anticoagulant, most platelets are activated a few minutes after contacting the tube walls, which initiates the coagulation cascade [2, 3, 7, 9].

CGF (concentrated growth factor), the third-generation platelet concentrate introduced by Sacco in 2006, contains more growth factors and has a harder fibrin structure than first-generation PRP and second-generation PRF (platelet-rich fibrin) [11]. CGF has a higher adhesive strength, tensile strength, higher viscosity than the other platelet preparations. CGF has a difference in centrifugation speed which permits the isolation of much larger and denser fibrin matrix richer in growth factors [19].

PRF was designated as L-PRF (leukocyte- and platelet-rich fibrin) and the preparation protocol had been modified later by the developer of PRF, to produce advanced PRF (A-PRF) and injectable PRF (i-PRF) as well as several other groups of products [10].

Successful clinical results have been reported with L-PRF, but some physicians worried about a possible health hazard with glass-evacuated blood collection tubes with silica activators. O'Connell described the unavoidable silica contact.

Despite these findings and the successful results in clinical studies, the initial L-PRF method was modified by changing the structure of the tubes by using biocompatible material, titanium to eliminate the negative effects of silica from dry glass or glass-coated plastic tubes. It was observed that titanium induced platelet aggregation similar to glass tubes, and the clot produced in titanium tubes was clinically identical to that in glass tubes. Although T-PRF and the L-PRF methods are quite similar, the titanium-induced platelet activation provided distinctive characteristics to T-PRF. The fibrin of T-PRF seemed more tightly woven and thicker than

There are two other classification systems proposed in the recent years, but are limited as they only refer to Platelet-Rich Plasma products and sports medicine applications [15].

Currently used centrifugation protocols for platelet rich fibrin products

Venous blood sample is collected in desired test tube and immediately centrifuged. There are various centrifugation processing-protocols that are currently being used.

that of the classic L-PRF. This difference may be due to a better hemocompatibility of titanium compared to glass, which could have potentially led to the formation of a more polymerized fibrin. Due to this structure, it was hypothesized that T-PRF may last a bit longer in the tissue. The SEM examination showed no difference between the two groups in platelet aggregation or the presence of leukocytes [3, 13, 20].

Modifications to centrifugation speed and time with the low-speed concept was shown to favor an increase in growth factor release from PRF clots which in turn may directly influence tissue regeneration by increasing fibroblast migration, proliferation and collagen mRNA levels [18].

It was recently hypothesized that by reducing centrifugation G-force, an increase in leukocyte numbers may be achieved within the PRF matrix. It was since shown that by decreasing centrifugation g-force (now termed advanced-PRF or A-PRF) an increase in total leukocyte numbers within PRF matrix scaffolds was observed.

Standard PRF contains a 3-dimensional fibrin matrix following centrifugation, however this is not ideal for injections as it is cumbersome to handle. A pioneer development of the low speed centrifugation method introduced the concept of injectable PRF (i-PRF), a liquid formulation of PRF without using anticoagulants. It maintains a liquid viscosity for roughly 15 minutes following centrifugation and interestingly can be injected in a similar method to PRP yet bears the added advantage of forming a fibrin clot shortly after injection. Also the recent study demonstrated the ability of i-PRF to release higher concentrations of various growth factors and to induce higher fibroblast migration and expression of PDGF, TGF- β , and collagen1 when compared to PRP thus provide a better environment for the regeneration and repair of the defects [22].

The low speed centrifugation concept (LSCC) enhances the regeneration potential of fluid PRF-based matrices. Consequently, the reduction of RCF by application of LSCC opens up new avenues for advanced PRF-matrices, in which the cell-cell communication between platelets and

leukocytes and that of these cells within the recipient tissue might result in improved wound healing and enhanced tissue regeneration. Thus, further preclinical and clinical studies are necessary to evaluate this concept to optimize clinical benefits [18, 23].

In a clinical setting, there is a need to increase the regenerative potential of bone substitute materials or bio-membranes. This could be achieved by adding autologous tissue engineering fluid systems. Accordingly, the presented plastic tubes and the RCF reduction allowed for the generation of a liquid injectable PRF-based matrices (i-PRF) without the use of anticoagulants. This i-PRF, prepared according to the LSCC, is highly enriched with platelets, leukocytes and growth factors, which could provide a significant benefit for the regeneration process [23].

In a study of a new centrifugation method, they aimed to change the direction of fibrin formation during the platelet aggregation, and make T-PRF much denser and more resistant. According to their hypothesis, it could make it possible to use in guided bone, and guided tissue regeneration more successfully. New autogenous product called Modified T-PRF was with superior fibrin network. Results showed that, fibrin formation was made more organised and denser with 2-way direction centrifugation [20].

