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REVIEW ARTICLE

Differences between first- and second-generation autologous platelet concentrates

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Abstract

Autologous platelet concentrates (APCs) applied alone or combined with other biomaterials are popular bioactive factors employed in regenerative medicine. The main biological rationale of using such products is to concentrate blood-derived growth factors and cells into the wound microenvironment to enhance the body's natural healing capacity. First-generation APC is represented by platelet-rich plasma (PRP). While different protocols have been documented for PRP preparation, they overall consist of two cycles of centrifugation and have important limitations related to the use of an anticoagulant first and an activator afterward, which may interfere with the natural healing process and the release of bioactive molecules. The second generation of platelet concentrates is represented by leukocyte and platelet-rich fibrin (L-PRF). L-PRF protocols involve a single centrifugation cycle and do not require the use of anticoagulants and activators, which makes the preparation more straight forward, less expensive, and eliminates potential risks associated with the use of activators. However, since no anticoagulant is employed, blood undergoes rapid clotting within the blood collection tube; hence, a timely management of L-PRF is crucial. This review provides an overview on the most documented protocols for APC preparations and critically discusses the main differences between first- and second-generation APCs in terms of cell content, protein release, and the formation of a 3D fibrin network. It appears evident that the inconsistency in reporting protocol parameters by most studies has contributed to conflicting conclusions regarding the efficacy of different APC formulations and has significantly limited the ability to interpret the results of individual clinical studies. In the future, the use of a standardized classification system, together with a detailed reporting on APC protocol parameters is warranted to make study outcomes comparable. This will also allow to clarify important aspects on the

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mechanism of action of APCs (like the role of leukocytes and centrifugation parameters) and to optimize the use of APCs in regenerative medicine.

KEYWORDS
autologous platelet concentrates, classification, L-PRF, PRGF, PRP, regenerative medicine

1 | INTRODUCTION

The use of different blood-derived products to stimulate the healing process started more than 40 years ago. In the beginning, platelet concentrates were used only in severe thrombopenia to prevent hemorrhages.[1] Over the years, an increased interest in the application of autologous platelet concentrates (APCs) for the regeneration of hard and soft tissues has been documented due to the adhesive properties of the fibrin matrix and the large amounts of growth factors stored inside the platelets that could favor the healing process.[2] Nowadays, APCs applied alone or combined with other biomaterials are a popular strategy in several medical fields, particularly in sports medicine, orthopedic surgery, dermatology, as well as dentistry, where their high concentration in growth factors is exploited to treat inflammatory conditions or to promote tissue regeneration.[3–7]

1.1 | APC generations

The first generation of platelet concentrates is represented by platelet-rich plasma (PRP), which was firstly documented at the end of the 90s.[8] Although a large variety of protocols has been proposed for PRP preparation (see Section 4), overall they all require two main steps, namely centrifugation and activation.

While PRP (and its derivatives) is still popular, it has important limitations related to the use of an anticoagulant first and an activator afterward, which may interfere with the natural healing process and the release of bioactive molecules.[9]

Moreover, the legal and regulatory requirements associated with PRP use are heterogeneous and country-specific. For instance, most European countries consider PRP as a "non-standardized medicinal product," for which good manufacturing practice (GMP) standards must be applied.[10,11] In particular, the Directive 2005/62/EC clearly states that good practice guidelines based on the principles of GMP must be implemented.

These shortcomings and the lack of uniformity in PRP preparation protocols among published studies led to the development of a second generation of platelet concentrates defined as an autologous platelet and leukocyte-rich fibrin material (L-PRF).[12,13]

In the literature, there is lack of clarity in relation to the use of the L-PRF and PRF terms. However, since the introduction of the APC classification in 2009 which distinguishes four main types of platelet concentrates, two groups of PRF preparations were identified: P-PRF (not extensively used) and L-PRF[14] (see Section 3). Therefore, throughout this article, we have adopted the term "L-PRF," unless the authors specifically referred to subsequent modifications of the original L-PRF protocols, like A-PRF, A-PRF+, i-PRF, etc.

L-PRF protocol involves the immediate centrifugation of blood samples through a single centrifugation cycle and does not require the use of anticoagulants and activators, which makes the preparation more straight forward, less expensive, and eliminates potential risks associated with the use of bovine thrombin[15] or other activators. Since anticoagulants are not employed, blood undergoes clotting over time within the blood collection tube, therefore it becomes critical for the clinician to begin centrifugation shortly following blood collection to separate blood layers. In contrast to PRP, L-PRF is characterized by a high concentration of leukocytes, which contribute to the immune and antibacterial responses.[16]

2 | BIOLOGICAL RATIONALE

Adequate blood supply is a prerequisite for wound healing, and it is one of the critical parameters that can influence the final outcome (regeneration or repair). As a matter of fact, hemostasis and fibrin clot formation are the first steps that trigger the wound healing cascade.[17] Platelets are the first cells populating the wound microenvironment and they contribute to the hemostasis stage of healing. Beginning with the adhesion to von Willebrand factor and collagen in the subendothelial matrix, platelets become activated. While the primary role of platelets is hemostasis, they can mediate inflammation via the release of substances such as proteolytic enzymes and cationic proteins from their granules. Degranulation of activated platelets leads to the release of more than 300 biologically active molecules, including several growth factors, such as PDGF, VEGF, IGF-2, EGF, and TGF-β, which contribute to the recruitment of immune cells and osteogenic precursors and serve as messengers and regulators that influence a variety of cell–cell and cell–extracellular matrix interactions.[18,19] Therefore, autogenous blood proteins (i.e., growth factors) are capable of supporting angiogenesis, as well as proliferation and maturation of the tissues.[20,21] During wound healing, platelets adhere to the injured site resulting in platelet–platelet interaction (aggregation) under the control of humoral mediators in plasma, such as epinephrine, thrombin, and mediators released from activated platelets (adenosine diphosphate - ADP and serotonin). Platelets and leukocytes play pivotal roles in both innate and adaptive immune responses.

Therefore, the main biological rationale of using an autologous blood product such as an autologous platelet concentrate is to concentrate and accumulate blood-derived growth factors, cytokines,

lysosomes, and cells into the wound microenvironment, and eventually into the soft and/or hard tissues undergoing healing, thus enhancing the body's natural healing capacity.

2.1 | Biology of platelets

Blood is composed of cells (erythrocytes and leukocytes) and cell fragments (platelets) suspended within a liquid plasma. The circulating platelets are non-nucleotide cell fragments released from large bone marrow megakaryocytes and regulated mainly by thrombopoietin hormone. In humans, normal platelet count varies between 150000 and 350000 cells/μL (150–400 × 10^9/L) of blood. The total number of platelets in the different APC preparations mainly depends on the preparation protocols that are applied.

Platelets contain three major types of granules, α-granules, dense or δ-granules, and lysosomes. The majority of platelet proteins/biomolecules are released from the platelet α-granule secretome. Platelet-derived mediators/proteins are summarized in Table 1. Platelets have abundant surface receptors classified as agonist receptors and adhesion receptors. While agonist receptors, including collagen, thrombin, and ADP recognize stimulatory proteins/molecules, adhesion receptors promote aggregation. Although platelets are active cells, they have a limited capacity of new protein synthesis and their lifespan is between 7 and 10 days. Activated platelets release their granule content including nucleotides, adhesive proteins, several growth factors (e.g., platelet-derived growth factor [PDGF], vascular endothelial growth [VEGF], transforming growth factor-1 [TGF-1], and epidermal growth factor [EGF]), angiogenic factors, and cytokines, which are of crucial importance to trigger the healing cascade. For instance, PDGF has the potential to recruit progenitor cells, induce fibroblast proliferation, migration, chemotaxis for macrophages, collagen synthesis, as well as secretion of cytokines.[2]

The interaction between platelets and the monocyte–macrophage system is a key mechanism in the context of wound healing. While few studies indicate that platelet interaction drives macrophages toward M1 phenotype with a prominent proinflammatory role,[22] other data suggest that collagen-activated platelets exert anti-inflammatory functions by increasing the anti-inflammatory cytokine IL-10 and reducing TNF-α secretion by macrophages in a prostaglandin E2- or CD40L-dependent manner.[23,24]

TABLE 1 Following the activation of platelets, the production of platelet-derived mediators linked with hemostasis, inflammation and infection occurs. The table summarizes the main mediators contained in and released by platelets.

Released from the granules of the platelets		
Type of platelet granule	Protein/Molecule	Function
α-granule	PF4 (CXCL4)	Recruitment and activation of other immune cells
	RANTES (CCL5), IL-8, NAP-2, MIP-1-α (Chemokines)	
	PDGF, EGF, VEGF, IGF-1, CTGF, FGF, HGF, TGF-β (Growth factors)	
	β-defensin	
δ-granule	Serotonin (5HT)	Platelet activation and thrombus formation T-cell migration and increase monocyte differentiation into dendritic cells
	ADP	
	Polyphosphates	
	Glutamate	
	Histamine	
	Calcium	
Lysosomes	Elastase	Diapedesis
	Collagenase	
	Cathepsin	
Produced from the platelets		
IL-1β		Angiogenesis, Chemotaxis, Cell adhesion Immunomodulatory activities
Thromboxane		
Nitric Oxide		
GP1bα		
Adhesion molecules (P selectin, Fibrinogen, Fibronectin, and Integrins)		

Abbreviations: ADP, adenosine diphosphate; CTGF, Connective tissue growth factor; EGF, Epidermal growth factor; FGF, Fibroblast growth factor; GP1bα, Glycoprotein 1b alpha; HGF, Hepatocyte growth factor; IGF-1, Insulin-like growth factor-1; IL-1β, Interleukin 1 beta; IL-8, Interleukin 8; MIP-1-α, Macrophage inflammatory protein 1-alpha; NAP-2, Neutrophil-activating peptide-2; PDGF, Platelet-derived growth factor; PF4 (CXCL4), Chemokines such as platelet factor IV; RANTES, Regulated upon activation, normal T-cell expressed and secreted; TGF-β, Transforming growth factor-β; VEGF, Vascular endothelial growth factor.

