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Review Article

Platelet Concentrates: A Literature Review Focusing on New Technologies

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Abstract

Platelet aggregates are solutions derived from autologous blood submitted to the centrifugation and utilized to aid the tissue regeneration. The protocols of obtaining such solutions depend on several factors such as the radius of the centrifuge's rotor, the strength and the time of rotation, and the tube's material that are utilized. Taking in account the variability of the factors that interfere in the distribution of the growth factors present, the platelet aggregates evolve in each new method created, also altering its properties and there for its clinical applications, being able to associate to bone grafts, injected, utilized in wound covering, and there for, capable to aid in the regeneration of varied tissues. This review proposes to reunite and describe details about the procedures that compose the different types of platelet concentrates and their respective utilities in the sub-areas of healthcare in general. For this reason, a bibliographical research was made in chronological order taking upon as its starting point the creation of the fibrin glue and following until the most recent studies of which describe modern alternatives for regenerative therapy and coagulation simulation, in addition to publication of structural texts for clarification of the homeostasis mechanism, platelet anatomy and function, growth factors, and laboratory good practices when the procedure of the blood gathering and processing and subsequent application of the platelet aggregate created. It is hoped that this work may clarify the main divergences among the platelet aggregates, focusing in growing technologies.

Keywords: Regenerative Therapy; L-PRF; C-PRF; Alb-PRF; Current Applications; Historical

Introduction

Haemostasis is a physiological process which its function is to interrupt hemorrhage and consists didactically of 2 stages: primary haemostasis, in which occurs the interaction between endothelial collagen and the surface platelet receptors through the Von Willebrand factor; and the secondary haemostasis in which the platelet granules are de-granulated and the coagulation cascade begins [1]. On the other hand, the coagulation consists in a sequence of biochemical processes described in 1964 by Macfarlane and by Davie and Ratnoff, that through the intrinsic and extrinsic paths, contribute for the formation of the clot, an agglomerate of hemorrhages, platelets and fibrin, this being a result of the factor X activation linked to the cofactor Va and to the calcium (derived from the platelet granules) forming the prothrombinase complex, in which converts prothrombin into thrombin and, lastly, changes fibrinogen into fibrin, a molecule capable of stabilizing the platelet aggregate [2]. Platelet concentrates are surgical additives created from the blood centrifuge to simulate the stages of coagulation and aid in the tissue regeneration through the growth factors [4].

The history of the platelet concentrate begins in 1970, when Matras published the first article about fibrin glue to aid in the healing of the skin wounds, but it was from Marx studies in 1998 that the first generation of platelet concentrates came to light as such, composed by Platelet Rich Plasma and Growth Factors Rich Plasma created by Anitua E, in1999 [6]. Subsequently, Choukroun registered the second generation of platelet concentrates with the Platelets Rich Fibrin (PRF), obtained through a protocol simpler than the PRP [7]. However, only in 2019, Miron R. J., *et al.* described the parameters for the standardization of the centrifuge protocols since countless reports utilized relative centrifuge forces causing great confusion. According to him, the specifications of the platelet concentrates procedure say about the radius of the centrifuge, tube angle, rotations per minute, speed of the centrifuge in RCF (CF minimum, RCF-clot or RCF-maximum), compositions and size of the tubes and the model of the utilized centrifuge [8]. This work had as a goal to describe the protocols, properties and applications of the platelet concentrates of the PRP to the PRF and its variations.

Proposition

This review proposes to describe a retrospective of the obtaining protocols of the platelet concentrates focusing in the most modern formulations and detailing of the Platelet Rich Fibrin applicability in Dentistry.

Literature Review

Platelets are disc shaped blood cells synthesized in the bone marrow [9], containing 3 types of granules: dense granules, alpha granules, and lysosomal granules [10]. The importance of the platelets for the regenerative medical science and the tissue engineering are the alpha granules, that store seven growth factors important for the healing of the wounds: platelet derived growth factor (PDGF), transforming growth factors beta 1 and beta 2 (TGF-β1 e TGF-β2), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin type 1 growth factor (IGF-1), and hepatocyte growth factor (HGF) [11].