Applications of PRF

PRF consists of an autologous leukocyte-platelet-rich fibrin matrix, composed of a tetra molecular structure, with cytokines, platelets, cytokines, and stem cells within it, which acts as a biodegradable scaffold that favors the development of microvascularization and is able to guide epithelial cell migration to its surface. Also, PRF may serve as a vehicle in carrying cells involved in tissue regeneration and seems to have a sustained release of growth factors in a period between 1 and 4 weeks, stimulating the environment for wound healing in a significant amount of time [7].

PRF (A-PRF or L-PRF) can either be used as a clot, membrane, injectable liquid (i-PRF), plug, or the membrane can be cut up in fragments. PRF can either be applied in stand-alone, additive, or in combination therapies [17].

T-PRF and CGF can be used in similar ways as L-PRF or A-PRF [11, 20, 21].

A PRF membrane is as natural as the host tissue, while heterologous membranes are considered as foreign bodies by the host tissues and interfere with the natural tissue healing process. Membrane. A PRF membrane is as natural as the host tissue, while heterologous membranes are considered as foreign bodies by the host tissues and interfere with the natural tissue healing process [17].

The use of PRF as an adjunct in wound healing and periodontal regeneration has shown promising results. It has been successfully used for correction of osseous defects in periodontics, oral and maxillofacial surgery and implant dentistry.

Uses of PRF

- For accelerated hard and soft tissue [5, 7].
- For accelerated bone healing in implant surgery [3, 5, 7].
- Using the fibrin plug or membrane as a filler material in extraction sockets [3, 5, 17]. Avulsion sockets: stable blood clot for neovascularization and accelerated tissue regeneration. This can be used to improve wound

healing in immunocompromised and diabetic patients [24, 25].

- PRF can also be used as a protective barrier membrane to seal off and promote healing of oro-antral communications following extractions [3, 7]; to close a palatal connective tissue harvesting site [3, 5, 17].
- For bone augmentation [3, 5].
- The PRF membrane is typically used in combination with other biomaterials in bone augmentation and grafting sites as a graft material or barrier membrane. The purpose of PRF is to activate and facilitate the healing and regenerative capacity of the host tissue, by providing a strong fibrin scaffold, major growth factors and allowing space for tissue regeneration. Using PRF as a protective barriers on bone graft sites helps to avoid perforations of the weakened gingival tissues and to prevent associated contamination of the bone graft below [3, 17].
- Sinus lifts (also in implantology): As sole material, or in combination with graft materials [3, 5, 17, 24, 26].
- Using a mix of PRF with a bone graft, placing in bone defects, or, in cases of immediate implants, covering it with several PRF layers, noting good clinical results [7].
- In periodontics to correct intra-bony defects [3, 7]. Gingival recession [3, 5, 27, 28], guided bone regeneration [3, 7], periapical lesions etc. [24].
- It has also been used for regeneration in open apex [3, 29], regenerative pulpotomies, 3 [30], periapical surgeries etc. [5, 24].
- As a vestibuloplasty wound bandage [17].
- In tissue engineering [3], for *in vitro* cultivation of human periosteal cells for bone [24].
- As a potential scaffold in pulp revascularization procedures of necrotic immature permanent tooth: as it is rich in growth factors, it seems to enhance cellular proliferation and differentiation, augmenting angiogenesis, acting as a matrix for tissue growth, and regulating the inflammatory reaction [3, 7].
- In multiple extractions to preserve the alveolar ridge height [3].
- Bone regeneration around immediate implants, inside the alveolar defect [3]. Periodontal/peri-implant defects [3].
- Reconstruction of large bone defects after cancer surgery [7].
- In plastic surgery, PRF clots are often directly used to fill cavities or mixed with an adipocyte graft in a liposuction [7].
- In the membrane form, it could be useful for small otologic surgery [7].
 - The use of L-PRF for the treatment of MRONJ is really effective, especially when it is performed with a simultaneous application of L-PRF and morphogenetic protein-2 (BMP-2), even in patients submitted for long periods of time to therapy with intravenous bisphosphonates. However, success will depend on several factors such as the previous existence of infection or the clinical stage in which the patient is [31].

- In treatment of chronic skin wounds, skin ulcers [32].

I-PRF

- Mixing autogenous bone or bone substitutes (allografts) with i-PRF (PRF Liquid) for use in GBR procedures transforms particulate bone into a easily to handle gel consistency. PRF liquid (i-PRF) can be injected above, or PRF (A-PRF or L-PRF) membrane placed above the GBR or GTR membrane to act as a interposition barrier to protect and stimulate the bone compartment, and as a healing membrane in order to improve the soft tissue healing and remodeling, and thus avoid soft tissue dehiscence [17].
- In the Treatment of Degenerative Temporomandibular Joint Disease [22].

