Review

Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF)

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The topical use of platelet concentrates is recent and its efficiency remains controversial. Several techniques for platelet concentrates are available; however, their applications have been confusing because each method leads to a different product with different biology and potential uses. Here, we present classification of the different platelet concentrates into four categories, depending on their leucocyte and fibrin content: pure platelet-rich plasma (P-PRP), such as cell separator PRP, Vivostat PRF or Anitua's PRGF; leucocyte- and platelet-rich plasma (L-PRP), such as Curasan, Regen, Plateltex, SmartPReP, PCCS, Magellan or GPS PRP; pure plaletet-rich fibrin (P-PRF), such as Fibrinet; and leucocyte- and platelet-rich fibrin (L-PRF), such as Choukroun's PRF. This classification should help to elucidate successes and failures that have occurred so far, as well as providing an objective approach for the further development of these techniques.

History and techniques

In transfusion medicine, platelet concentrates were originally used for the treatment and prevention of haemorrhage due to severe thrombopenia, which is often caused by medullar aplasia, acute leukaemia or significant blood loss during long-lasting surgery. The standard platelet concentrate for transfusion has been named platelet-rich plasma (PRP) and classically contains 0.5×10^{11} platelets per unit.

The use of blood-derived products to seal wounds and stimulate healing started with the use of fibrin glues, which were first described 40 years ago and are constituted of concentrated fibrinogen (polymerization induced by thrombin and calcium) [1]. Nowadays, fibrin glues prepared from human plasma, such as Tisseel (Baxter, USA), are widely used. Autologous fibrin glues are considered the best choice to avoid contamination risk, but their use remains very limited owing to the complexity and the cost of their production protocols [2].

Consequently, the use of platelet concentrates to improve healing and to replace fibrin glues, as first described by Whitman *et al.* [3], has been explored considerably during the last decade. Platelets contain high quantities of key growth factors, such as PDGF-AB (platelet-derived growth factor AB), TGFβ-1 (transforming growth factor β-1) and VEGF (vascular endothelial growth factor), which are able to stimulate cell proliferation, matrix remodelling and angiogenesis. The use of these growth factors to enhance healing is an interesting option, but commercial interests might obscure a lack of true clinical benefits in some cases. Indeed, these concepts have spurred their commercial exploitation with the development of a wide range of preparation protocols, kits and centrifuges. Most of these products were called PRP, the same name as the original transfusion platelet concentrates, which does not allow distinction between the different systems and protocols.

All available PRP techniques have some points in common: blood is collected with anticoagulant just before or during surgery and is immediately processed by centrifugation. The time for platelet concentrate preparation is variable but is always completed within an hour. A first centrifugation step is designed to separate the blood into three layers, red blood cells (RBCs) are found at the bottom, acellular plasma (PPP, platelet-poor plasma) is in the supernatant and a 'buffy coat' layer appears in between, in which platelets are concentrated (Figure 1). The next steps vary among the numerous protocols but are an attempt to discard both the RBC layer and the PPP to collect only the 'buffy coat' layer. Finally, the obtained platelet concentrate is applied to the surgical site with a syringe, together with thrombin and/or calcium chloride (or similar factors) to trigger platelet activation and fibrin polymerization.

Choukroun's PRF (platelet-rich fibrin) is the latest development of these protocols. Here, blood is collected without any anticoagulant and immediately centrifuged. A natural coagulation process then occurs and allows for the easy collection of a leucocyte- and platelet-rich fibrin (L-PRF) clot, without the need for any biochemical modification of the blood, that is, no anticoagulants, thrombin or calcium chloride are required. This open-access technique is the most simple and also the least expensive protocol developed so far. However, some confusion is likely because different suppliers use similar nomenclature for their distinct products (such as Vivostat PRF and Fibrinet Platelet-Rich Fibrin Matrix [PRFM]).