Centrifuges are equipments that apply rotational force to separate components of different densities of a solution. Measuring the rotational force one utilizes "rpm" (rotations per minute) and "rcf" (relative centrifugal force) or G Force (the force of gravity), represented by the letter g in lowercase after the number that indicates how many times greater is this force in relation to Earth's gravitational force [14].

	Facilitating the epithelial cells chemotaxis.	
VEGF	Vascular endothelial cells proliferation, retinal pigment epithelium cells, pancreatic duct epithelium cells; angiogenic, embryogenic, and carcinogenic; increases vascular permeability facilitating the arrival of inflammatory cells on the location of the lesion.	Costa P. A. and Santos P. (2016) Carmona Ramirez J. U. (2006)
IGF-1	It promotes the cellular proliferation and prevents the apoptosis; facilitates the carcinogenesis and the tumor metastasis.	Felippe Junior J. (2011)
HGF	Proliferation of endothelial, epithelial and hepatocyte cells.	Costa P. A. and Santos P. (2016)

Table 1

Source: Authorial.

The G Force is calculated as follows

RCF or G Force = 1,12 x R x (RPM/1000)

R being the centrifuge rotor's radius, in which the measurement can be obtained in 2 ways.

- Maximum Radius: measured from the center of the centrifuge to the test tube's bottom (RCF maximum; may be altered due to the tube's angle).
- Minimum Radius: measured from the center of the centrifuge to the test tube's cap center (RCF minimum).

It can also be utilized the distance between the middle of the fibrin clot to the rotor's center, named clot radius or "rClot" and results in the RCF-Clot [8].

Considering this, we can infer that the G Force applied to the solution during the centrifuging is directly proportional to the centrifuge's radius, and, therefore, it also interferes in the obtaining protocols of the platelet concentrates [15].

The type of the centrifuge is an extremely relevant factor when it comes to the variable (Swing Out) or fixed angle of the tube, seen that if the angle of the tube varies, the maximum radius follows [15]. Besides that, the vibration and temperature variation, when it comes to hardware quality of the centrifuges, may also interfere in the quality of the platelet concentrates [7].

In order to perform the usual blood collection, either for medical exams or the production of platelet concentrates, there is a dif-

Figure 1: Illustration of the variation of the tube's angle influencing the maximum radius.

Source: "Protocolos e Técnicas laboratoriais de rotina: Aplicações em biologia molecular, microbiologia, cultivo celular e farmacognosia" de Valéria Louzada Leal e Cia. Image 46, p. 81.

ference among the tubes through the cap colors. Regarding the protocols performed for platelet concentrates attainment, there are 3 main colors: tubes with white caps are additive free, tubes with the blue caps have sodium citrate, and the tubes with the red caps have the clotting activators and may be substituted by glass tubes that contain silica in its structure [16]. There are still companies that have their own rule for the tubes cap colors, these companies must define the characteristics of each one and how to operate.

Figure 2: Illustration of the pattern of color of the tubes used in the production of the platelet concentrates.

Source: Authorial.

There are many protocols, but as a general rule, in the production of the PRP, the venous blood is collected, distributed in blue cap tubes containing anticoagulant, and centrifuged. On the first rotation, named Soft Spin (1300 rpm for 10 minutes), the blood is separated in 3 layers, the upper layer being called platelet-poor plasma (PPP), the intermediary layer composed by PRP and the last containing red blood cells (RBC). From that, there are two possibles paths: remove the PRP layer and utilize it directly, characterizing the LPRP, or centrifuge it again in order to purify it from the leukocytes, characterizing the P-PRP. Then the products are added to the tube, this time with the red cap, with bovine thrombin or calcium chloride in order to activate the degranulation of the platelets. [17].