2.2 | Protein release pattern and mechanism of action

The preparation protocol of APCs influences their composition and protein (i.e., growth factors and cytokines) release pattern.

As previously discussed, the main differences between first and second-generation APCs are the use of anticoagulants and the subsequent use of a coagulation activator (e.g., $CaCl_2$), which are necessary only in first-generation products. Conversely, the immediate single centrifugation of blood samples without anticoagulant application (second-generation products) results in the formation of a fibrin scaffold (L-PRF) containing cells (including a large number of leukocytes) and several natural cytokines and growth factors.

The mechanism of action of all APCs is essentially based on the growth factors stored in platelets' granules and on their release after platelet activation, which triggers the healing cascade. In other words, the rationale of using APCs is to reach an elevated supraphysiological dose of growth factors to enhance the natural wound healing process.[25] Besides growth factors, blood proteins such as fibrin, fibronectin, and vitronectin act as cell adhesion molecules and they function as biological mediators by regulating cell proliferation, chemotaxis, and differentiation. During the healing process, platelets act in coordination with leukocytes in the wound microenvironment by having cellular cross-talks via cytokines, including IL-1β, IL-4, IL-6, and TNF-α, as well growth factors.[26]

Previous studies have clearly indicated that the content of growth factors in PRP (first-generation product) can significantly vary based on the system used for its preparation.[27,28] A study also showed that growth factor and catabolic cytokine concentrations are influenced by the cellular composition of PRP.[29] More specifically, platelets increase anabolic signaling, while leukocytes increase catabolic signaling molecules.

Likewise, L-PRF production systems (second-generation products) can significantly impact on their content and protein release. Twenty-four different L-PRF production protocols involving six different centrifugation speeds and four different times were tested in order to assess cell numbers and cellular concentrations (namely leukocytes, red blood cells, platelets, neutrophils, lymphocytes, and monocytes).[30] Based on this study, protocol time seemed to have a greater impact on the final cell layer separation when compared to speed. The optimal centrifugation speed and time for solid L-PRF ranged between 400 and 700 g for 8 min. Remarkably, patient's baseline cell count (hematocrit) significantly influenced cell layer separation and this finding was particularly relevant at lower centrifugation speeds. Recently, Wang et al.[31] investigated the growth factors, cytokine and matrix metalloproteinase (MMP) release rate, as well as total release over time (6, 24, 72, and 168 h) from L-PRF in vitro and ex vivo. This study showed that L-PRF can sustain the release of growth factors and cytokines for up to 7 days. Interestingly, growth factors can activate cells to produce and release additional mediators, thus amplifying the cell-to-cell communication and optimizing the wound environment for healing.

Few studies have also compared the protein release profile of different generations of APCs. Schar et al.[32] compared the concentration and kinetics of growth factors release from L-PRF and leukocyte- and platelet-rich plasma (L-PRP) obtained from 11 donors. They showed that L-PRF had higher amounts of released TGF-β1, longer term release of growth factors and stronger induction of cell migration as compared to L-PRP.

Dohan Ehrenfest et al.[33] also suggested that leukocyte content and fibrin architecture are key APC properties that influence the local release of platelet growth factors. In particular, they compared the release of TGF-β1, VEGF, and PDGF-AB from a pure platelet-rich plasma (P-PRP) gel (namely plasma rich in growth factors—PRGF) and L-PRF gel membranes in culture medium and they showed that during 7 days, L-PRF membranes slowly released significantly larger amounts of all these growth factors. Moreover, they reported a different release pattern, since P-PRP gel membranes completely dissolved after less than 5 days, while L-PRF membranes were still intact at 7 days. In both APCs, vitronectin was the sole molecule to be released almost completely after 4 h, thus suggesting that this molecule is not trapped in within the fibrin matrix.

The growth factor release of PDGF-AA, PDGF-AB, PDGF-BB, TGF-β1, VEGF, EGF, and IGF from PRP, L-PRF, and advanced-PRF (A-PRF) was assessed by another study at different time points (15 min, 60 min, 8 h, 1 day, 3 days, and 10 days).[34] Remarkably, the longest protein release was reported for A-PRF, whereas PRP was characterized by a fast delivery. Another crucial aspect is the protein quantity that is released throughout time by APCs. The same study showed that while PRP released higher proteins at earlier time points, L-PRF stimulated a continuous growth factors release over a 10-day period. Moreover, A-PRF showed higher amounts of total growth factors released as compared with L-PRF.[34]

One study also assessed the effect of different processing methods on the release of PDGF-BB, VEGF, and TGF-β1 from non-Ca^{2+}-activated PRP, Ca^{2+}-activated PRP, L-PRF, and A-PRF.[35] In particular, the study investigated whether freeze–thaw–freeze (FTF) cycles, incubation at room temperature, or incubation at 37°C for 1 h with a further 16 h at 4°C, would impact on growth factor release. The results indicated that growth factor levels were significantly increased in the non-Ca^{2+}-activated PRP with FTF incubation, whereas in the L-PRF preparations there was a significant disadvantage to using room temperature incubation for releasing growth factors. They also confirmed that A-PRF released higher concentrations of PDGF-BB, VEGF, and TGF-b1 compared with the conventional L-PRF preparation method. Conversely, an in vitro study by Castro et al.[36] showed no differences in terms of growth factors release, cellular content, and dimensions between L-PRF, A-PRF, and A-PRF+ membranes.

Despite APCs have been used for several decades, few aspects related to APC use have not been fully clarified, such as the duration of platelet activity and whether released proteins remain locally or circulate via bloodstream following their release. Future studies are warranted to compare protein composition and release before and after the application of first and second-generation APCs.

Interestingly, in recent years, the role of platelet-derived extracellular vesicles in immunomodulation has been investigated. Although exosomes of platelet origin seem promising for regenerative applications, factors such as isolation methods, storage conditions and, most importantly, clinical safety need to be elucidated before their translation into clinic.[37]

3 | CLASSIFICATION OF APCs

Whereas each platelet concentrate presents with major specificities and has its own identity, in the past years, efforts have been made to cluster APCs into families. The application of a classification system carries the advantage to promote the use of a "common vocabulary" among researchers, thus facilitating communication, replication, and quantitative summaries (meta-analyses) of published data.

While we continue to distinguish between first and second-generation APCs, the classification system proposed by Dohan Ehrenfest et al. in 2009[14] is considered the first important milestone in this respect and is still widely reported (Figure 1). This system considers only two parameters, namely cell content (primarily in terms of leukocytes) and fibrin architecture, and it defines four main families of products:

- Pure platelet-rich plasma (P-PRP), or leukocyte-poor platelet-rich plasma, which includes products without leukocytes and with a low-density fibrin network after activation. One popular and well-codified P-PRP preparation is plasma rich in growth factors (PRGF)[38];
- Leukocyte- and platelet-rich plasma (L-PRP), which includes preparations with leukocytes and with a low-density fibrin network after activation. This is probably the family with the largest number of commercial or experimental systems and it is widely employed in many medical fields, including orthopedics, and for extraoral wound care.[39,40]
- Pure platelet-rich fibrin (P-PRF) or leukocyte-poor platelet-rich fibrin, which includes preparations without leukocytes and with a high-density fibrin network.
- Leukocyte- and platelet-rich fibrin (L-PRF), which includes preparations with leukocytes and with a high-density fibrin network.

The aforementioned system was validated by a multidisciplinary consensus conference in 2012[41] and was further acknowledged by the POSEIDO (Periodontology, Oral Surgery, Esthetic and Implant Dentistry Organization) the following year.[42]

Other classification systems have been proposed over the years, but they are limited to PRP (first-generation products) (Figure 1). In particular, Mishra et al.[43] classified four types of PRP based on platelets and leukocytes concentration:

- type 1 contains an increased concentration of platelets and white blood cells over baseline and is not activated by an exogenous activator;
- type 2 contains both increased platelets and white blood cells and is activated with thrombin and/or calcium;
- type 3, also known as "platelet-leukocyte gel," contains only an increased concentration of platelets without any white cells and is not activated prior to application;

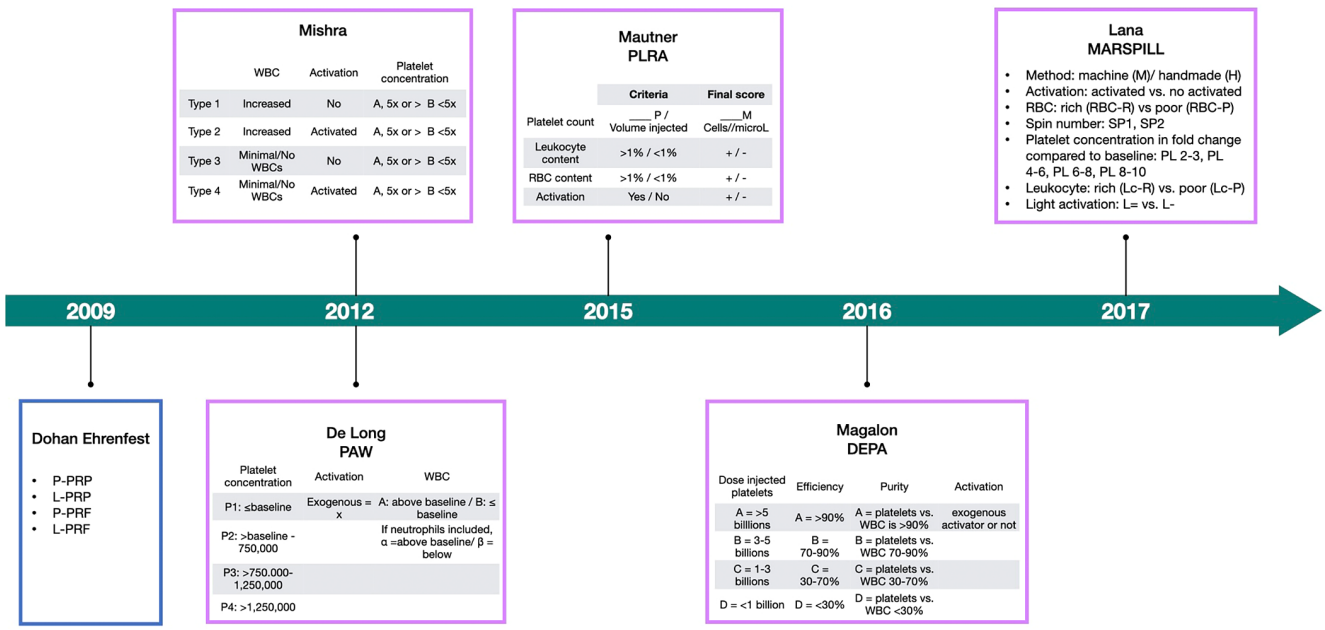

FIGURE 1 Timeline summarizing the different APC classification systems proposed over the years. While Dohan Ehrenfest 2009 is the only one considering both first and second-generation APCs, all the other systems are limited to PRP.