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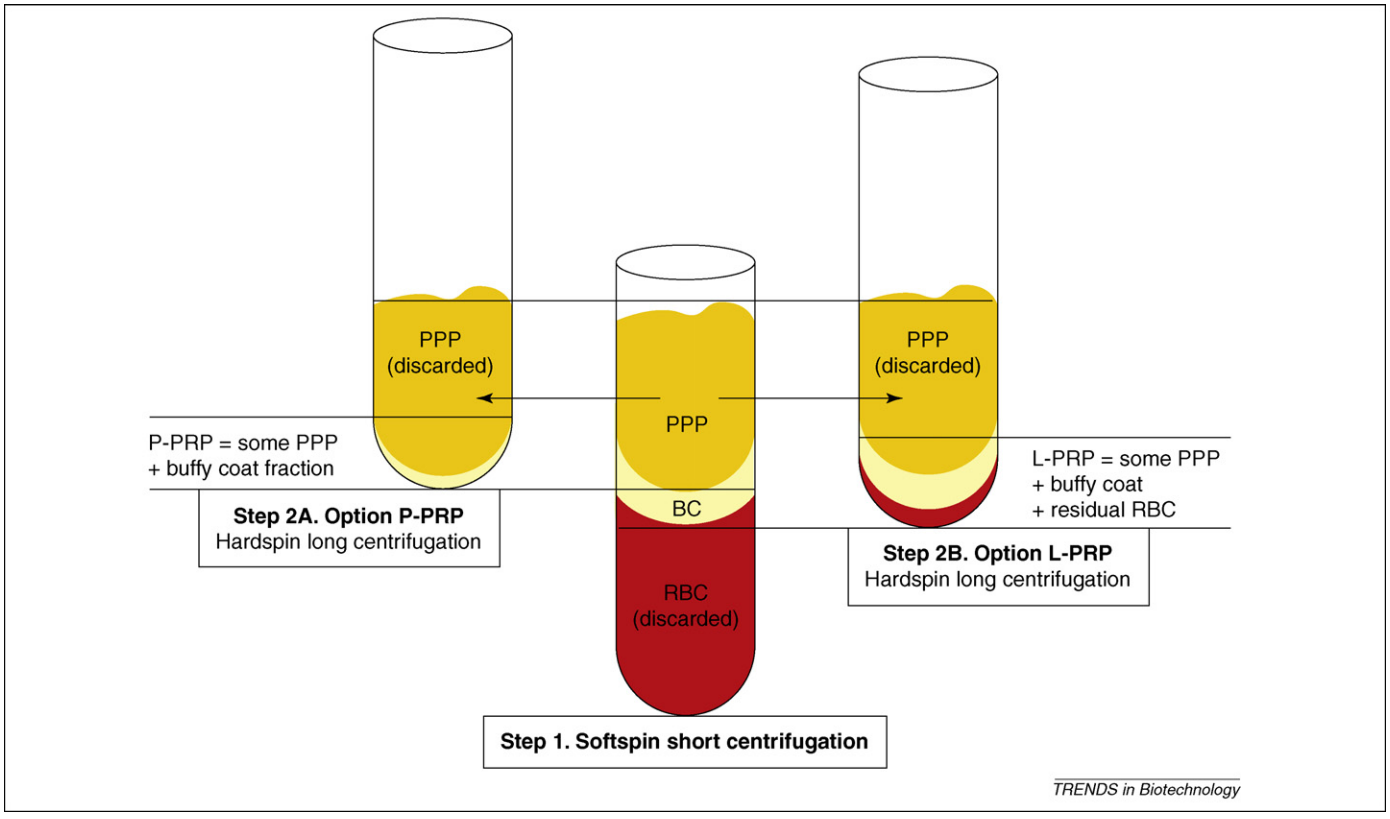

Figure 1. Classical manual platelet-rich plasma (PRP) protocol using a two-step centrifugation procedure [8,16]. Step 1: Whole blood is collected with anticoagulants and briefly centrifuged with low forces (softspin). Three layers are obtained: red blood cells (RBCs), 'buffy coat' (BC) layer and platelet-poor plasma (PPP). BC is typically of whitish colour and contains the major proportion of the platelets and leucocytes. Step 2A: For production of pure PRP (P-PRP), PPP and superficial BC are transferred to another tube. After hardspin centrifugation (at high centrifugal force), most of the PPP layer is discarded. The final P-PRP concentrate consists of an undetermined fraction of BC (containing a large number of platelets) suspended in some fibrin-rich plasma. Most leucocytes are not collected. Step 2B: For production of leucocyte-rich PRP (L-PRP), PPP, the entire BC layer and some residual RBCs are transferred to another tube. After hardspin centrifugation, the PPP is discarded. The final L-PRP consists of the entire BC, which contains most of the platelets and leucocytes, and residual RBCs suspended in some fibrin-rich plasma. Therefore, the final product greatly depends on the means of BC collection. The transfer step is often performed with a syringe or pipette, with only eyeballing as measuring tool. Because the manual PRP process is not clearly defined, this protocol might randomly lead to P-PRP or L-PRP.

Definition of relevant parameters and classification

Three main sets of parameters are necessary for a clear classification of platelet concentrates (Table 1). The first set of parameters (A) relates to the preparation kits and centrifuges used. The size of the centrifuge (parameter A1), the duration of the procedure (parameter A2) and the cost of the device and kits (parameter A3) are significant factors when considering the repetitive use of these techniques in daily surgical practice. The ergonomy of the kit and the complexity of the procedure (parameter A4) are also key parameters because complex procedures are in danger of being unusable or potentially misused, leading to irreproducible results. For these reasons, automatized systems have been developed and are commercially available. These parameters (A) define the practical characteristics of each technique.

The second type of parameters (B) relates to the content of the concentrate. The final volume of usable concentrate (parameter B1) depends on the initial blood harvest and can define the potential clinical applications of a preparation protocol. The efficiency in collecting platelets (parameter B2) and leucocytes (parameter B3) and their preservation during the entire process (parameter B4) define the basic pharmacological relevance of the product and indicate its potential applications.

The third set of parameters (C) relates to the fibrin network that supports the platelet and leucocyte concentrate during its application. The density of the fibrin network is mainly determined by the concentration of the fibrinogen (parameter C1) during preparation [4]. Most protocols lead to a low-density fibrin gel, which allows for convenient surgical application but lacks a true fibrin support matrix. By contrast, a high-density fibrin network means that the platelet concentrate can be considered as a biomaterial, and the fibrin matrix itself might have potential healing effects [5]. The fibrin polymerization process (parameter C2) needs to be evaluated, taking into account the ratios between fibrinogen and thrombin concentrations, as well as the biomechanical properties of the final fibrin network. Fibrinogen is activated by thrombin, which initiates polymerization into fibrin. However, the fibrin fibrillae can be assembled in two different biochemical architectures: either via condensed tetramolecular or bilateral junctions or via connected trimolecular or equilateral junctions [4]. Bilateral junctions are provoked by a drastic activation and polymerization, for example with high thrombin concentrations, that leads to a dense network of monofibres similar to a fibrin glue, which is not particularly favourable to cytokine enmeshment and cellular

Table 1. Definition of the key parameters to be evaluated in each platelet concentrate protocol