Choukron officially separated the PRF from the PRP in 2001 in his work entitled "The opportunity in perio-implantology: The PRF", but only in 2006, alongside Dohan, it had been clarified that the L-PRF has a much simpler technique than the PRP, only being the blood free of anticoagulants [7], reviewed by Miron R. J. in 2019, establishing 400g (clot radius) or 700g (total radius), depending on the centrifuge's radius, for 12 minutes. Therefore, the blood separates in 3 layers: an upper layer of PPP, an inferior layer of RBC, and an intermediary layer, consisting of L-PRF [17]. This new technique had appeared as an alternative to bovine thrombin use, since it was observed a defence reaction against the bovine factor Va enabling a crossed reaction between the created antibodies and human factor Va, causing coagulopathies [34]. In contrast, the P-PRF, found only by the commercial name of Fibrinet PRFM, that despite its name, it is characterized as PRP once it is obtained with the trisodium citrate use as anticoagulant during the first rotation and the calcium chloride that triggers the degranulation of the platelets and begins the coagulation [17,19]. Therefore, the different properties between the PRP and the PRF are observed as result of the handling of the solutions, it means, the anticoagulant use followed by the application of cascade triggers of coagulation (thrombin and calcium) in the PRP case and the natural polymerization without additives for the PRF, which characterizes it as a platelet concentrate of second generation [40]. Idealized by Anitua E, in 1999, it consists in a solution of concentrated growth factors created from 5ml of centrifuged blood at 160g for 6 minutes with sodium citrate as an anticoagulant. So the blood is separated in the same 3 layers, they are collected and the two superior layers are transferred to a new tube containing calcium chloride to activate the coagulation cascade and after 15 to 20 minutes they form the PRGF gel [20]. It is a simplification of the PRP protocol which utilizes calcium chloride instead of bovine thrombin, besides the different force and time of the rotation, and mainly it has made the entry of the PRP into clinics possible through the use of portable centrifuges [20,21].

In 2010, Sacco L. idealized a material based in Choukroun's PRF protocol but trying to harness all components of all the separated layers during the centrifugation, seen that they are freely available in the blood and they are all necessary. This material was given the name of CGF (Concentrated Growth Factors), set through the rotation speed alternation, however, always below 300 RCF [42].

In 2014, Ghanaati, S., *et al.* introduced a new technique for the creation of what became A-PRF (Advanced Platelet Rich Fibrin), from the rotation at 276g for 14 minutes [23,24], but the mentioned protocol has been changed to 208g for 8 minutes by the same group in 2018, and it became A-PRF+ [25], belonging, therefore, to the centrifugation derivatives group low-speed-low-time, a concept posited by Choukroun J and Ghanaati S in 2019 in his study entitled "Reduction of relative centrifugation force within injectable platelet-rich-fibrin (iPRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: the first introduction to the low speed centrifugation concept" through which they discovered that the decrease of centrifugation speed increases the concentration of inflammatory cells and create the iPRF [26].

The liquid or injectable (iPRF) was cited by Mourão., *et al*. in 2015, who associated to the bone graft, generating an agglutinated dough (Stick Bone) capable of acting in the bone regeneration as the PRP, however, without the use of anticoagulants, just activated through membrane obtained in red tubes with silica [28].

In 2013, Tunali., *et al*. created the T-PRF, produced in titanium tubes at 3500 rpm for 15 minutes and characterized by the largest elasticity and traction module [23,38].

In turn, C-PRF (concentrated platelet rich fibrin) or bio-PRF or H-PRF, developed in 2019 by Miron R. J., *et al*. through the L-PRF protocol but utilizing only 0,3 - 0,5ml of the white layer nearest to the red cells [8], and utilizing the concept of "Horizontal Centrifugation" emphasized by Lourenço., *et al*. one year prior as a technique to increase the production and extend the release of growth factors and cytokines in comparison to the fixed angle centrifuge [30], since utilizing it in a 90° angle, the maximum radius increases and therefore the necessary number of rotations per minute is lower, so that the same G Force is applied to the solution.

In 2018, Mourão., *et al.* created the Alb-CGF according to the works of Kawase T., *et al*. (2014), in which the heating of the membranes reduced its degradation time without harming its biocompatibility and its effects [37]. After performing the CGF fabrication protocol in the liquid state, the PPP was separated and heated at 75° for 10 minutes denaturing the albumin, and so it was stored for 10 minutes more in room temperature so that it was once again mixed to the CGF, resulting in an opaque and moldable membrane. Even after 7 days of its production, the Alb-CGF showed the same concentration of VEGF and FGF2 (Growth Factors) comparing it to the first hour [28]. The Alb-CGF was the predecessor of the Alb-PRF, created in 2020 by Miron R. J., *et al*. however utilizing the C-PRF instead of the CGF [29].