- type 4 contains only an increased platelet concentration and is activated with thrombin and/or calcium.

The main novelty introduced by this system is the evaluation of platelet concentration, with type A PRP being five times or more the blood concentration of platelets, and with type B PRP being less than five times the blood concentration of platelets. However, since platelet concentration depends on the volume of liquid serum used to keep the platelets in suspension and the serum quantity may vary significantly between protocols, this parameter is not overall recognized as relevant.

In the same year, DeLong et al.[44] proposed the PAW system to classify PRP, which considers three parameters: the absolute number of platelets (P), the platelet activation method (A), and the presence/absence of white cells (W). More specifically, the total white blood cell (WBC) content is identified as either above (A) or below/equal to (B) baseline levels. Hence, systems designated with an "A" are buffy coat systems, whereas plasma-based systems fall under "B." A subcategory for neutrophil count has also been introduced for systems that concentrate WBCs. If neutrophils are included in the buffy coat, then α ("above") is added. If neutrophils are filtered out, then ß ("below") is added. This means that according to this classification buffy coat systems will have a designation of either Aα or Aß. Although the PAW classification recognized the potential importance of neutrophil content in PRP, red blood cells (RBC) were not addressed and the evaluation of WBCs and neutrophils into "above baseline" and "below baseline" categories may represent an oversimplification of the impact of WBC and neutrophil content on PRP activity and efficacy.

In order to overcome the limits of the previous PRP classification systems, Mautner et al.[45] proposed a new and more comprehensive classification named PLRA, which accounts for platelet count, leukocyte presence, RBC presence, and use of activation.

Another and more recent effort to standardize the reporting on PRP has been published by Magalon et al.,[46] who proposed the DEPA (Dose, Efficiency, Purity, Activation) classification system, which is based on:

- Dose of injected platelets, which is calculated by multiplying the platelet concentration in PRP by the obtained volume of PRP and should be measured in billions or millions of platelets. Based on this parameter, four categories can then be identified: A, very high dose of injected platelets (>5 billions); B, high dose of injected platelets (from 3 to 5 billions); C, medium dose of injected platelets (from 1 to 3 billions); and D, low dose of injected platelets (<1 billion).
- Efficiency of production, is based on the recovery rate in platelets (also called platelet capture efficiency) and corresponds to the percentage of platelets recovered in PRP from the blood. Based on this parameter, four categories can be distinguished: A, high device efficiency (recovery rate >90%); B, medium device efficiency (recovery rate from 70% to 90%); C, low device efficiency (recovery rate from 30% to 70%); and D, poor device efficiency (recovery rate < 30%).
- Purity of the PRP, which corresponds to the relative composition of platelets, leukocytes, and RBCs in the obtained PRP and is categorized as follows: A, very pure PRP if the percentage of platelets in the PRP compared with RBC and leukocytes is >90%; B, pure PRP if the percentage of platelets in the PRP compared with RBC and leukocytes is from 70% to 90%; C, heterogeneous PRP if the percentage of platelets in the PRP compared with RBC and leukocytes is from 30% to 70%; and D, whole blood PRP if percentage of platelets in the PRP compared with RBC and leukocytes is <30%.
- Activation process, which requires reporting on the addition of an exogenous clotting factor to activate platelets or not.

Finally, in 2017, Lana et al.[47] reviewed all the available classification systems on PRP and proposed a new and articulated classification, the MARSPILL, that considers different parameters used during PRP preparation and focuses especially on peripheral blood mononuclear cells (PBMCs). In this respect, this classification is focused on the differentiation of platelet-rich plasma and rich mononuclear cells or platelet-rich plasma and poor mononuclear cells, related to the use of leukocytes, but with a special attention to the mononuclear cell population. Moreover, eight parameters are assessed as follows:

- Method, which refers to whether PRP is prepared in an automated manner (M, machine) or if it is handmade (H);
- Activation, which distinguishes between activated and nonactivated PRP;
- Red blood cells content, which would distinguish between rich (RBC-R) and poor (RBC-P);
- Spin number (SP1 for 1 spin and SP2 for 2 spins);
- Platelet concentration in terms of fold change compared to baseline (PL: 2–3; PL: 4–6; PL: 6–8; and PL: 8–10 folds compared to baseline);
- Leukocyte content, which distinguishes between leukocyte-rich (Lc-R) or -poor (Lc-P) PRP.
- Light activation, which would distinguish between PRP light activated (L+) or not (L).

Noteworthy, for second-generation APCs characterized by the presence of leukocytes and with a high-density fibrin network (L-PRF), there is no proposed classification system comparable to the ones developed for PRP. Nevertheless, the initial L-PRF protocol proposed by Choukroun et al.[12,13,16] that required 10 mL of blood sample to be collected without anticoagulant in a glass-coated plastic tube, which is then immediately centrifuged at 3000 rpm (approximately 400 *g*) for 10 min has been adapted over the years and new modifications of the original L-PRF have been introduced,[2] as described in Section 4.1.3.

4 | PROTOCOLS FOR APC PREPARATION AND THEIR IMPACT ON BIOLOGICAL AND MECHANICAL PROPERTIES

Several commercially available systems are used to produce first and second-generation APCs. More specifically, APC protocols can

be evaluated in relation to (i) the preparation kit, including the centrifugation process and addition or not of activator; (ii) platelet and leukocyte content of the final preparation; and (iii) fibrin network of the final preparation.[14]

Table 2 summarizes the main characteristics of APC protocols documented in the literature.

4.1 | Protocols overview

4.1.1 | P-PRP (first generation)

For the production of P-PRP, a specific volume of the patient's autologous venous blood is collected with an anticoagulant to prevent spontaneous coagulation, which is then processed by centrifugation at low forces to separate it into three layers: (a) acellular plasma or platelet-poor plasma (PPP), which is rich in fibrinogen; (b) the buffy coat layer (BC), containing platelets and few leukocytes; and (c) red blood cells (RBCs).

The supernatant consisting of the buffy coat and plasma is aspirated, pulled, and transferred to another centrifuge tube for a second centrifugation (without anticoagulant) that aims at concentrating platelets at the bottom of the tube, from where they are collected and suspended in plasma. The activator is eventually used to induce degranulation of the platelets and fibrin polymerization, thus resulting in the formation of a platelet gel rich in various growth factors.[14,48]

Plasmapheresis using a cell separator was historically the first method for the formulation of autologous plasma concentrates but is not widely used in practice as it often requires the assistance of a hematologist and the resulting P-PRP may contain residual RBCs and leukocytes.[14]

One of the most popular protocol modifications for P-PRP is PRGF (plasma rich in growth factors), firstly described by Anitua in 1999.[38] Whole blood is collected with an anticoagulant, prior to administration of anesthesia, and undergoes a single stage centrifugation at 460 g for 8 min to separate the three layers—(a) plasma, (b) BC, and (c) RBC. The upper plasma layer consists of two fractions, namely F1 (upper one fifth in tube) termed P-PGF (plasma poor in growth factors) and F2 (subsequent one fifth in tube) termed P-RGF (plasma rich in growth factors). These fractions are collected and transferred into separate collection tubes. Activator (calcium chloride) is added to induce fibrin polymerization and the F2 fraction will form a gelatinous substance within 10 min. Leukocytes, which are contained in the BC layer, are discarded during fractioning. As a result, PRGF does not contain white blood cells that have been shown to negatively impact autologous fibrin formation and the homogeneity of the final product.[49,50]

Following this protocol, an unstable PRGF gel formation occurs within 15–20 min, which should be applied immediately to the clinical site.[14,38] This PRGF protocol, as defined by Anitua, has been marketed as PRGF ENDORET®, which is available both with anticoagulant and activator (KMU15) and in a formulation that does not require neither anticoagulant nor activator (KMU16). Avoiding the use of bovine thrombin as an activator (KMU16), eliminates the biosafety risk for the patient and contributes toward a controlled transfer of biological mediators,[49] although the timing of fibrin formation is less predictable.

This protocol allows for the obtainment of variable autologous preparations rich in growth factors from the same patient, depending on the degree of activation and coagulation of the collected sample.

The PRGF protocol by Anitua has evolved since its original publication for optimization of its physical and biological properties. In particular, Anitua et. al[51] reported that by reducing the amount of sodium citrate and calcium chloride to 0.4 mL and to 20 μL, respectively, it is possible to obtain a statistically significant higher enrichment and recovery of platelets, as well as a higher growth factor concentration. This updated protocol also allows a reduced clotting and clot retraction time.

