

Narrative Review

CURRENT METHODS FOR THE PREPARATION OF NON-TRANSFUSIONAL BLOOD-DERIVED COMPONENTS

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ABSTRACT

Non-transfusional hemocomponents (NTHs) are autologous blood-derived products rich in growth factors that may play an important role in bone regeneration by promoting mesenchymal stem cell proliferation and differentiation. Despite their increasing clinical use, standardized classification and preparation protocols remain lacking, making their application and comparison difficult. In this review, we summarize the literature on NTH preparation methods, comparing platelet concentrates, gels, and lysates according to their preparation protocols, biological properties, and clinical practicality. Particular attention is given to differences in centrifugation techniques, cellular composition, and fibrin architecture, all of which may influence growth factor release and regenerative potential. A clearer understanding of these differences may help clinicians select the most appropriate NTH for clinical use. Further categorization and standardization are needed to optimize the use of NTHs and promote greater consensus in regenerative medicine.

KEYWORDS: *non-transfusional hemocomponents, growth factors, regeneration, biomaterials, platelet-rich plasma*

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INTRODUCTION

NTHs are autologous products obtained by processing the patient's blood. They may be prepared as solutions or gels and can be applied or injected at the wound site to support healing. Several types of NTHs are available, including platelet concentrates, platelet gels, and platelet lysates, which are widely used in oral surgery (1).

The use of autologous blood-derived products began many years ago with fibrin glues (concentrated fibrinogen), while other NTHs subsequently were subsequently developed from these technologies. Platelet concentrates were initially used in patients with severe thrombocytopenia. Because standardized terminology was lacking, early studies used terms such as "platelet-fibrinogen-thrombin mixture" (2), "platelet-derived wound healing factors" (PDWHF) (3), and "gelatin platelet" (4). The term "platelet-rich plasma" was subsequently introduced by Marx et al. (5) and became widely adopted.

Over the years, the introduction of specific commercial products represented an important development in this field. Commercially available systems also contributed to the development of more specific terminology. Some modern techniques, such as Choukroun's platelet-rich fibrin (PRF), differ substantially from the first generation of platelet-rich plasma (PRP). Platelet gels are derived directly from platelet concentrates.

Platelet lysate is another product derived from platelet concentrates. It is obtained by inducing platelet lysis through repeated freeze-thaw cycles or sonication, producing a solution rich in growth factors (GFs).

In Italy, platelet concentrates and gels obtained through simple centrifugation procedures are not considered blood derivatives. The preparation of blood components is legally restricted to transfusion services; however, according to DM 2/11/2015 and its subsequent modification in DM 01/08/2019 (6), decentralized production may be possible subject to specific regulation and accreditation (7). This review describes and compares different methods used to prepare NTHs.

MATERIALS AND METHODS

A narrative literature review was conducted to examine current methods for the preparation and characterization of non-transfusional blood-derived components (NTHs). The literature considered included publications addressing platelet concentrates, platelet gels, platelet-rich plasma (PRP), platelet-rich fibrin (PRF), PRF derivatives, and platelet lysates.

The selected literature was analyzed with particular attention to the main factors that influence the characteristics of the final products, including blood collection procedures, use or absence of anticoagulants, centrifugation protocols, relative centrifugal force (RCF), number and duration of centrifugation steps, platelet and leukocyte content, fibrin architecture, platelet activation methods, and growth-factor release. Commercial preparation systems and relevant regulatory aspects were also considered where reported in the literature.

The findings were organized according to the principal types of NTHs and their preparation methods, with the aim of comparing their biological characteristics, practical advantages, and limitations and of identifying areas in which greater standardization and consensus are needed.

PLATELET CONCENTRATES AND GELS

Classification of platelet concentrates

A classification system for platelet concentrates was proposed in 2009 (8) and confirmed at the POSEIDO consensus conference in 2013 (9). These classifications categorize platelet concentrates according to leukocyte content and fibrin organization. Four main categories can be distinguished: pure PRP (P-PRP), leukocyte-rich PRP (L-PRP), pure PRF (P-PRF), and leukocyte-rich PRF (L-PRF) (1). This classification provided simple and pragmatic terminology but did not incorporate other important parameters, such as platelet concentration and leukocyte count or differential (Table I).