The most recent protocol developed is the F-PRP (Folder PRF), from Csonge., *et al.* (2021), obtained through the centrifugation protocol of 375 rpm for 10 minutes in a Steinberg centrifuge (CGOLDENWALL 80-2). After the separation of the plasma, the substance was stored in metal flasks (Dent-Art-Technik Gy), for 8 minutes until the fibrin filaments start to appear and then, in its gelatinous state, the membrane was folded 4-5 times, increasing its thickness with the goal of increasing the resistance to resorption and, as a consequence, its durability. The study achieved a positive result, seen that in comparison with the A-PRF, the F-PRF lasted 6 days more [32].

Figure 3: Illustration of the blood processing through centrifuging.
Source: Autoria.

As cited before, the mais difference between the PRP and the PRF is the use of anticoagulant, however this discrepency goes beyond than just the fabrication protocol. During the fibrinogenesis, the fibrin fibrils may organize themselves in 2 different ways: double stranded linear fibrils and branched trimolecular fibril junctions [21]. The organization of the fibrin network on the platelet aggregates depend on the amount of thrombin existing during the clotting, where the presence of the thrombin in small amounts result in a grater prevalence of trimolecular junctions, characterized as flexible networks capable of sustaining cytokines and enabling celular migration, while high concentrations of thrombin result in double strands, composing a stiffer fibrin network with less capability of cytokine entanglement and not so favorable for celular migration [39].

Figure 4: Double fibrin strands.

Source: Dohan and Choukroun., *et al.* 2006, E 37-44, Figure 4.

Figure 5

Source: Dohan and Choukroun., *et al*. 2006, E 37-44, Figure 5.

Besides the molecular structure, the platelet concentrates also differentiate themselves by the containing amount of molecules, derived from the platelet activation. In the absence of anticoagulants, the massive platelet activation occurs and subsequent the release of cytokines retained by the trimolecular fibrin network of the PRF, in contrast to PRP, which the fibrin matrix retains whole platelets and extrinsic cytokines [40]. In the study of 2017, Miron R. J., *et al.* discussed about the properties of the PRF in particular, that due to its three-dimensional fibrin network where the growth factors responsible for its angiogenic capabilities, immune system control, epithelial cell proliferation, and the recruitment of stem cells are found [29].

Figure 6: Computerized model of the molecular structure of the fibrin glue without the other structures.

Source: Dohan and Choukroun., *et al*. 2006, E 45-50, Figure 8.

Figure 7: Computerized model of the molecular structure of the PRP, (1) platelets and (2) extrinsic cytokines, therefore, not integrated with the structure.

Source: Dohan and Choukroun., *et al.* 2006, E 45-50, Figure 9.

In conclusion, a physiological concentration of thrombin, implies in a progressive polymerization capable of forming a more flexible fibril network that allows grater cellular migration and adhesion of cytokines released gradually, which stretches its life spam [40]. Besides that, the different molecular structures from the sur-

Figure 8: Computerized model of the molecular structure of the PRF

Source: Dohan and Choukroun., *et al.* 2006, E 45-50, Figure 10.

gical additives derivatives of the autologous blood provide different properties, and so, in each new protocol created, the platelet concentrates cover action mechanisms according to the eventual cellular migration and the concentration of growth factors, in addition to its applicability.

The usage of the PRF is very well supported by the studies *in vitro* showing dose-dependent stimuli in gingival fibroblasts, dermal prekeratinocytes, preadipocytes, and maxillofacial osteoblasts [4]. Miron., *et al.* in 2017 gathered information about the effects of the PRF in wound healing of soft tissue *in vitro*, *in vivo*, and clinical studies all in one work. In summary, of the 48 articles that comply the inclusion criteria, 44 obtained positive resulta regarding the use of the PRF for wound healing of soft tissue, 6 being *in vitro*, 11 *in vivo* and 27 clinical studies.