Other applications

Platelet rich fibrin (PRF) has extensively been utilized in dental medicine but many practitioners may be surprised to learn that it has far more often been applied in general medicine for a variety of indications highlighted in this chapter. While PRF was first utilized for the treatment of hard-to-heal leg ulcers, it remains interesting to point out that PRF has also been shown to improve a variety of leg and hand ulcers, utilized for facial soft-tissue defects, chronic rotator cuff tears, acute traumatic ear drum perforations, orthopedics, tendon injuries, management of knee osteoarthritis laparoscopic cholecystectomy, plastic surgery, superficial rhytids, acne scars, vaginal prolapse repair, urethrocutaneous fistula repair, and liposuction surgical procedures [33].

PRF Lysate

A newer application of PRF based products is the PRF Lysate. In this, after PRF preparation, it is incubated at 37 °C in a humidified atmosphere of 5% CO₂/95% air and the exudate thus collected has been referred to as PRF lysate. It is said to be a good source of several growth factors. In a study, it has used to reverse the damage caused by chronic UV radiation exposure to human dermal fibroblasts by significantly increasing the proliferation rates, migration rates, and collagen deposition equal to those of normal fibroblasts. This is a relatively new application and not many studies are there on the same. Further studies may investigate potential applications of PRF lysate [34].

Practical guidelines for optimizing the quality of PRF [17]

PRF is a living biomaterial that requires a good knowledge and skills on how to produce, prepare, conserve and use it most effectively and efficiently [4, 6, 15]. Incorrect use could lead to damaged dry product leading to inconsistent clinical results. The most critical factor affecting the success of PRF in healing and regenerative therapy is the quality of PRF preparations. Following are some practical guidelines how to optimize the quality of PRF, increase clinical efficiency and consistency, and how to prevent common mistakes.

- Limit centrifuge vibration during PRF preparation.
- Patients receiving anticoagulants, it is suggested to centrifuge blood for longer periods, or increase the waiting time after centrifugation (approximately 5-10 minutes).
- Standardized and efficient preparation of PRF using standard PRF box.

- The exudate collected at the bottom of the box is rich in proteins (Vitronectin and Fibronectin). This solution can be recovered with a syringe and used to hydrate biomaterials, flush the surgical site, wet the implant surface and to preserve harvested autogenous bone blocks, rather than using saline.
- Conservation of the PRF membrane: Conserve the PRF clot in its centrifugation tube. As long as the serum has not been flushed away from the clot, the growth factor content remains stable. It is a good way to gain 5-15 minutes. PRF membranes remains usable many hours after preparation, as long as the PRF is prepared correctly and conserved in physiologic conditions.
- Optimal preparation and selection: the platelet-rich region is adjacent to the red thrombus. It is necessary to preserve a small RBC layer at the PRF clot end. Not to squeeze out all of the plasma contained in the original PRF clots.
- Optimizing and preserving growth factor release: PRF membranes should always be preserved in a wet serum environment. PRF can be used a long time after preparation, as long as the material is conserved in the adequate conditions.
- Handling the PRF (Clinical application) the elastic consistency of the PRF membrane also allows the clinician to punch a hole in the membrane to drape over a healing abutment before suturing the flap [17].

Discussion

PRF has increasingly grabbed the attention of clinicians world-wide because this biomaterial is of natural origin (autologous/derived from the patients' own blood); can be produced chair side; is easy to make, readily available; easy to use within the daily clinical routine; widely applicable in dentistry, whilst being financially realistic for the patient and the health care system [2, 3, 5, 10, 17, 18, 19, 21, 24, 33].

With L-PRF being more user friendly and economic, this arsenal is finding wider applications in surgical field. The introduction of i-PRF will also find suitable applications, where injectable form of platelet concentrate is required. Looking at the current trends PRP and L-PRF are most commonly used and have been researched upon. Newer advances such as A-PRF, i-PRF, t-PRF, CGF and sticky bone concept have been reported in single or few cases but no long term or controlled trial have been done to prove the advantage of their advancement over conventional PRP and PRF. So clinicians should use the advancements with caution [3, 5, 7, 11, 13, 14, 19, 23, 26, 33].

Conclusion

The use of PRF biomaterial for accelerating tissue healing and regeneration has increasingly gained the attention of medical and dental practitioners in the whole world because of its natural origin. The PRF can be used as a membrane (A-PRF or L-PRF, T-PRF, CGF), gel (i-PRF), plug or the membrane can be cut in fragments, and applied. But, further research is required to find the best bio-material for PRF processing which would enhance the biologic properties of PRF.

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