Table I. From the POSEIDO classification of platelet concentrates (AP, automated protocol; MP, manual protocol).

P-PRP (Leucocyte- and Platelet-rich Plasma) before activation/ P-PRP gel (after activation) L-PRP (Leucocyte- and Platelet-rich Plasma)/ L-PRP gel (after activation)	-Blood collected with anticoagulants -Exogenous coagulation factors for activation	AP: Cell separators, Vivostat PRF MP: Anitua's PRGF, Nahita PRP AP: SmartPREP, PCCS PRP, GPS PRP, Magellan PRP MP: Curasan PRP, Friadent PRP, Regen PRP, Plateltex PRP
P-PRF (Leucocyte- and Platelet-rich Plasma) ★ L-PRF	-Blood collected without anticoagulants -Immediate centrifugation -Intrinsic coagulation pathway	MP: Fibrinet PRFM MP: Choukroun's PRF and derivatives

★For P-PRF (i.e., PRFM), the highly structured fibrin network is obtained using an anticoagulant (sodium citrate).

In these preparations, blood is collected using a specific disposable kit and then centrifuged. Processing times and centrifugation forces vary among protocols, and these parameters are sometimes defined empirically, without clear standardization (Fig. 1).

**Fig. 1.** Venous blood draw.

PRP TECHNIQUES

PRP techniques generally involve collecting blood into tubes containing an anticoagulant to prevent platelet activation, followed by centrifugation, often in two cycles ranging from approximately 15 minutes to 1 hour. During the first, “soft-spin” centrifugation, the blood separates into three layers: red blood cells (RBCs) at the bottom, acellular plasma (PPP; platelet-poor plasma) at the top, and the buffy coat between them. PPP contains circulating plasma proteins, including fibrinogen, but, unlike the buffy coat, contains relatively few platelets. A second, longer and faster “hard-spin” centrifugation is then performed on the PPP and buffy coat, with or without a portion of the RBC fraction.

Subsequent steps vary among protocols, but the procedure generally involves removing the RBC and PPP fractions to isolate the buffy coat. This fraction may then be activated with thrombin, calcium chloride, or other activators, resulting in platelet activation and fibrin polymerization, with the conversion of fibrinogen to fibrin and the formation of a gelatinous clot. These gels have been shown to exhibit adhesive properties and increased tensile strength, which may contribute to clot stabilization and regenerative processes (10-12). The supernatant obtained during these procedures may also have potential applications in tissue repair (13).

P-PRP techniques

P-PRP can be produced using automated protocols, such as plasmapheresis with a dedicated cell separator, or automated systems such as the Vivostat PRF centrifuge (Vivolution, Denmark). However, the cost and labor-intensive nature of these procedures may limit their use in routine clinical practice. Moreover, some of these systems have been reported to have limitations in platelet collection efficiency and platelet preservation (14).

More convenient manual protocols, such as Anitua's PRGF (15) and the Nahita PRP system (16), have been developed as alternatives. PRGF, also known as plasma rich in growth factors, is produced by soft centrifugation of anticoagulated blood. The fraction located beneath the superficial plasma layer, referred to as plasma poor in growth factors (PPGF), and above the buffy coat is collected by pipetting, taking care to avoid the buffy coat, which contains leukocytes and platelets. The collected fraction is then activated with 10% CaCl_2 . However, difficulties in accurately collecting the desired fraction, a lower platelet concentration than that of other PRP preparations (17), and the formation of an unstable and fragile PRGF gel supported by a low-density fibrin network similar to fibrin glue represent important limitations of this technique (Fig. 2).