The PRF revealed to be an important ally to the osteonecrosis treatment [50], pain and swelling reduction after the third molars extraction [11], decrease in adverse reactions of inflammation and soft tissue healing [56], lifting of the maxillary sinus associated with bone allograft [25,35], horizontal bone gain [48], treatment of chronic leg ulcers [22], preservation of the ridge height after tooth extractions [19], improvement of the bone micro-structure in extraction alveolus [27], bone reconstruction of the endonasal cranium [61], better stabillity of the dental implants and less marginal bone resorption after the dental implant procedure [6], besides being capable of recruiting neutrophils that act in the pathogen elinmination [29], increase of the homeostasis after dental extraction in patients subjected to the anticoagulant treatment [5].

Discussion

Simonpieri A., *et al*. in 2012, described the literature about PRP and concluded that it is a relatively intriguing protocol, seen that there are conflicting studies and in many of those, it isn't clear which type of PRP was utilized, L-PRP or P-PRP, associated or not with bone graft and in several animal models, with very different biologies than the human kind. Boyapati L. and Wang H. L., 2006, on the other hand, attribute these variabilities to disorganized studies, they assumed that there are correct studies, but insufficient, studies with platelet concentrates variation and association to different materials [36]. Besides that, Kawase T., Mubarak S. and Mourão C. F. A. B. in 2020, presented the hypothesis saying the platelet concentrates don't possess a stable position in the regenerative medicine business because the PRP was tested for the first time directly in humans, overpassing the *in vitro* and animals testing, characterizing in an instable and variable evidence, allowing the platelet concentrates obsolescence, and enabling it to be seen as an alternative medicine or a myth with no basis [37].

In light of these facts, the second generation of platelet concentrates postulated by Dohan and Choukroun in 2006, the PRF, represents not only one alternative biochemically more favorable for not including anticoagulants in its preparation, but also for being sustained by evidences since its creation through a sequence of five articles that expatiate on its technical concepts and evolution (Part I), its biological characteristics associated with the platelets (Part II), the possible function of the leukocytes in the solution (Part III), clinical effects in the healing of tissues (Part IV), and the histological evaluation of the association between the PRF and the bone allograft for lifting of the maxillary sinus (Part V).

The L-PRF possesses a more homogeneous and organized literature than the PRP, presenting dose-dependent stimuli for the migration and differentiation of the osteoblasts, associated or not to the bone graft, and promoting a faster, safer, and better-quality bone regeneration, mainly because of the presence of leukocytes [35].

Bisphosphonates are antiresorptives used for metabolic diseases that cause bone disorders, such as osteoporosis and bone cancer metastasis, while Bisphosphonate related osteonecrosis of the jaw (BRONJ) is characterized as an area of the necrotic bone exposed or not on patients without oncology treatment history by radiation in the head and neck region [21].

In case of application of platelet concentrates to prevent MRONJ, for Fortunato 2020, there wasn't significant improvement compared to isolated surgical treatment after the tooth extractions in patients submitted to the antiresorptives treatment. However in 2016 Asaka., *et al.* had already performed through what was proven to be the PRF able to prevent damages to the healing caused by the use of antiresorptives and Şahin O., *et al*. in 2020 obtained success in their studies in which they used LPRF in extraction alveolus to prevent MRONJ, besides Giudice who in 2020 concluded that the PRF is an excellent alternative for the healing of wounds in these patients.

There are few studies like Jankovic's (2012) and Danielsen's (2010) that the use of PRF for the soft tissue regeneration didn't obtain a significant result. In return, studies such as Mourão (2020) and Alpan and Cin (2020), in which the PRF promoted the healing and pain decreased in extraction alveolus areas and in donor areas of the palate, are more frequent.

Lins, Brandão and Rocha, reported in their study in 2021 where the PRF was effective to improve the signs of aging and to fill the dark circles and nasolabial folds, besides they claimed that the PRF excels over the PRP in orofacial aesthetic procedures for not having anticoagulants, being a safer and stable option. According to Abuaf., *et al.* (2016), the PRF induced to the collagen synthesis type I through the stimuli of the dermal fibroblasts.

A study conducted by Areewong K., Chantaramungkorn M., and Khongkhunthian P. in 2019 showed that the use of the PRF for the preservation of the extraction alveolus didn't present any significant difference compared to the regular healing without auxiliaries, while in the study of Canellas., *et al.* (2019), the LPRF was significant to avoid the resorption of the extraction alveolus. Dohan Ehrenfest explained such differences in 2009 when he determined that the attainments of the PRF are always attainments of leukocytes and the distribution of these cells along the solution explains the double contradictory effect in the proliferation of the osteoblasts.