Fibrinet® platelet-rich fibrin membrane (PRMF) by Cascade Medical (New Jersey, USA) is another system that leads to the formation of pure platelet-rich plasma (P-PRP). This protocol shares similarities with L-PRP protocols, but the resulting product contains low numbers of leukocytes due to the use of a separator gel. After the first centrifugation, the BC and PPP layers are transferred to a separate tube containing calcium chloride, which immediately stimulates the clotting cascade. The transfer is facilitated by a proprietary connection system. The second centrifugation leads to the formation of a stable PRFM clot. Similar to Anitua's PRGF protocol,[38] the addition of calcium chloride stimulates the platelet and fibrinogen concentrates, but the resulting Fibrinet® PRFM forms a denser fibrin matrix, which is attributed to the dynamic clotting during the second centrifugation process.[14,52]

The Nahita single-centrifugation system (Nahita, Navarra, Spain) is also a system for P-PRP preparation utilizing a slightly higher centrifugal acceleration at an increased duration, which is ergonomic and does not damage platelets during collection and processing.[53]

4.1.2 | L-PRP (first generation)

In order to obtain L-PRP, the same first step of soft spin centrifugation is undertaken as for P-PRP. Subsequently, PPP, the BC layer, and fragments of residual RBCs are separated via a hard spin centrifugation. After discarding the PPP, the resulting L-PRP concentrate consists of the complete BC (containing most leukocytes) and residual RBCs in a fibrin-rich plasma.[14]

Curasan® (Kleinostheim, Germany) and Friadent-Schutze® (Vienna, Austria) are two-stage centrifugation protocols for the preparation of L-PRP that follow the methodology previously described. A small volume of L-PRP is produced with the above protocols, which is not only high in platelets and leukocytes, but also contains a residual number of RBCs.[14] The Curasan® system's platelet concentration efficiency is approximately one third when

TABLE 2 Main characteristics of APC protocols available documented in the literature.

	System/Protocol	Clinical formulation[a]	Anticoagulant	First step centrifugation	Second step centrifugation	Activator	Platelet concentration[b]	Leukocyte collection
P-PRP	Cell separator PRP	Unclear	Yes	3000 g?	3000 g?	*No information*	High	No
	Fibrinet PRFM	Clot	Trisodium citrate	1100 g for 10 min	1500 g for 15 min	Calcium chloride		No
	Anitua's PRGF	Clot/Gel/Membrane	Sodium citrate	460 g for 8 min	-	10% Calcium chloride	Low	No
	Nahita PRP	Clot	Trisodium citrate, Citrate, Acid citrate dextrose	280 g/1500 rpm for 7 min	-	Calcium chloride	Low	No
L-PRP	PCCS PRP	Unclear	Acid citrate dextrose	3000 rpm for 3 min 45 s	3000 rpm for 13 min	*No information*		Yes
	SmartPReP PRP	Unclear	Acid citrate dextrose	*No information*	*No information*	Thrombin-calcium citrate		Yes
	SmartPRep2 APC+	Unclear	*No information*	*No information*	*No information*	Thrombin-calcium citrate		Yes
	Symphony II PCS	Unclear	Calcium citrate	*No information*	*No information*	Bovine thrombin and 10% calcium chloride solution	High	Yes
	Magellan PRP	Unclear	Calcium citrate	*No information*	*No information*	Bovine thrombin and 10% calcium chloride solution		Yes
	Secquire Cell Separator	Gel	Calcium citrate	3500 rpm for 9 min	3500 rpm for 6 min	Bovine thrombin and 10% calcium chloride solution	High	Yes
	Accelerate PRP	Gel	Calcium citrate	3500 rpm for 9 min	3500 rpm for 6 min	Bovine thrombin and 10% calcium chloride solution		Yes
	GPS PRP	Unclear	Calcium citrate	3200 rpm for 15 min	same as first	Bovine thrombin and 10% calcium chloride solution		Yes
	Curasan PRP	Unclear	*No information*	2400 rpm for 10 min	3600 rpm for 15 min	Bovine thrombin and Calcium Chloride	Low	Yes
	Friadent PRP	Unclear	Acid citrate dextrose	2400 rpm for 10 min	3600 rpm for 15 min	Bovine thrombin and calcium chloride		Yes
	Regen PRP	Unclear	Acid citrate dextrose	1500 g for 10 min	-	Autologous thrombin obtained by treating patient's blood with calcium chloride and ethanol		Yes
	Plateltex PRP	Unclear	Sodium citrate	180 g for 10 min	1000 g for 10 min	Batroxobin dissolved in calcium gluconate		Yes
	ACE PRP	Unclear	Trisodium citrate acid citrate dextrose	160 g/1300 rpm for 10 min	400 g/2000 rpm for 10 min	Calcium chloride		Yes
P-PRF	Vivostat PRF	Sealant	Yes	25 min, (rpm unclear)	Unclear	No	Low	No

TABLE 2 (Continued)

	System/Protocol	Clinical formulation[a]	Anticoagulant	First step centrifugation	Second step centrifugation	Activator	Platelet concentration[b]	Leukocyte collection
L-PRF	Choukroun's L-PRF(as described by Dohan et al.[13,16])	Clot/Plug/Membrane	No	3000 rpm for 10 min	-	No	High	Yes
	A-PRF	Clot/Plug/Membrane	No	1500 rpm for 14 min	-	No	High	Yes
	A-PRF+	Clot/Plug/Membrane	No	1300 rpm for 8 min	-	No	High	Yes
	I-PRF	Liquid	No	700 rpm for 3 min	-	No	High	Yes
	T-PRF	Clot/Plug/Membrane	No	2800 rpm for 12 min	-	No	High	Yes
	C-PRF	Liquid	No	2400–3300 rpm (time not clearly stated)	-	No	High	Yes
	CGF	Clots/Gel/Membrane	No	30 s acceleration, 2700 rpm for 2 min, 2400 rpm for 4 min, 2700 rpm for 4 min, 3000 rpm for 3 min, and 36 s deceleration and stop	-	No	High	Yes

Note: The details of some established protocols could not be yielded during our literature search, and this has been indicated in the table as "no information."

Abbreviations: g, G-force; rpm, revolutions per minute; s, seconds.

[a]The clinical formulation is not always clearly stated in the literature and, thus, uncertainty remains on the formulation of the final products for some of the included protocols.

[b]For platelet concentration, no objective conclusions could be drawn to qualify the platelet content of all protocols in comparison to each other as no such information is available in the literature.

compared to similar automated protocols (such as the PCCS), which could possibly be attributed to a higher degree of platelet disruption during the preparation process or dissimilarities in the platelet adhesion to the collection bags.[54] Additionally, the Friadent-Schutze® system has been found to be inferior to the SmartPReP® system in respect to platelet collection efficiency and ergonomics.[55] A number of other systems are also available in the market.

For instance, the Regen PRP (Regen Laboratory, Mollens, Switzerland) protocol requires collection of blood in a tube with acid citrate dextrose (ACD) and a proprietary separator gel that improves the degree of platelet and leukocyte collection, while the Plateltex® (Bratislava, Slovakia) system utilizes lyophilized batroxobin, a fibrinogen cleaving enzyme for fast fibrin clot formation, allowing L-PRP to be formed in 10 min. However, the composition of the final L-PRP prepared with these manual systems is often unclear and may vary, with reproducibility being operator dependent.[14]

The double-centrifugation system ACE® (Surgical Supply and Surgical Science Systems, Brockton, MA) is based on the basic L-PRP protocol and results in an increased concentration of platelets and larger platelet aggregates, when compared to the Nahita system. However, greater sample variability in respect to platelet concentration was noted with the ACE protocol, which could be associated with this technique-sensitive manual protocol. Greater volumes of the patient's blood are also required with this system.[53]

The PCCS® (Platelet Concentrate Collection System) (3i Implant Innovations Iberica S.l., Barcelona, Spain), the SmartPReP® by Harvest Corp (Plymouth, USA), and the SmartPReP®2 *APC*+TM System (Harvest Technologies, Plymouth, USA) are automated protocols that consist of two side-by-side containers that allow collection and centrifugation. The PCCS system produces L-PRP similar in consistency to the preparation produced by Curasan®. Whole blood is extracted with anticoagulant and placed into the first compartment (polyvinyl chloride) for the first centrifugation. The PPP and BC are then transferred into the second chamber via opening the clamp and air pressure, where the second centrifugation takes place. Any PPP remaining is transferred back into the first chamber when 35cm^3 of air is pumped into the valve.[54] The SmartPRep® system allows for the automatic transfer of the PPP and BC into the second compartment based on changes in weight and centrifugation speed. Similar to SmartPRep®, SmartPReP®2 *APC*+ uses a specialized filter to separate plasma from RBCs. Following the completion of the first centrifugation, plasma concentrate is collected in the inferior portion of the plasma chamber and the remainder is filled with PPP, which is then discarded. L-PRP is obtained by resuspending the platelet concentrate through the centrifugation process multiple times. Thrombin-calcium citrate activator is then added to activate the platelets at the site of application.[48] The Gravitational Platelet SeparationTM II System® (GPSTM II System) (Cell Factor Technologies/Biomet Orthopedics, Warsaw, Ind) is also a variation of the two-compartment, two-stage centrifugation protocols. Three systems are commercially available, depending on the amount of blood drawn (27 mL, 55 mL, and 110 mL), which is then mixed with calcium citrate as an anticoagulant. However, after the first centrifugation, PPP is discarded, and the second centrifugation is completed with the RBCs. To obtain L-PRP, platelets are first suspended by shaking the tube for 15–30 s prior to aspiration of the BC layer on the surface of the RBC base. The PRP is then activated prior to application.[14,48]

The SymphonyTM II Platelet Concentrate System® (PCS) (DePuy ACE, Warsaw, Ind) shares numerous similarities with the SmartPReP®2 *APC*+ and the Gravitational Platelet SeparationTM II System®. This automated system has a high platelet concentration efficiency and facilitates the production of L-PRP with increased growth factor release.[48]