Key parameters	Subparameters	Definition
A: Preparation kits and centrifuge (for processing of 50 mL of whole blood)	A1: Size and weight of the centrifuge type required for the method	• Heavy (and cumbersome) • Light (and compact) • Heavy but potentially light (i.e. a commercialized system is heavy, but technique could be performed with a smaller centrifuge)
	A2: Duration of procedure (from blood harvest to surgical application)	• Quick (less than 20 min) • Long (between 20 and 60 min) • Very long (more than 1 h)
	A3: Cost (initial cost of equipment and repeated costs for reagents and kits)	• Very inexpensive, less than 5 euros • Inexpensive, between 5 and 50 euros • Expensive, more than 50 euros
	A4: Ergonomy of the kit (including required manipulations) and complexity of procedure	• Very simple (+ +) • Simple (+) • Complex (−) • Very complex (− −)
B: Platelets and leucocytes	B1: Final volume of platelet gel material (relative to initial blood harvest)	• Large, more than 25% of the blood sample • Small, less than 25%, • Variable, if additional fibrin-rich PPP can be preserved to increase volume above 25%
	B2: Platelet collection efficiency B3: Leucocyte collection efficiency	• Excellent, more than 80% • Good, between 40 and 80% • Low, less than 40% • Sometimes unknown • No leucocytes, when technique eliminates most leucocytes
	B4: Preservation of the platelets and leucocytes	• Healthy • Damaged • Unknown • Activated, when coagulation is induced during the centrifugation process
C: Fibrin	C1: Fibrinogen concentration and fibrin density	• High density • Low density
	C2: Fibrin polymerization type	• Strong, mainly trimolecular or equilateral junctions • Weak, mainly tetramolecular or bilateral junctions

migration. On the contrary, a slow physiological fibrin polymerization yields a higher percentage of equilateral junctions, which allow the establishment of a flexible fibrin network with multifibre assembly that is capable of supporting cytokine enmeshment and cellular migration [6]. Moreover, this organization will also provide elasticity to the fibrin matrix comparable to that of a solid biomaterial. Fibrinogen collection efficiency and polymerization type define the material characteristics of the concentrate.

Using these sets of parameters, actual available methods can then be classified in four main categories, depending on the pharmacological (B parameters) and material (C parameters) characteristics of the obtained product (Table 2): pure PRP (P-PRP), leucocyte-rich PRP (L-PRP), pure PRF (P-PRF) and, finally, leucocyte-rich PRF (L-PRF). In each category, the concentrate can be produced by different processes (A parameters), either in a fully automatized setup or by manual protocols.

Leucocyte-poor or pure platelet-rich plasma (P-PRP)

Pure platelet concentrates for topical use were first developed as an additional application of the classical transfusion platelet units and were first reported for maxillofacial surgery [3,7].

Automated protocols for P-PRP: plasmapheresis with a laboratory cell separator and Vivostat PRF

The first method of producing platelet concentrates for topical use was the so-called plasmapheresis, which uses a cell separator, either in a discontinuous flow set up (in which the patient stays connected to the machine and the blood filtering continues until the desired quantity of platelets has been collected) or starting from a bag of harvested blood with anticoagulant [8]. These cell-separator devices employ a differential ultracentrifugation (3000 g in general). The different blood components, such as platelets, leucocytes and RBCs, are first separated from the PPP, which can then be re-infused to the patient. When the integrated optical reader detects the first buffy elements in the serum, these are automatically collected into a separate bag as the platelet concentrate (PRP). As soon as the optical reader detects elements of RBCs, platelet collection is interrupted and RBCs, mixed with leucocytes and some residual platelets, are directed towards a third separate collection bag before eventual re-infusion. This method allows around 40 mL of PRP to be obtained from 450 mL of whole blood. With discontinuous flow, in which the patient stays connected to the machine, up to 300 mL of PRP could be collected [8]. Despite the use of this sophisticated technology, the final PRP always contains

Table 2. Characteristics and classification of the main platelet concentrates protocols available

PC class	Method (and relevant Refs)	Main characteristics									
		A: Process				B: Content				C: Fibrin	
		A1: Centrifuge type	A2: Duration	A3: Cost	A4: Ergonomy	B1: Volume	B2: Platelet collection	B3: Leucocyte collection	B4: Preservation	C1: Density	C2: Polymerization
P-PRP	AP	Cell separator PRP [8]	Heavy	Very long	Expensive	– –	Small	Excellent	No leucocytes	Damaged	Low	Weak
		Vivostat PRF [9]	Heavy	Long	Expensive	+	Small	Low	No leucocytes	Damaged	Low	Weak
	MP	Anitua's PRGF [10–14] Nahita PRP [15]	Heavy but potentially light	Long	Inexpensive	–	Variable	Low	No leucocytes	Unknown	Low	Weak
L-PRP	AP	PCCS PRP [9,14] SmartPReP PRP [9,16,18] Magellan PRP [19] GPS PRP [20]	Heavy	Long	Expensive	+	Variable	Good	Good	Unknown	Low	Weak
	MP	Friadent PRP [16] Curasan PRP [8] Regen PRP Plateltex PRP [17] Ace PRP [15]	Heavy but potentially light	Long	Expensive	–	Variable	Good	Good	Unknown	Low	Weak
P-PRF	MP	Fibrinet PRFM [9]	Heavy but potentially light	Long	Expensive	+	Large	Good	No leucocytes	Healthy, activated	High	Strong
L-PRF	MP	Choukroun's PRF [21–30]	Light	Quick	Very inexpensive	+ +	Large	Excellent	Good	Healthy, activated	High	Strong

Abbreviations: AP, automated protocols; MP, manual protocols, PC, platelet concentrate.

residual RBCs and leucocytes. In addition, this protocol is cumbersome and labour-intensive and often requires the help of haematologist. Although it is the more accurate method from a technical point of view [8], its use in daily practice remains rare. Recently, more compact systems have been developed that can be used more easily, both for autotransfusion during surgery and for topical application (for example the Electa cell separator, Sorin group, Italy).