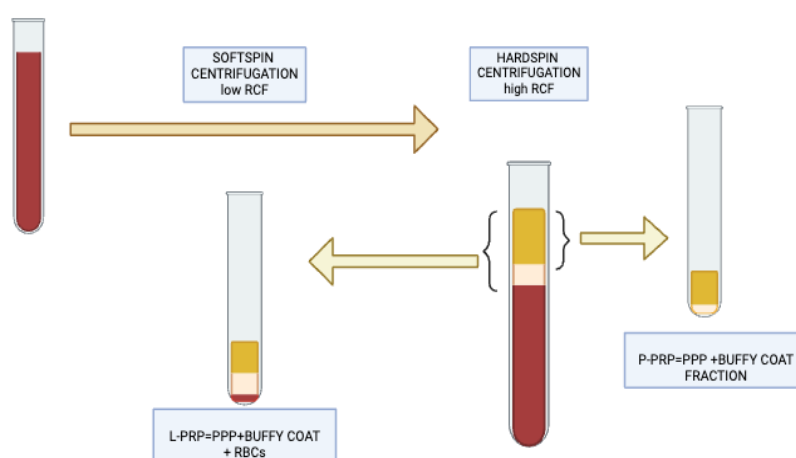


Fig. 2. PRP preparation. Whole blood is centrifuged in the presence of an anticoagulant. After a shorter “soft-spin” centrifugation, three layers are produced: PPP, buffy coat, and RBCs. To produce P-PRP, the PPP and superficial buffy coat are transferred to another vial, with most leukocytes excluded. After a second, longer “hard-spin” centrifugation and removal of part of the PPP, P-PRP is obtained. For L-PRP, both the buffy coat and PPP are collected in the second vial, together with a portion of the residual RBC fraction.

The inclusion of leukocytes in NTHs has long been debated. Some studies have reported deleterious effects on the fibrin scaffold and the development of a pro-inflammatory environment (18), which may compromise regenerative processes. In manual protocols, complete separation of leukocytes from the buffy coat is not possible; however, modern systems have been shown to substantially reduce leukocyte concentrations (19). Conversely, studies investigating the complex interactions between neutrophils and activated platelets have shown that these cells can generate important anti-inflammatory molecules (20, 21). The role of leukocytes in regenerative processes therefore warrants further investigation.

L-PRP techniques

L-PRP techniques, characterized by a high leukocyte concentration, account for a large proportion of commercially available systems, including Curasan (Kleinostheim, Germany), Friadent-Schutze (Vienna, Austria), Plateltex (Bratislava, Slovakia), and Regen PRP (Regen Laboratory, Mollens, Switzerland). Automated systems, such as SmartPreP, PCCS, GPS, and Magellan, provide greater standardization by reducing manual processing and the associated variability.

In these techniques, blood is collected into vials containing anticoagulants. After the first centrifugation, the blood separates into its constituent layers. PPP and the buffy coat, together with a residual portion of the RBC fraction, are then collected and subjected to a second, hard-spin centrifugation to concentrate the platelets. Following this second centrifugation, the preparation can be activated using different agents, including bovine thrombin, calcium chloride or

calcium gluconate, lyophilized purified batroxobin, or collagen.

Thrombin appears to have a substantial effect on the mechanical properties of PRP clots and, consequently, may influence their regenerative properties (22-24). Nevertheless, some studies have reported reduced osteoinductive and chondroinductive potential when thrombin is used for activation (25). To reduce the risks of hemorrhage, coagulopathy, and immune reactions, some protocols recommend replacing bovine thrombin with autologous thrombin (26).

Despite the large number of available methods, the literature still lacks a universally accepted classification system. Marx (27) proposed one of the first qualitative definitions of PRP. Based on analyses of the dose-response relationship, significant efficacy was reported when the platelet count reached at least 4- to 5-fold above baseline (28). In this context, a “benchmark” therapeutic PRP concentration of 1 million platelets/ μ L has been proposed.

Several other factors, including platelet damage, differences in platelet capture efficiency among devices (17, 27), the risk of premature platelet activation and consequent release of GFs into discarded PPP, and differences in platelet concentration and purification (29, 30), are critical variables that are often insufficiently reported in clinical studies. In addition, variations in centrifugation parameters can produce different PRP preparations with different platelet yields (14, 28-36). Particular attention should therefore be paid to device parameters, especially relative centrifugal force (RCF), rather than RPM alone. RCF can be calculated as $RCF = 1.118 \times 10^{-5} \times R \times RPM^2$, where R is the rotor radius in centimeters and RPM is the rotational speed in revolutions per minute. Because RCF depends on both rotor radius and rotational speed, reporting RPM alone may make comparisons between protocols and their replication difficult. A comprehensive description should therefore include all relevant centrifugation parameters (37-39).

PRF TECHNIQUES

In PRF techniques, blood is collected without anticoagulants and immediately centrifuged at a moderate centrifugal force. After centrifugation, the blood separates into three layers, with a fibrin clot forming in the middle of the tube. Platelet activation and fibrin polymerization occur naturally, without the addition of exogenous activators, resulting in the formation of a dense fibrin network.