Further automated systems to produce L-PRP have been developed over the years, including the Magellan Autologous Platelet Separator® (Medtronic Biologic Therapeutics and Diagnostics, Minneapolis, Minn), which results in improved reproducibility, as operator error and cross-contamination are controlled. The patient's blood and the anticoagulant are placed in a collection chamber, which is in turn positioned in a closed system. Following centrifugation, L-PRP can be collected and applied to the surgical site.[48] The Secquire Cell Separator® (PPAI Medical, Fort Myers, Fla) demonstrated an improved platelet concentration efficiency and is facilitated by the use of specifically designed ports that allow the RBCs and PPP to be discarded after the first and second centrifugation, respectively.[48] Similarly, the AccelerateTM Platelet-Rich Plasma (PRP) Gel® (Exactech Inc., Gainesville, Fla) system utilizes a specifically designed tube for the mixture of blood with anticoagulant, which is in turn placed within a centrifuge capsule adapter located in a second centrifuge capsule adapter. The L-PRP product is delivered through an extraction port following the centrifugation process.[48]

4.1.3 | L-PRF (second generation)

A novel APC formulation termed platelet-rich fibrin was pioneered in 2001 by Choukroun et al.,[12] which utilizes platelet concentrates without anticoagulants and contains a high concentration of host immune cells (leukocytes) promoting local wound healing and contrasting infection. Neutrophils are the primary mediators to respond at the tissue injury site by performing phagocytosis of any pathogens and necrotic tissue with the aim to prevent infection. Secondary to neutrophils, monocytes are recruited, which differentiate into macrophages and perform numerous functions, including neutrophil phagocytosis, wound debridement, upregulation of cytokines and growth factors, and angiogenesis stimulation. Overall, leukocytes contribute to both soft and hard tissue repair as part of the tissue healing response.[56,57] Therefore, the philosophy behind L-PRF is based on the hypothesis that the incorporation of leukocytes might improve wound healing and tissue regeneration due to the previously mentioned leukocyte functions, in addition to the presence of a mechanically strong fibrin scaffold. Another important advantage in this second-generation APC

is that anticoagulants are not employed; hence, the healing cascade is not negatively impacted/disturbed, as platelet activation and fibrin clot formation are initiated immediately.[58] The patient's autologous venous blood is collected in dry silica-coated or glass tubes and is immediately centrifuged, resulting in the formation of three layers: (a) RBC base layer; (b) acellular plasma or platelet-poor plasma (PPP); and (c) L-PRF clot in the middle. The L-PRF clot constitutes a complex three-dimensional scaffold of a dense fibrin matrix and contains platelets, leukocytes, growth factors, and fibronectin.[12] Progressive polymerization results in the increasing incorporation of cytokines in the homogeneous fibrin network.[14] One reported advantage of L-PRF is that its high-density fibrin network favors the slow and gradual release of growth factors overtime, as compared to PRP.[34,59]

However, it must be noted that venous blood collection and subsequent transfer to centrifuge tubes is time dependent and must be completed as soon as possible (preferably within 1 min). Prolonging the process will result in diffuse fibrin polymerization, which will make the obtained L-PRF unsuitable for clinical use.[2]

Modifications to the PRF protocol as originally described by Choukroun[12,13,16] have led to the formulation of numerous second-generation autologous platelet concentrate systems, with different characteristics and suitable for various clinical uses.[36,60]

In particular, advanced PRF (A-PRF) was described after reducing the centrifugal force to 1500 rpm for 14 min and using glass-based vacuum tubes.[61] Decreasing the centrifugation speed has been suggested to limit cell loss and increase the number of leukocytes in the PRF product, as white cells are not transferred to the bottom of the tube.[61]

A further development of A-PRF named A-PRF+ was later proposed by Fujioka-Kobayashi et al.[62] by lowering the centrifugal speed to 1300 rpm and centrifugal time to 8 min. They reported that A-PRF+ has a significantly increased level of released growth factors compared to A-PRF and L-PRF, as a higher number of leukocytes is entrapped within the matrix. However, the clinical benefit in regard to cellular content and growth factor release deriving from A-PRF and A-PRF+ is still inconclusive, as the evidence is contradictory.[61–63]

An injectable flowable form of PRF (i-PRF) was also developed.[64] This injectable form of PRF can be produced by using blood without anticoagulant and by centrifugation at 700 rpm for 3 min in plastic tubes (hydrophobic tubes with no coatings to decelerate blood clotting). Blood constituents are separated within the first few minutes of the procedure, with deposition of a liquid layer (containing plasma, clotting factors and platelets) in the upper part of the tube allowing for easy aspiration and application of PRF in liquid form. The i-PRF remains in a liquid consistency for 15–30 min after the completion of centrifugation.[58]

Liquid fibrinogen is a further flowable L-PRF formulation, which is obtained via a single-step centrifugation process at 2700 rpm/408 g for 3 min using collection tubes with an inert inner surface.[65] The superior yellow layer (liquid fibrinogen) is aspirated without the red cells, which can subsequently be kept in the aspiration syringe for 20–30 min, as after this interval, slow spontaneous coagulation will occur. Liquid fibrinogen actively participates in the release of growth factors, namely TGF-β1, VEGF, BMP-1, and PDGF-AB, and can thus be considered a bioactive agent.[66] Liquid fibrinogen can be used in the preparation of L-PRF blocks, which have shown clinical benefits for the horizontal augmentation of resorbed alveolar ridges (Corteliini et al., 2018).

Titanium PRF (T-PRF) has been described by Tunali et al.[67] by centrifuging the blood sample at 2800 rpm for 12 min and by using medical grade titanium tubes. This protocol is based on the hypothesis that titanium tubes may be more effective at activating platelets than the glass tubes originally used by Choukroun.[12] The authors have demonstrated that fibrin in T-PRF is thicker and the fibrin network in T-PRF covers a larger area in comparison to L-PRF. It has thus been hypothesized by the group that T-PRF may last longer in tissues than L-PRF due to its structure.[68] However, the potential superiority of T-PRF in clinical applications requires further validation.[68]

Another modification of L-PRF is concentrated growth factor (CGF).[2] CGF is obtained through a specific protocol of centrifugation which requires a special programmed centrifuge (Medi-fuge MF200, Silfradent srl, Forli, Italy) and specific plastic tubes coated with silica microparticles for blood collection.[69,70] This protocol allows to separate blood in three different fractions: the upper white part, the lower red part, and the intermediate. CGF has a gelatinous consistency with platelets localized in BC and releases growth factors for almost 8 days.[69] In particular, it contains autologous osteoinductive growth factors derived from platelets and an osteoconductive fibrin matrix.[71]

Finally, a recent development in L-PRF technology is represented by C-PRF, which was introduced by Miron et al.[72] in 2020. C-PRF is isolated from the buffy coat layer during L-PRF preparation (0.3–0.5 mL layer above the RBC layer). C-PRF has a 10-fold increased platelet and leukocyte concentration compared to conventional L-PRF, but the clinical benefits have not yet been confirmed.[72,73]

Remarkably, in recent years horizontal centrifugation of PRF (H-PRF) was shown to result in better cell layer separation and to minimize cell accumulation on the distal surfaces of centrifugation tubes, which may prevent proper cell layer separation.[74] H-PRF demonstrated a significantly better antibacterial effect than L-PRF.[75]

It is not clear how the aforementioned modifications of L-PRF may impact on the clinical performance of the product. However, a recent in vitro study showed no significant difference in the ratio of activated whole platelets between L-PRF, A-PRF, CGF, and H-PRF.[76]

4.1.4 | P-PRF (second generation)

The Vivostat® P-PRF Preparation Kit (Vivolution A/S, Birkeroed, Denmark) is regarded as an advanced cell separator that allows the manufacturing of P-PRF. According to this protocol, whole blood is drawn from the patient, with the addition of citrate, which is then processed for approximately 25 min to produce a fibrin sealant matrix, without the need to use activators. The P-PRF can then be applied to the surgical site. However, it has been demonstrated that

platelets processed with this system are disrupted, the cytoplasmatic matrix leaks, and the content of intracellular vesicles is reduced.[77]

4.2 | Centrifugation

Centrifugation is the process that allows various blood components to be separated into different layers based on their densities. Particles with higher density, such as erythrocytes, will accumulate at the base of the tube, whereas those with the lowest density, such as white blood cells and platelets will precipitate in the superior layers. The formation of layers via centrifugation is dependent on numerous parameters, including duration, speed (revolutions per min), and g-force. Therefore, altering these parameters will largely affect the properties and composition of different preparations of APCs.[78] While it is beyond the remit of this review to discuss the basic principles of centrifugation, we have herein provided a summary of how this important protocol step can impact on the biological and mechanical properties of APCs.

4.2.1 | PRP (first generation)

Perez et al.[79] assessed the influence of centrifugal time on PRP by a two-stage centrifugation protocol on the concentration, integrity, and viability of platelets, among other empirical parameters. They showed that increasing the time of the centrifugation process from 6 to 10 min mildly enhanced platelet recovery and reduced the concentration of white blood cells in the superficial layer of the P-PRP sample after the first centrifugation. Additionally, platelet recovery efficiency after the first centrifugation increased when the centrifugal acceleration increased from 50 to 70 *g* and decreased in the 190–820 *g* range. After validation with 20 donors, the study concluded that a centrifugal acceleration to 100 *g* for a duration of 10 min after the first spin provided the optimum platelet recovery (80%) when applied to 3.5 mL of whole blood. Following the second centrifugation at a centrifugal force of 400 *g* for 10 min, a x5-fold increase in platelet concentration compared to baseline was noted (after removing two third of the plasma volume) and platelets remain inactivated.[79]

A similar platelet recovery was reported in another study[80] following the first centrifugation at 230–270 *g* for 10 min, but a reduced white blood cell recovery was noted (4.1%–5.8% compared to 5%–10%). A x7.4-fold increase in platelet concentration was reported following a second centrifugation at 2300 *g* for 10 min, discarding one tenth of PPP and adding EDTA. However, the latter also reduced platelet recovery efficiency to 35%.[80]

Conversely, an increased platelet efficiency (92%) was achieved in a subsequent study[81] when using an acceleration of 900 g for 5 min on 9 mL of whole blood for the first centrifugation, while 1500 *g* for 15 min for the second centrifugation yielded 84% platelet efficiency, a x4.2-fold increase compared to baseline. However, the group did not assess the integrity of platelets as part of their protocol.