The Vivostat PRF centrifuge (Vivolution, Denmark) can be considered as an advanced cell separator, and it was originally designed to produce the Vivostat Fibrin sealant. The use of a specific preparation kit with this centrifuge allows the production of a leucocyte-poor platelet concentrate for surgical use. However, Vivostat PRF has been used in only a few published studies, and this system is cumbersome and very expensive for daily practice. Moreover, its platelet collection efficiency is rather low and platelets are damaged during the process [9].

Manual protocols for modified P-PRP: Anitua's PRGF

One of the first platelet concentrate protocols (PRGF, which stands for either plasma rich in growth factors [10] or preparation rich in growth factors [11]) was described in 1999 by Anitua and has been commercialized by BTI (BioTechnology Institute, Vitoria, Spain). In this protocol, venous blood is collected and centrifuged in several small tubes to obtain the three typical layers: RBCs, 'buffy coat' and acellular plasma. The upper part of the acellular plasma is called plasma poor in growth factors (PPGF) and is discarded from each tube by careful pipetting to avoid creating turbulences. The remaining plasma is termed PRGF and is collected with a pipette, using only 'eyeballing' as a measuring tool (Figure 2). Several pipeting steps, each associated with possible pipeting and handling errors, are necessary to collect the entire PRGF fraction of the patient, after which fibrin polymerization is induced by a 10% calcium chloride solution. After 15 to 20 min, an unstable PRGF gel is formed that will need to be used immediately.

There are some inconsistencies in the PRGF protocol. In the original description of the protocol [10], most of the plasma (after discarding a small fraction as described above) was collected, including the 'buffy coat' layer that contains most of the platelets and leucocytes. However, in later applications of this method [12,13], the authors claim that the buffy coat layer was not collected. The objective of this approach was to avoid the collection of leucocytes, but it seems to be technically imprecise and in danger of yielding irreproducible results. Moreover, it also leads to a low platelet collection efficiency because platelets and leucocytes are found together in the intermediate layer after lowspin centrifugation [14]. Platelet preservation has not been examined, but the soft centrifugation should keep platelets in good shape. Anitua's PRGF method is an inexpensive manual protocol for the preparation of leucocyte-poor PRP. However, the lack of ergonomy and reproducibility of the procedure is problematic. Other similar protocols can now be found in the literature, for example the Nahita PRP [15].

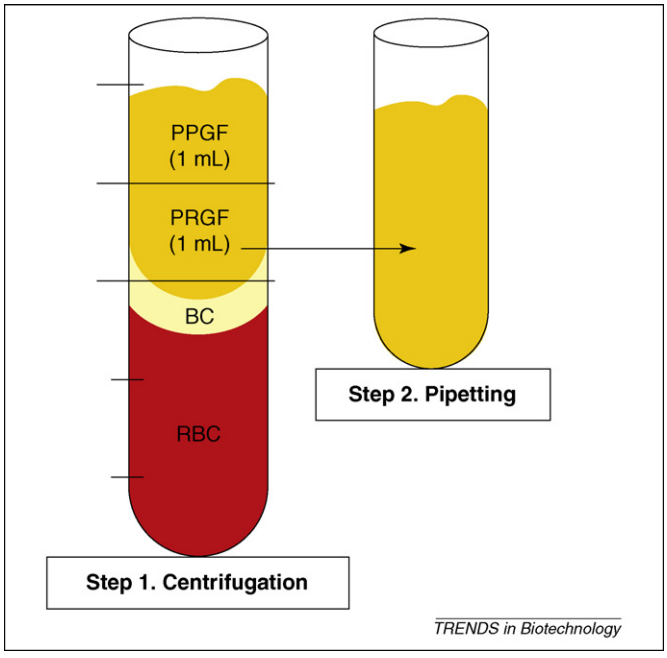

Figure 2. Commonly described protocol for Anitua's PRGF [10–14]. Step 1: Citrated blood (in 5 mL tubes) is softly centrifuged (8 min at 460 g) and separated into three layers: RBC base, buffy coat (BC) and acellular plasma. Acellular plasma contains the empirically defined layers plasma poor in growth factors (PPGF) and plasma rich in growth factors (PRGF). Step 2: The PPGF layer (1 ml) is discarded, and the PRGF, just above the BC, is collected by careful pipetting. PRGF from all sample tubes is collected into one tube and calcium chloride is added for clotting.

Leucocyte- and platelet-rich plasma (L-PRP)

The initial objective of developing alternative easy-to-handle methods was to make it possible to use platelet concentrates in daily practice without having the support of a transfusion laboratory. Without a cell separator, elimination of leucocytes becomes more difficult, and the resulting platelet concentrates therefore contain a high quantity of leucocytes, which were not initially desired. However, through changes in the collection parameters, the same protocols might also be used to produce pure leucocyte-free PRP.

Manual protocols for L-PRP: Curasan, Friadent-Schütze, Regen and Plateltex

Two similar protocols, each using a two-step centrifugation procedure, were marketed by Curasan (Kleinostheim, Germany) [8] and Friadent-Schütze (Vienna, Austria) [16], respectively. Each method follows the first principal step described above and shown in Figure 1, in which a first centrifugation step separates the blood components into three layers of RBCs, 'buffy coat' and PPP. The PPP and buffy coat layers are then carefully collected, avoiding RBC contamination, and transferred to another tube, where they are subjected to a second centrifugation step at high speed, which separates the sample again into its components. After the second centrifugation step, most of the PPP layer is discarded using the 'eyeballing' method. The PRP concentrate obtained with this method is composed of a high quantity of platelets, leucocytes and circulating fibrinogen, but it also contains residual RBCs. The concentrate is applied with bovine thrombin and calcium chloride.