Baslarli O., *et al*. in 2015 concluded that the L-PRF doesn't act stimulating the osteoblasts to form the bone and Kotsakis G. A., *et al*. the next year, clarified that it works through its growth factors accelerating the initial healing stages and the combination of the fibrin, fibronectin, and thombospondin acting as a provisional extra cellular matrix, that increases and improves the bone formation. The *in vivo* study of Tovar N., *et al.* from 2021 showed that the L-PRF membrane as a result of the rotation of 600 RCF, has the potential of greater bone repair in association to the PLGA scaffolding in bone defects of critical size.

Studies such as Costa N. S., Santos W. L. and Martins L. G. T. (2019) and Pucetti., *et al.* (2021) had inconclusive results about the use of PRF for the lifting of the maxillary sinus, because according to them, although being a safe technique, low cost, and without contraindications, doesn't present clinical relevance about the technique without the PRF. Amaral Valladão also performed a retrospective study in which the Sticky Bone, in other words, an agglutinated iPRF and the particulate bone, covered by a L-PRF membrane stimulated the growth of the horizontal bone, but it wasn't observed vertical gain. On the other hand, studies were conducted with the goal of evaluating the effectiveness of LPRF as the only grafting material for the maxillary sinus and LPRF associated to the heterogeneous bone to the same purpose, concluding that the PRF is the satisfactory material for vertical bone gain [55] besides the increase of the angiogenesis associated to a greater bone neoformation [56], repair of the sinus membrane [57] and the possibility of implant installation in a shortened post-surgery period [58].

Diana C. performed in 2018 a study with 31 individuals to evaluate the influence of the PRF in the osseointegration of implants of immediate loads, but the hypothesis was rejected once no significant difference was observed between the study group and the control group. This result goes against the studies of Oncü, him being one of the pioneers in the investigation of platelet concentrates role in the implant osseointegration. According to him and his contributors, the PRF accelerates the osseointegration and increases the stability of the implants in the initial healing period [60-62]. The PRF was also validated for osteoporosis cases in the prosthetic treatment with dental implants, with and without antiresorptives treatment [63].

Ravi S. studies from 2020 suggest that platelet concentrate most favorable is the one that possesses a constant and lasting release of growth factors over time, therefore the development of new technologies of platelet concentrates must base itself in the search for the additive that corresponds to these requirements, having an extended time until its resorption and the interruption of its effect.

Conclusion

There is no consensus about the attainment of the platelet concentrates protocols following exactly the parameters proposed by Miron R. J. in 2019. An attached chart (ATTACHMENT 1) was created using the idealized technique by its creators in the cases of L-PRF, i-PRF, A-PRF, T-PRF, C-PRF, Alb-CGF, Alb-PRF and F-PRF referenced in the attachment. As P-PRF is only found in the PRFM format (Platelet Rich Fibrin Matrix), with the commercial name Fibrinet® Autologous Fibrin and Platelet System, the presented protocol was indicated by the manufacturer, being characterized as PRP for the inclusion of anticoagulants. In case of the P-PRP and L-PRP, the protocol indicated on the chart was evaluated by Vendramin F. S., Franco D., and Franco T. R. I. which the result was the increase of 570% of platelet concentration in relation to the blood.

The most modern formulas of platelet concentrates are the A-PRF+ (2018), the C-PRF (2019), Alb-PRF (2020) and F-PRF (2021), however it cannot be stated if the quality of one overpasses the other because there are not enough studies to do so.

What distinguishes themselves are their protocols: the A-PRF+ obtained through low-speed-low-time method, the C-PRF, through the horizontal centrifugation and using only 0,3 to 0,5ml above the RBC layer, the Alb-PRF, which utilizes the heating technique from the PPP to increase its durability due to the albumin and the F-PRP which consists of the PRF membrane folded 4-5 times to increase its thickness.

The development of new protocols of platelet concentrates attainment searches for a greater durability of its effect, increasing the period of growth factors release associated with the role of the fibrin.

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