PRGF contains a 2–3 times higher platelet concentration than in physiologic conditions, which has been identified as the optimum concentration for tissue regeneration stimulation.[82] PRGF products have demonstrated mitogenic, differentiating, angiogenic, anti-inflammatory, and antibacterial effects against *Staphylococcus epidermidis*, *Staphylococcus aureus*,[83] and *Escherichia coli*.[84] In the study by Anitua et al.[85] where PRGF was prepared following a single-stage centrifugation at 460 g for 8 min followed by activation with 10% calcium chloride, a x 2.67 (+/− 0.60)-fold increase in platelet concentration was reported, while no leukocytes could be detected in the final PRGF product.

The impact of centrifugation parameters has been explored also in relation to L-PRP. In an in vivo study, L-PRP produced with a two-stage centrifugation had the highest platelet concentration and demonstrated higher levels of phosphorus, selenium, and serotonin compared to single-spin L-PRP. Filtrated L-PRP did not have a significantly different platelet concentration to single-spin L-PRP, but it had increased levels of calcium and decreased counts of copper, magnesium, sodium, selenium, and potassium.[86] Additionally, filtrated L-PRP had a decreased concentration of nucleosides, methionine, pentoses, creatinine, creatine, isoleucine, tyrosine, arginine, lactic/malic/pyruvic, and succinic acid compared to double-spin and single-spin L-PRP. In this study, tibial bone defects treated with double-spin L-PRP showed enhanced bone regeneration, thus suggesting that a medium platelet concentration (obtained by double-spin L-PRP in this study) could enhance osteoblast proliferation, as compared to a high concentration medium, which impedes the process, and a low platelet concentration medium, which has no significant effect. These compositional differences in L-PRPs are also supported by the findings of further studies that showed statistical differences in the platelet counts between double-spin and single-spin PRPs, with the former demonstrating a higher platelet concentration.[87,88] Furthermore, Tamimi et al.[53] showed that using double centrifugation (ACE system) compared to single centrifugation (Nahita system) for the preparation of L-PRP, results in an increased platelet concentration (336% vs. 227%).

4.2.2 | L-PRF (second generation)

Decreasing the relative centrifugal force, duration and speed of centrifugation was shown to increase total platelet counts in L-PRF formulations. Mamajiwala et al.[89] reported this pattern for samples from three separate age groups (20–34 years, 35–49 years, and 50–65 years). Additionally, they assessed antimicrobial activity by measuring the widest diameter of the inhibition zone on agar plates. For all groups, the highest platelet concentration and optimum antimicrobial activity was noted after centrifugation at 1400 rpm for 8 min and the lowest when centrifugation was completed at 3500 rpm for 14 min. It is also important to note that all L-PRF membranes for the three age groups demonstrated statistically significant lower platelet counts compared to the whole blood. In regard to the fibrin network, the age group 20–34 years consistently showed a denser

fibrin network pattern, with the highest density following centrifugation at 3500 rpm for 14 min. This result was of statistical significance ($p < 0.05$) when compared to the group that underwent centrifugation at 1400 rpm for 8 min (lowest fibrin network density). The age group 50–65 years also demonstrated the same statistically significant fibrin network density result between the two centrifugation groups.[89]

Another study[61] confirmed that decreasing the centrifugation speed will increase platelet counts, but unlike Mamajiwala et al.,[89] it showed that increasing the centrifugation time also enhances the platelet concentration of L-PRF clot when prepared according to Choukroun's protocol.[12,13,16]

A more recent study additionally concluded that at lower g-forces, the L-PRF product has a more even distribution and concentration of platelets across the whole structure, when compared to higher centrifugation forces.[90]

The effect of centrifugation parameters on the distribution of neutrophilic granulocytes was also investigated by different studies.[61] In particular, single-stage centrifugation at 1500 rpm for 14 min resulted in a distribution of this cell type 68 ± 24% along the length of the A-PRF clot, in comparison to a distribution of 25 ± 12% following centrifugation at 2700 rpm for 12 min (L-PRF). This is an important finding, as recent studies have shown that neutrophilic granulocytes can contribute toward tissue regeneration and do not act only as early inflammatory cells.[61] It was also demonstrated that A-PRF has a looser fibrin structure with increased interfibrous space compared to the L-PRF, which is in agreement with the data collected by Mamajirwala et al.[61]

An increase in age has been associated with a less dense fibrin pattern,[91] but controlling the centrifugation parameters has been suggested to improve the fibrin network density. As L-PRF preparations are often used for their regenerative potential in the field of periodontology,[92] a denser fibrin network is preferred, and therefore the effect of age on the quality of the L-PRF should be considered.[89]

Histologic analysis of two L-PRF preparations showed that centrifugation at 2700 rpm, 719 *g* for 12 min produced a less porous fibrin scaffold, with fewer inter-fibrinous spaces than PRF prepared at 1500 rpm, 222 g for 14 min. The former structure was also associated with increased resistance to cellular penetration into the L-PRF-based matrix, which can be seen as a barrier membrane. However, having a fibrin scaffold with increased porosity facilitates cell penetration and thus enables host vascularization, as endothelial cells can migrate into this matrix and promote angiogenesis.[93] This is in contrast to the recommendation by Mamajiwala et al.[61]

Additionally, centrifugation speed may negatively impact cellular viability by affecting the release of signaling molecules and thus the interaction with host cells directing angiogenesis.[93] When a low or medium g-force is used for centrifugation in i-PRF production, the preparation is characterized by higher numbers of platelets and leukocytes, in addition to increased growth factor release (VEGF, ECF, PDGF-BB, and TGF-β), which correspond to an enhanced angiogenesis effect compared to when a high relative centrifugal force is applied.[26,94] Remarkably, lower centrifugation speeds (approximately 200 *g*) demonstrated to induce an increased growth factor release from L-PRF preparations when compared to higher centrifugation speeds in multiple studies.[34,62,90,95] This is partially in contrast with observations by Castro et al.,[36] whose in vitro results indicated lack of differences in terms of growth factors release, cellular content, and membrane dimensions when comparing L-PRF, A-PRF, and A-PRF + obtained with two centrifuges with adapted relative centrifugal forces (RCF). The authors suggested that adapting RCF for different centrifuges is crucial to adequately compare different protocols and this is why conflicting results may be found in the literature. However, they also indicated that a lower g-force reduced membrane tensile strength.

It should also be noted that the choice of collection tube might significantly impact on L-PRF formulations, as silica-coated plastic tubes have been associated with more diffusive platelet distribution at higher centrifugation speeds when compared to glass tubes.[96] At low centrifugation speeds, homogenous distribution of platelets was reported in tubes consisting of both materials. However, the same group previously demonstrated that silica particles can shed from the inner surface of the tube during centrifugation, leading to their incorporation in the L-PRF formulation.[97] Since silica nanoparticles have been criticized for their potential in vitro cytotoxicity,[98,99] certain groups advocate for their use only in vitro,[96] to avoid introducing chemical additives to the preparation.[100] Nevertheless, if particles detach, it is likely that they will precipitate during centrifugation and gather at the bottom of the tube. A recent study reported that the selection of centrifugation tube can have a very pronounced effect on the size of the L-PRF clot, even more than the centrifugation device itself.[90]

Conclusions: In summary, there is no consensus on which are the optimal centrifugation parameters in order to obtain the most favorable platelet concentrate in terms of platelet recovery efficiency and optimum growth factor release. However, there is evidence indicating that centrifugation can impact on platelet and growth factor release, cellular viability, and mechanical properties of the APC products. Therefore, it is important that detailed parameters on the centrifugation protocols are reported by researchers.

4.3 | Leukocyte content

4.3.1 | Antimicrobial effect and pain perception

There is controversy in the literature with regard to the incorporation of leukocytes in APC preparations. Some groups have advocated that their presence can negatively impact the therapeutic outcome owing to the stimulation of the inflammatory process via the proteases and acid hydrolases they contain.[101] This is supported by a recent study that reported the superior effects of P-PRP on bone regeneration compared to L-PRP and which the authors attributed to the activation of the NF-κB pathway via pro-inflammatory cytokines stimulated by the leukocytes in the L-PRP formulation.[102] The group also hypothesized that the activation of the NF-κB pathway may also be associated with the increased manifestation of pain associated with L-PRP application.

On the other end, contrasting evidence does support the importance of leukocyte content in APC preparations, including in the injectable formulations, due to the associated stimulatory effect on growth factor release,[43] anti-pain mediators, release[103] and antimicrobial activity,[104] which have been demonstrated by several studies. In particular, in an in vitro study, Moojen et al.[105] reported the antibacterial effects of L-PRP against *Staphylococcus aureus*, that was highest in the initial hours post-application, rendering it suitable for use for antimicrobial prophylaxis. Additionally, Bielecki et al. reported a strong antimicrobial capacity of L-PRP against MSSA (*methicillin-susceptible Staphylococcus aureus*) and MRSA (*methicillin-resistant Staphylococcus aureus*), but no correlation was demonstrated between L-PRP and the inhibition of *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.[103] Therefore, the group concluded that further investigation on the antibacterial mechanisms of L-PRP is required due to the complex and diverse role of platelets and leukocytes.[84] A more recent study reported similar findings in respect to the antibacterial effects of L-PRP against MSSA and MRSA, together with antimicrobial activity against *Pseudomonas aeruginosa* and *Enterococcus faecalis*. No activity was reported against *Escherichia coli* and *Klebsiella pneumonia*.[106] Remarkably, as the leukocyte content may fluctuate in different L-PRP preparations, their properties and effectiveness in clinical applications can vary; this is an additional aspect of L-PRP formulations that requires further investigation.[103]

A recent study has demonstrated the antibacterial capacity of L-PRF against key periodontal pathogens, namely *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *and Aggregatibacter Actinomycetemcomitans*.[107] This is in contrast with another in vitro study that indicated that PRP was able to inhibit *Porphyromonas gingivalis* and *Aggregatibacter Actinomycetemcomitans*, but not L-PRF.[108] Remarkably, Feng et al.[75] showed that both H-PRF and L-PRF had antimicrobial activity against *S. aureus* and *E. coli*, but H-PRF demonstrated a significantly better antibacterial effect (inhibition rate), which the authors attributed to the higher content of immune cells in H-PRF.