Similar methods for PRP generation have also been developed with Plateltex (Bratislava, Slovakia) and Regen PRP (Regen Laboratory, Mollens, Switzerland). The Plateltex protocol uses specific gelifying agents, such as calcium gluconate and lyophilized purified batroxobin, an enzyme that cleaves fibrinopeptide, to induce fibrin polymerization without bovine thrombin and gelling in around 10 min [17]. The Regen method employs a separator gel within the centrifugation tubes with the aim of improving the collection of platelets and leucocytes.

All these protocols require substantial manual procedures, meaning that the preparation process is time-consuming, and furthermore, they only lead to small volumes of L-PRP. Some of the PPP fraction can be preserved: it contains fibrinogen and allows an increase in the final L-PRP volume. Adapted kits can be quite expensive if used frequently. The final product exhibits a low density fibrin matrix, which is strong enough for application as a fibrin glue but quickly dissolves. Platelets and leucocytes are typically well preserved and concentrated in these protocols, but the success of the method depends on the operator and results are not always reliably reproduced. Numerous modifications of this basic protocol with regard to centrifugation forces and time and the type of anticoagulants have been used in different studies or commercialized (for example, Ace PRP) [15]. In most publications on these techniques, the composition of the final PRPs used is often unclear. For example, if the buffy coat layer is not completely collected, the platelet collection efficiency decreases, and P-PRP can sometimes be produced instead of L-PRP (Figure 1).

Automated protocols for L-PRP: SmartPReP, PCCS, GPS and Magellan

Automated systems for L-PRP have been developed in the form of PCCS (Platelet Concentrate Collection System) by 3I (Palm Beach Gardens, USA) [9,14] and SmartPReP by Harvest Corp (Plymouth, USA) [9,16]. The centrifuges used have been designed to take a customized collection and centrifugation device, which consists of two connected compartments. In the PCCS method, citrated whole blood is transferred into the first compartment and centrifuged briefly to obtain the three layers (RBC, buffy coat, PPP). Then, by opening of a tubule and using air pressure, the superficial layers (i.e. PPP and buffy coat) are transferred to the second chamber and centrifuged again but for a longer period. Finally, using the same air pressure system, most of the PPP layer is transferred back into the first compartment and thus discarded. The final product is rich in leucocytes and has similar characteristics to the manual Curasan PRP described above.

The SmartPReP protocol requires even less manipulation. The two-chamber device is designed to automatically transfer the upper layers (PPP and buffy coat) into the second chamber based on variations in weight and centrifugation speed. SmartPReP is a multifunction system [18]: using a specific collection and separation kit, this centrifuge can also be used to concentrate stem cells from bone marrow aspirates.

The Magellan APS (Autologous Platelet Separator) by Medtronic (Minneapolis, USA) can be considered as a

further advance of the previously described cell separator with optical reader. However this device is compact, similar to a table centrifuge, and designed for small blood samples of up to 50 mL. The obtained platelet collection efficiency is high, but cell preservation is unknown [19]. Data sheets provided by the company indicate that the leucocyte content is also high.

GPS (Gravitational Platelet Separation System) by Biomet Biologic (Warsaw, USA) is another variation of a two-chamber centrifugation device with two-step centrifugation protocol [20]. The main difference is that the PPP is discarded after the first centrifugation using a syringe and tubules, and the second centrifugation step is performed with the RBC layer. The final PRP concentrate is collected by aspiration of the buffy coat layer on the surface of the RBC base. The procedure is thus inversed, but the final result seems to be similar.

The main drawbacks of all these techniques are that they require expensive and cumbersome centrifuges and collection/preparation kits. Moreover, the final concentrates dissolve quickly, similar to a fibrin glue. Their use in daily practice remains uncommon and the PCCS is no longer available.

Leucocyte-poor or pure platelet-rich fibrin (P-PRF) concentrates

In this category, there is only one method available. The Fibrinet PRFM kit by Cascade Medical (New Jersey, USA) contains two tubes, one for blood collection and another for PRFM clotting, together with a transfer device. A small amount of blood (typically 9 mL) is drawn into a collection tube, which contains tri-sodium citrate as an anticoagulant and a proprietary separator gel, and centrifuged for six minutes at high speed. The three typical layers of RBCs, buffy coat and PPP are obtained. Buffy coat and PPP are easily transferred to a second tube containing $CaCl_2$ with the help of a specifically designed tube connection system. The clotting process is triggered by the presence of $CaCl_2$ and the tube is immediately centrifuged for 15 min, after which a stable PRFM clot can be collected. The company claims that the system produces a 'natural' platelet concentrate owing to the absence of bovine thrombin. However, this claim is doubtful because the blood is mixed with anticoagulant and separation gel, leading to what could be considered unnatural conditions.

This protocol is similar to other typical L-PRP protocols, such as the Curasan method. The main difference is that only very low amounts of leucocytes are collected owing to the specific separator gel used in the method. However, the platelet collection efficiency is high and the preservation of the platelets during the procedure seems to be acceptable [9]. Platelet activation and fibrin polymerization are triggered using only calcium chloride, the same method as in Anitua's PRGF protocol. However, the fibrin matrix in Fibrinet PRFM is denser and more stable than that in PRPs, probably due to the dynamic clotting during the second centrifugation step, which is more efficient than a static PRP polymerization. The simultaneous processing of large sample numbers with the Fibrinet method remains difficult and is expensive in daily practice. In addition, fundamental or clinical studies demonstrating the efficiency of Fibrinet PRFM are not yet available.