Kour et al.[109] compared the antimicrobial potential of PRP, L-PRF, and i-PRF and concluded that even though all three APC formulations showed some antibacterial activity against *Porphyromonas gingivalis and Aggregatibacter Actinomycetemcomitans*, i-PRF followed by PRP showed increased activity against *Porphyromonas gingivalis* compared to L-PRF. These findings are similar to the results from Karde et al.[110] reporting that i-PRF has a significant inhibitory effect on oral pathogens compared to PRP and L-PRF.

It should also be noted that leukocytes have antinociceptive properties and can lead to a decrease in the clinical perception of pain via chemokines, anti-inflammatory cytokines, and opioid peptides during the initial stages of the inflammatory process.[111]

4.3.2 | Fibrin and leukocyte interrelationship

In comparison to L-PRP, L-PRF consists of a denser fibrin network and manifests enhanced fibrin polymerization, which is strongly correlated with the cells and growth factors encased in its fibrin polymer molecular architecture, with leukocytes being of particular importance.[112] It must be noted that in L-PRF, the interrelationship between fibrin and leukocytes is significant; this is attributed to the fact that fibrin itself has an effect on the healing cascade,[113] by being an important determinant for neo-angiogenesis and thus allowing the flow of leukocytes via the formation of new vessels.[114]

4.3.3 | Effect on growth factor release

As previously mentioned, leukocytes have been proposed as an important source of growth factor release, especially VEGF, which is an angiogenesis-associated growth factor,[115] and thus the role of leukocytes in the process of angiogenesis is presumed to be significant.[14] The slow release of growth factors and matrix proteins, namely VEGF, TGFβ-1, PDGF-AB, and TSP-1 from L-PRF over 7 days has been previously documented.[14] The study concluded that leukocytes embedded in the L-PRF formulation act as an active source of growth factors (VEGF, TGFβ-1); upon decrease of the levels of these growth factors in the formulation, leukocytes are capable of stimulating the production of further VEGF and TGFβ-1 to maintain the desired levels. However, the details of this negative feedback mechanism require further investigation.[14] Other groups have also reported the stimulatory effect of leukocytes on the secretion of growth factors associated with the healing process, namely VEGF, TGFβ-1, PDGF, IGF, EGF, TGF, BMP-1, and bFGF.[36,66,116] Zimmermann et al.[117] compared L-PRP to P-PRP in regard to growth factor release and reported increased availability of PDGF-AB, PDGF-BB, and TGFβ-1 in the presence of leukocytes in L-PRP, in support of previous findings.

Conclusions: In summary, the general consensus among several research groups is that multiple leukocytes, especially lymphocytes, can have a significant regulatory role in the healing and inflammatory processes and, therefore, they should not be eliminated from the preparations, unless new evidence suggestive of negative effects emerges.[111] While the strong antimicrobial properties of leukocyte-containing APCs have been demonstrated in multiple studies, yet their impact on growth factor release, as part of the healing cascade, involvement in pain perception and association with the stimulation of the inflammatory process require further investigation to ascertain the net therapeutic benefit of leukocyte incorporation in APC preparations.

4.4 | Use of activator for PRP/PRGF (first generation)

4.4.1 | Types of activators and effect on PRP/PRGF properties

Endogenous or exogenous activation of platelets is required in PRP preparations prior or at the time of application, whereas the addition of activators is not necessary to obtain L-PRF formulations. It

involves the release of growth factors via the degranulation of platelets and the cleavage of fibrinogen to initiate the clotting cascade.[118] The most documented activators are calcium chloride, thrombin, a combination of calcium chloride and thrombin, and collagen type I.[9]

The activation strategy of PRP/PRGF formulations should be clearly reported, as its effect on platelet aggregation will dictate PRP/PRGF physical form and growth factor kinetics, thus influencing its clinical application. For instance, thrombin leads to the rapid aggregation of platelets, which is associated with a significant retraction of the PRP gel[119] and a potentially decreased growth factor availability at the operation site over time, since it leads to an immediate rather than a sustained release.[120] Collagen type I has been reported to have a similar efficiency to thrombin in regard to activating platelets and stimulating growth factor release.[44] On the contrary, calcium chloride addition to PRP results in the formulation of a more porous fibrin matrix, with a slower and more gradual release of growth factors over time, whereas the addition of calcium chloride/thrombin leads to the formulation of an insoluble gel capable of sustained growth factor release.[44]

Cavallo et al.[9] investigated the effect of PRP activated with different methods on three growth factors and two inflammatory mediators, alongside their release kinetics. When PRP was activated with thrombin, thrombin and calcium chloride and collagen type I, there was rapid release of PDGF and TGFβ-1 that remained stable over 24 h. It should be noted that collagen was deemed a weaker platelet activator in this study, as there were lower levels of bioactive molecules released upon application of this particular activation methodology. Contrary to other growth factors, VEGF showed a progressively increasing release trend from 15 min to the 24 h interval. A gradual growth factor release trend up to 24 h was noted for calcium chloride-activated PRP.[9] The sustained growth factor release associated with the application of calcium chloride as an activator was key in the development of PRGF, in an effort to produce a superior PRP/PRGF.[85] A previous study supported the findings by Cavallo et al., as bovine thrombin-activated PRP showed higher release of PDGF-AB in the initial 24 h, compared to collagen-activated PRP.[119] However, no significant difference was reported between the two groups over the 10-day period. The maintained release of VEGF over a 10-day time frame was also reported, with no significant difference between the two groups in respect to the cumulative growth factor release. However, activation with thrombin showed higher TGFβ-1 being eluted, which was most notable during the first 5 days.[119] A further study highlighted the potentially superior growth factor release pattern when collagen type I was applied due to the sustained release of growth factors over 7 days, rather than their immediate release, as observed with thrombin activation.[121]

Regarding clot formation, evidence showeed that the PRP clot can be formulated within 30 min following calcium chloride application and within 15 min following thrombin and calcium chloride/thrombin activation.[9] Conversely, no clot formation occurred during the 24 h observation period for the collagen type I-activated PRP,[9] which is in contrast to the findings by Fufa et al., who reported clot formation within 24 h, albeit significantly less retraction was observed as compared to when PRP was activated with bovine thrombin.[119]

4.4.2 | Adverse events

Adverse effects have been documented in association with the aforementioned activation protocols. Calcium chloride has been occasionally reported to cause discomfort and a burning sensation, consequently to a low pH.[44] Additionally, the application of bovine thrombin has the potential to cause undesirable immune responses and rare life-threatening coagulopathies,[122,123] to interfere with the osteoconductive potential of materials used in orthopedic surgery[124] and to negatively impact the proliferation and viability of cells in vitro.[125] The incidence of adverse events associated with the use of thrombin derived from bovine plasma has led to the development and application of human-derived and recombinant thrombin formulations.[106] However, it should be noted that bovine thrombin is still widely used and the addition of a major volume of thrombin as an activator enhances the antimicrobial properties of L-PRP, with bovine thrombin showing enhanced inhibition for MRSA, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* compared to autologous thrombin.[106]

Collagen type I is a safer activator alternative to thrombin due to its endogenous nature, biocompatibility, and biological inclusion in the intrinsic coagulation cascade.[126]

Conclusions: In summary, there is no consensus in regard to the optimal method of activation of PRP. Currently, the choice of activator is most frequently made on the basis of ease of use and practicality for the operator.[9] However, risk of adverse events is an important aspect that clinicians should take into account when selecting the activator and which needs to be explained to the patients.

4.5 | Anticoagulants for PRP/PRGF

Anticoagulants are added to PRP/PRGF formulations as part of their preparation protocols with the aim to prevent coagulation prior to centrifugation via the restriction of the clotting cascade.[58]

The most commonly used anticoagulants are EDTA (ethylenediaminetetraacetic acid), citrate-based anticoagulants (calcium citrate, sodium citrate, and acid citrate dextrose), and heparin.

However, it must be noted that additives, such as anticoagulants, have been shown to affect the composition and efficacy of PRP due to pH alteration,[44] which can in turn negatively influence in vitro proliferation.[127]

Whole blood collection with EDTA was shown to result in higher platelet counts, followed by sodium citrate (SC) and anticoagulant citrate dextrose (ACD).[128–130] However, after the first centrifugation, samples processed with SC yielded a higher platelet recovery of 81.21%, as compared to 76.15% for EDTA.[128] Several studies have demonstrated changes in morphology and activation of platelets by

EDTA.[80,130,131] This finding is also supported by a study by do Amaral et al.,[128] as an increased mean platelet volume was reported for the PRP group activated with EDTA after centrifugation, but not for the SC and ACD groups. It is also important to note that the use of EDTA has been criticized for its cytotoxicity[130] and it is regarded as an environmental pollutant.[132] Nevertheless, certain groups advocate its suitability for use as an anticoagulant if doses are maintained at subtoxic levels.[130] ACD and SC use as an anticoagulant has yielded higher stimulatory effects on mesenchymal stromal cell (MSC) proliferation compared to EDTA.[128] Increased MSC proliferation in the presence of citrate (CTAD and ACD) anticoagulants, alongside enhanced TGFβ-1 release, has also been confirmed by another study.[133]

Heparin can also be used as an anticoagulant in PRP preparations, but inferior properties compared to other methodologies for anticoagulation have been reported in the literature, including reduced platelet counts.[130]

In conclusion, there is still controversy in the literature regarding the optimal anticoagulant to use in PRP acquisition and preparation, as research groups advocate the implementation of different methodologies for anticoagulation.

To eliminate this problem, Choukroun et al.[12] introduced the second generation of APCs, which eliminated the use of anticoagulants and activators and thus encouraged natural clot formation, as described in previous sections. In L-PRF preparations, platelet activation and the initiation of the clotting cascade begin upon contact of the platelets with the inner surface of the collection tube, secondary to the activation of autologous thrombin, ultimately leading to the formation of a fibrin clot in the center of the tube.[2] Therefore, immediate application after blood draw is advised for L-PRF, as prolonging this process will result in deterioration of its structural integrity.[134]

5 | CLINICAL HANDLING AND FORMULATIONS

For APC preparations, it is generally recommended that whole blood is collected just prior to the onset of the surgical procedure. This is in consideration of the fact that platelets will accumulate at the surgical site to initiate clotting and healing, thus somehow reducing the whole blood platelet count. In addition, during surgery intravenous fluid will dilute whole blood, further reducing platelet numbers..[135]

For both PRP/PRGF and L-PRF, four different therapeutic biological preparations, namely supernatant, liquid, clot, and membrane, can be obtained depending on the degree of coagulation and activation.[13,61,101,136]

5.1 | PRGF (first generation)

The different formulations are suitable for different applications, with the PRGF supernatant being utilized as a cell culture medium and eye solution,[101,137] the liquid for surgical applications and to activate implant surfaces,[138] the gel/clot for tissue engineering and ulcer management,[139] and the membrane for alveolar ridge preservation, periodontal regeneration, and soft tissue repair.[101] Additionally, the agglutination of PRGF derivatives with biomaterials make this APC formulation suitable for numerous therapeutic applications, while a more sustainable growth factor release pattern is further enhanced by the presence of a biomaterial.[101] Following centrifugation according to the manufacturer instructions for PRGF preparation, whole blood can be separated in different fractions useful for targeted applications, as previously described. F2, containing the greatest distribution of platelets and growth factors, can be combined with a graft to produce "sticky bone," whereas F1 can be utilized as a fibrin membrane and is often applied over the clot to mimic a barrier;[140] however, an incubation at 37°C for 4 min is required. Within the Endoret® kit, a specific membrane shaper can be used for F1, which helps compressing the plasma clot and which also presents a weight that can be placed on the lid to obtain a more homogeneous membrane.

Khorshidi et al.[141] compared the mechanical properties of L-PRF and PRGF membranes obtained from 10 healthy volunteers and they showed that L-PRF membranes present increased tensile strength and toughness, as assessed through universal testing machine. It was also concluded that L-PRF membranes have easier handling properties.

5.2 | L-PRF (second generation)

The centrifugation process should begin no longer than 1 min following the onset of whole blood collection to avoid jeopardizing the morphology and size of the final formulation, due to diffuse fibrin polymerization.[60,142] This is further supported by a study involving equine blood which indicated that when whole blood collection is heterogeneous and prolonged, and the centrifugation temperature fluctuates outside the range of 21°C–30°C, then the L-PRF formulation will neither be homogeneous nor support the sustained growth factor release pattern or fibrin architecture previously discussed.[143] As described in the previous sections, the L-PRF clot is formed in the center of the collection tube after completion of the centrifugation process. The RBC layer is often closely intertwined with the fibrin clot containing the L-PRF exudate, which is populated with growth factors, thus careful separation with specialized instruments resembling a spatula is usually undertaken.[73] Upon gentle compression of the L-PRF clot for 5 min, a strong L-PRF membrane is formed, which has a high concentration of growth factors, leukocytes, and platelets. L-PRF membranes have favorable biological properties, namely strength, sustained release of high quantities of growth factors over 7–14 days, and provide a fibrin matrix architecture that can be populated by a plethora of cells, which is useful in the process of tissue regeneration. Additionally, L-PRF has demonstrated greater resistance to dissolution post-application compared to PRP, due to the slow remodeling of the fibrin matrix with the gradual

incorporation and preservation of platelets and leukocytes in the fibrin architecture.[13,144] This process can take place via compression of the L-PRF clot in two gauzes[14] or in a customized compression box/system that allows the formation of multiple L-PRF membranes simultaneously as well as various formulations, including L-PRF plugs.[73]

The stability of L-PRF formulation relies on its storage in a sealed compartment at room temperature to prevent dehydration. If stored under these conditions, L-PRF can remain unchanged for 2.5–3 h.[73] This is in contrast to other evidence that has shown that L-PRF formulations should be applied to the surgical site immediately after completion of the formulation protocol, with a nonabsorbable impermeable material and cuvette being used in sterile conditions to compress the L-PRF clot and thus enhance growth factor release.[2,145] This is also in line with studies that reported deterioration of the L-PRF clot after 40–60 min post-preparation[95] and the descent of the clot in the tube post-centrifugation, where it starts to merge with the erythrocyte layer.[112]

As for all other platelet concentrates, L-PRF and i-PRF can be combined with a bone graft to produce an L-PRF bone block. A bone graft obtained in this way is gelatinous in structure and can be easily adapted in bone and periodontal defects.

6 | CONCLUSIONS AND FUTURE PERSPECTIVES

It is well established that platelets play an important role in the wound healing process, as they participate in hemostasis, inflammation, angiogenesis, and trigger the cascade of events that lead to tissue healing. As a matter of fact, during the healing process, platelets are able to release a number of growth factors and mediators that promote cellular cross-talks and act coordinately with leukocytes to stimulate the recruitment and differentiation of mesenchymal stem cells and other target cells involved in the healing process.[26]

As such, the rationale behind the use of APCs in tissue regeneration is predominantly based on reaching a supraphysiological dose of growth factors and mediators to enhance the natural wound healing process.[25,134]

Despite APCs have been extensively documented for tissue regeneration/healing purposes for several decades, the inconsistency in reporting protocol parameters has contributed to conflicting conclusions regarding the efficacy of the different APC formulations and has significantly limited the ability to interpret the results of individual clinical studies.

In the future, the use of an internationally approved and standardized classification system should be pursued to make study outcomes comparable and to promote the use of a standardized vocabulary among researchers and clinicians.

Moreover, it is recommended for all studies employing APCs that the platelet content both in terms of concentration and quantity delivered to the target site is clearly specified, together with the efficiency of production based on the recovery rate in platelets. Besides providing details on platelet content, researchers should clearly indicate the presence of leukocytes (including percentage of neutrophils) and of red blood cells. Other important details to report include the centrifugation protocol, which should specify duration, speed (revolutions per min) and g-force employed, and choice of blood collection tube. For first-generation APCs, it is then crucial to provide clear details in relation to the activation process as well as on any anticoagulants employed.

The relevance in reporting all the aforementioned parameters relies on the impact that each of these factors might have on platelet and growth factor release, cellular viability and on the mechanical properties of the resulting APC products.

While we have started to learn more about the importance of these parameters in the past years, there is still no consensus on which protocol would allow to obtain the most favorable platelet concentrate. The efforts of research groups to use a standardized nomenclature and to meticulously report protocol details will be crucial to progress in the optimization of APC products.

Overall, the use of first-generation APCs has been declining over the years due to their demanding preparation protocols in terms of time efficiency and ease of application, cost-effectiveness of alternative preparations, and controversial outcomes in their clinical application.[146] Nevertheless, a specific and well-codified modification of P-PRP, which is PRGF, has attracted interest by a number of research groups in several medical fields.[147] Remarkably, PRGF is available both with anticoagulant and activator (KMU15) and in a formulation that does not require neither anticoagulant nor activator (KMU16). The latter eliminates the risk of adverse events for the patient and contributes toward a controlled transfer of biological mediators.[49] Moreover, avoiding the use of anticoagulants/activators has the important advantage of eliminating the possible classification of the platelet concentrate as a medicinal product, with all the associated regulatory restrictions.

Conversely, in the past years, there has been a progressive development and widespread use of second-generation APCs, which are characterized by simpler preparation methodologies and do not require the addition of anticoagulants and activators, which may be considered a blood manipulation.[14] However, since anticoagulants are not employed, it is important to note that blood undergoes rapid clotting within the blood collection tube; therefore, it becomes critical for clinicians to begin centrifugation shortly following blood collection to separate blood layers.

Remarkably, L-PRF offers the possibility to create robust autologous membranes that can possibly be used as barriers,[148] although in case of large/challenging GBR procedures, its combination with a barrier membrane is still advised.[149–151]

In the past decade, several modifications in the preparation protocol of L-PRF have been introduced (particularly changes in centrifugation time, speed and g force), However, it is important to emphasize that there is currently no robust scientific evidence that these protocol adaptations can lead to significantly different clinical outcomes.

The role and importance of leukocytes within APCs remains a question of debate among different research groups. In particular, the lack of leukocytes in P-PRP and P-PRF products is based on the idea that their presence can negatively impact the therapeutic outcome owing to the stimulation of the inflammatory process via the proteases and acid hydrolases they contain.[101,102] On the contrary, the rationale of the high leukocyte content in L-PRF and L-PRP formulations is based mainly on their documented antimicrobial activity[84,106] and potentially reduced pain perception.

Future studies are needed to clarify whether leukocyte presence has a tangible and clinically relevant impact on growth factor release, pain perception, and inflammatory process, as heterogeneous data are currently available in the literature.

Finally, it is also worth mentioning that overall APCs may be classified differently in different countries. For instance, in the United States, the FDA guidance and 21CFR Part 1271 do not apply to the use of PRP/L-PRF, as they are considered a blood component, not a human cellular or tissue product. On the contrary, in Europe, a product like PRP is considered as a medicinal product. The different classification and the different country-specific regulations can affect the regulatory pathway and requirements for approval in using APC products in dentistry, which may be more or less straightforward. This, in turn, may directly influence clinicians in the selection of a specific platelet concentrate compared to another, regardless of their properties, but simply because of the set of regulations in place that facilitate the use of one product over another.

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