Leucocyte- and platelet-rich fibrin (L-PRF) concentrates: Choukroun's PRF

Choukroun's PRF protocol is a simple and free technique developed in France by Choukroun *et al.* [21]. It can be considered as a second-generation platelet concentrate because the natural concentrate is produced without any anticoagulants or gelifying agents [22]. Venous blood is collected in dry glass tubes and centrifuged at low speed (Process protocol, Nice, France) [23]. In the absence of anticoagulants, platelet activation and fibrin polymerization are triggered immediately. Therefore, after centrifugation, three layers are formed: the RBC base layer, acellular plasma top layer and a PRF clot in the middle (Figure 3). The PRF clot forms a strong fibrin matrix with a

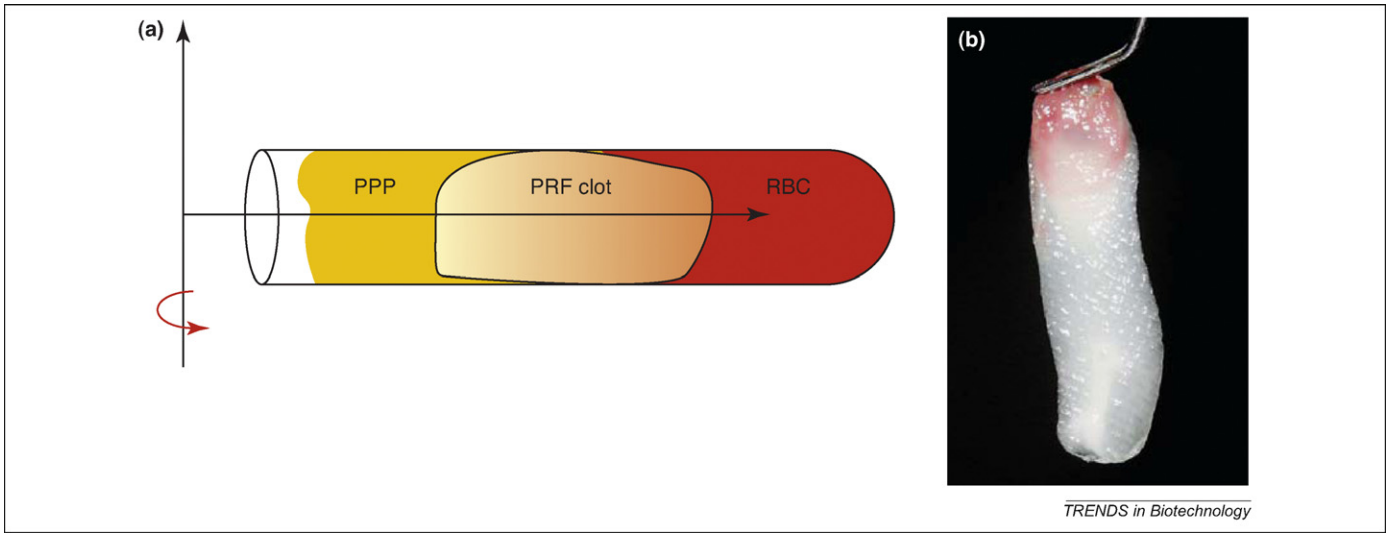

Figure 3. Choukroun's platelet-rich fibrin (PRF) method [21–30]. **(a)** Blood is softly centrifuged immediately after collection without anticoagulants, and coagulation starts quickly. Blood is separated into three components with the formation of a strong fibrin clot in the middle of the tube. This clot acts as a plug that traps most light blood components, such as platelets and leucocytes, as well as circulating molecules, such as growth factors and fibronectin. This method leads to the natural production of a dense leucocyte-rich PRF (L-PRF) clot. **(b)** After compression of the L-PRF clot, it can be used easily as a membrane (actual length shown: 3 to 4 cm).

complex three-dimensional architecture, in which most of the platelets and leucocytes from the harvested blood are concentrated [24,25]. When pressed between two gauzes, the PRF clot becomes a strong membrane, and some applications of this autologous biomaterial have been described in oral [26], maxillofacial [27,28], ENT (ear, nose, throat) [29] and plastic surgery [30].

Unlike the PRPs, Choukroun's PRF does not dissolve quickly after application; instead, the strong fibrin matrix is slowly remodelled in a similar way to a natural blood clot. Platelets and leucocytes are collected with high efficiency in this method and leucocytes are preserved throughout. However, platelets are activated during the process, which leads to a substantial embedding of platelet and leucocyte growth factors into the fibrin matrix [24,25].

This method allows the production of a high quantity of L-PRF clots simultaneously using either a specific centrifuge that takes eight tubes or any modified laboratory centrifuge, making it possible to produce even more clots for larger surgeries. Another advantage of this method is its low cost and the great ease of the procedure, which allows the production of many concentrates quickly and by natural means, that is, without the use of chemicals or unnatural conditions. Therefore, this method seems to be most suitable for widespread use in daily practice and is actually the main technique in some countries, including France, Italy and Israel. Efforts should be undertaken to further develop this protocol in the near future.

P-PRP or L-PRP? Potential applications and controversies

Most studies involving the use of PRPs have employed different in-house protocols in which the basic two-step centrifugation process was varied with regard to centrifugation forces (from 160 g to 3000 g) and time (from 3 to 20 min for the first centrifugation step). The definition of these parameters frequently seems to be empirical, and cross-examination of these technical data is an impasse. Moreover, it is very difficult to judge whether the actual experiments have been performed with P-PRP or with L-PRP [31]. P-PRPs seem to be the most frequently tested platelet concentrates *in vitro* and *in vivo*, but an accurate analysis of the entire literature is impossible.

The first published *in vitro* studies demonstrated a general tendency of PRPs to stimulate the proliferation of several cell types, including osteoblasts [32,33], fibroblasts [34], tendon cells [35], chondrocytes [36], periodontal ligament cells [37] and bone mesenchymal stem cells (BMSCs) [38]. However, contrasting results have also been reported [39,40], and this topic is still hotly debated in the literature, probably owing to the great number of PRP protocols that might lead to significantly different platelet concentrates. In addition, experiments were often performed with animal cell lines [41,42] or commercialized cell lineages [40,43], even though primary cultures of human cells [33,39,44] should have been used as the gold standard because of the high immunogenicity of any platelet concentrate, which will always contain some leucocytes. The effect of PRPs in differentiation is also controversial because some studies demonstrated a stimulation of osteoblastic differentiation [32,33,42], whereas others reported an inhibitory effect [39,41].

In vitro stimulations provoked by platelet concentrate preparation are only observed for a short time, which has led to discussion over their clinical long-term significance [45] because most authors consider them as being only effective *in vivo* over a short period [46]. The best proliferation results *in vitro* have been obtained with physiological platelet amounts, that is, for a PRP with a platelet concentration that was equivalent to 2.5 times the blood concentration [44]. Higher platelet concentrations seemed to induce negative effects. However, the data on the dose-dependent effects are limited and should be investigated further after the fibrin and leucocyte content of the platelet concentrate has been accurately defined.

Clinical studies have indicated that platelet gels can shorten recovery time, reduce surgery-related swelling and pain [47], accelerate the repair of the soft tissues [48] and increase bone regeneration in the short-term [45]. However, all these benefits could also be observed with the use of simple fibrin glues [2,18]. In most cases, PRPs are considered and used as a sophisticated autologous fibrin glue, and the literature remains unclear about the true clinical differences between these related fibrin products.

With regard to the use of platelet concentrates in biotechnological applications, it is worth noting that promising results have already been obtained for bone tissue engineering studies. BMSCs collected from the iliac crest were treated with PRPs as a cell culture and implantation medium [49] with the aim of replacing fetal calf serum in reimplanted materials to avoid contamination or immune response [50]. PRPs were shown to strongly stimulate proliferation of BMSCs but inhibited their differentiation [38,51]. This method for tissue engineering with BMSCs and PRP might be relevant in reconstructive craniofacial surgery [52] or in orthopaedics [53]. These invasive protocols were tested in human periodontal [54] and implant surgery [55], although they seem to be currently impractical for use in daily practice.

Most studies employing Anitua's PRGF (manual P-PRP) were performed by BTI, the dental implant company commercializing this product. Preliminary results on chronic ulcers have been encouraging but were also limited because only small wounds were treated, which did not entirely close [12]. Wider applications of this concentrate type in orthopaedic surgery, for example on tendons, has been proposed [13] but will need to be validated independently.

The main differences between the L-PRP and the P-PRP types of platelet concentrates are related to their leucocyte content and the potential effects of the leucocytes on proliferation, differentiation, immunity and infection. Due to the great variability in the different protocols, the obtained results have never been analyzed using the leucocyte content of the final concentrate as a key parameter. Thus, differences between P-PRP and L-PRP preparations have not yet been accurately documented.

Leucocytes and fibrin: the two key parameters

The literature dealing with platelet concentrates often ignores the impact of leucocytes and fibrin, which are

the two key parameters in our classification (Figure 4). Some authors even recommend, without any scientific evidence, the elimination of leucocytes [11]. However, several studies have already pointed out the key role of leucocytes in PRP [31], both for their anti-infectious action [56,57] and immune regulation [25,58]. Apart from an anti-infectious effect, leucocytes produce large amounts of VEGF [59]. Platelets are known to contain angiogenesis stimulators, such as VEGF and basic fibroblast growth factor, and inhibitors, such as endostatin and thrombospondin-1, in similar quantities [60]. Additional VEGF in PRP preparation, which stems from the leucocytes, might be crucially important for the promotion of angiogenesis.

When used during coronary artery bypass surgery, L-PRP significantly reduced occurrences of chest wound infection, chest drainage and leg wound drainage [61]. The leucocyte content did not seem to induce negative effects or to impair the potentially beneficial effects of PRP, even when used in joints [62]. Recent research showed that L-PRP was able to stimulate anabolism and remodelling capacities of tendons [63] and could be successfully used in injected form for the treatment of tendonitis [64]. Injected L-PRP could also be used for the treatment of long bone delayed healing [65]. An uncontrolled immune reaction of L-PRPs has not been reported and, on the contrary, their use was able to diminish pain and inflammation of the treated sites [62,64]. However, the respective effects of platelets and leucocytes in the platelet concentrates have not yet been analyzed, and the contribution of the leucocytes to the observed overall effect remains unclear, although synergistic effects can be hypothesized [58,62]. Further studies need to focus on this aspect of PRP function to clearly elucidate the relative contributions of the concentrate components, thereby helping to develop concentrates with specific and desired effects.

The density of the fibrin matrix and its composition is another key parameter of any platelet concentrate [43]. However, most studies addressing the biological effects of platelet concentrates focused on investigating platelet growth factors and ignored the cytokines in their environment or the influence of the fibrin matrix, which supports their release. The fibrinogen concentration

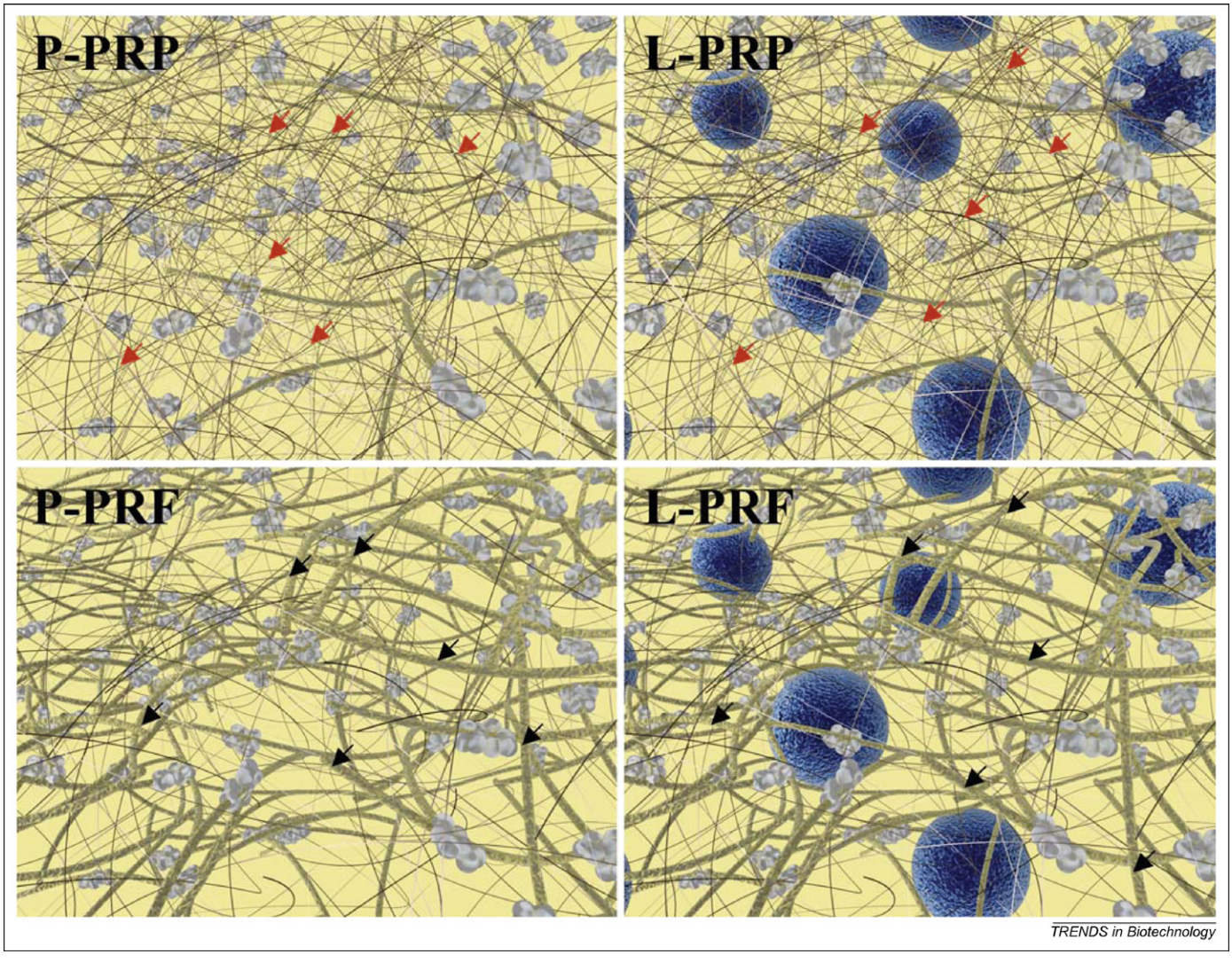

Figure 4. Schematic illustration of the matrix and cell architecture of the four categories of platelet concentrates. Two key parameters are important: leucocyte content (blue circles) and density of fibrin (yellow/light-brown fibres). Platelet aggregates (light-grey shapes) are always assembled on the fibrin fibres. In typical P-PRP and L-PRP preparations (top panels), the fibrin network is immature and consists mainly of fibrillae with a small diameter (red arrows) due to simple fibre polymerization. This fibrin network supports platelet application during surgery but dissolves quickly like a fibrin glue. In P-PRF and L-PRF preparations (bottom panels), fibrin fibres are thick (black arrows) due to multiple fibre assembly and constitute a resistant matrix that can be considered as a fibrin biomaterial.

varies considerably between the different methods; for example, in P-PRP protocols, the fibrinogen mainly originates from the platelet α granules after activation and the final fibrin concentration is low, whereas in other protocols, circulating fibrinogen is also collected and reinforces the final fibrin network. Platelet concentrates should be analyzed as a whole, as an assembly of platelets and leucocytes in a complex fibrin matrix. Platelet growth factors cannot be described alone as 'magic' molecules and should be considered from the matrix biology point of view.

Conclusions

The world of platelet concentrates for surgical use is actually a jungle of commercial proposals and unclear products. Under the same name, more than ten different autologous glues or biomaterials are available. The technological classification presented here aimed to provide an overview of the available systems and to categorize them with respect to three main parameters: fibrin density, leucocyte content and degree of standarization of the procedure. PRPs are often considered as improved fibrin glues; however, PRFs can be regarded as dense fibrin biomaterial with biomechanical properties. A high density fibrin clot can serve as a biological healing matrix by supporting cell migration and cytokine release, expanding the range of its potential applications greatly. The influence of the leucocytes on the biology of each product and its potential benefits should now be carefully analyzed because it could explain many controversial data from the literature. Finally, expensive and complex procedures are often unusable in daily practice and many will disappear. Simple and free systems, such as Choukroun's PRF, were developed by clinicians for clinicians and are anticipated to be major methods in the next years.

We feel that clarification is the first step in defining any clinical and biotechnological applications for each technique, and the development of these products is now completely dependent on an accurate and rational description of their structure and associated biology.

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