P-PRF techniques

P-PRF is a leukocyte-free NTH characterized by a highly organized fibrin network. The Fibrinet PRFM kit (Cascade Medical, New Jersey, USA) is the only commercial method reported to produce P-PRF (40). In contrast to other PRF techniques, blood is initially collected in vials containing sodium citrate. The buffy coat is then activated with $CaCl_2$ and subjected to a second centrifugation cycle to obtain a gel with a high platelet concentration (14). The resulting fibrin scaffold is reported to be more stable and mechanically stronger than that of PRP, owing to dynamic polymerization. However, the cost and complexity of the procedure are important disadvantages.

L-PRF and derivatives

Choukroun developed L-PRF in 2001 (41), and it is considered a second-generation platelet concentrate. In the absence of anticoagulants, platelets are activated and fibrin polymerizes naturally. After 12 minutes of centrifugation in a specific device (Intra-Spin System), a strong fibrin clot forms in the center of the tube. This product represented an important development, making the use of hemocomponents more accessible in routine clinical practice.

L-PRF is generally considered an optimized blood clot (42) that, like a sponge, retains most of the lighter blood components, including platelets, leukocytes, and GFs. Activated platelets and leukocytes become embedded within a highly organized fibrin network. The method is relatively inexpensive and involves a simple procedure, allowing natural fibrin polymerization without the use of exogenous activators or other artificial triggers.

As with PRP, preparation protocols play a fundamental role in determining the characteristics of the final product, although other factors are also involved. The quality and performance of the centrifuge itself can significantly affect cellularity and fibrin architecture in PRF (43). Ehrenfest et al. reported that even when the protocol, RCF, disposable tubes, and blood collection materials were identical, different PRF clots could be produced, demonstrating that PRF quality and centrifuge performance are closely interconnected (43).

Over the years, the original PRF preparation protocol has been modified by various authors, including Choukroun and Sacco, resulting in derivatives such as advanced PRF (A-PRF) (44), injectable PRF (i-PRF) (45-47), and concentrated growth factor (CGF) (48). These products are obtained by modifying centrifugation parameters, including speed and duration, as well as acceleration and deceleration profiles.

Fibrin architecture and leukocyte content substantially influence GF release, and important differences have been reported in studies comparing L-PRF and PRP membranes. L-PRF showed a more sustained release of TGF- β 1, PDGF-

AB, VEGF, thrombospondin-1, and fibronectin for at least 7 days. In addition, a stronger fibrin network was observed in L-PRF membranes than in PRP membranes, whereas GF release from PRP membranes appeared to be concentrated mainly within the first few hours (49). Diffusion has been hypothesized to predominate during the initial phase of GF release. Subsequently, a more prolonged release may occur as GFs associated with fibrin fibers and platelet membranes are liberated through degradation of these structures (50). The less stable matrix of PRP derivatives, resulting from incomplete polymerization, may be less effective at retaining GFs and blood proteins, potentially explaining the more rapid release of these components during the first few hours (51). Conversely, viable leukocytes may contribute to the production of additional GFs after the initial release, potentially contributing to the more sustained release observed in L-PRF (49).

Differences have also been reported among PRF derivatives. Scanning electron microscopy (SEM) analysis showed stronger and more intricate cross-linking of fibrin fibers in CGF clots than in A-PRF (52). It has been suggested that the alternating centrifugation speeds used during CGF preparation may optimize fibrinogen conversion and fibrin polymerization, resulting in a stronger and more stable matrix (53). The looser fibrin network observed in A-PRF may favor platelet accumulation, potentially explaining the higher GF release measured by ELISA compared with CGF (52).

Masuki et al. reported that platelet and GF concentrations in PRF were comparable to or higher than those in PRP preparations (54). Differences in cell distribution within the fibrin matrix have also been reported among A-PRF and other PRF protocols. The reduced centrifugation force used for A-PRF resulted in a higher concentration of leukocytes in the distal portion of the clot (44). Lower centrifugation forces and shorter centrifugation times reduce cell sedimentation and may consequently increase cellularity and GF release within the fibrin matrix (55). Kobayashi et al. (55) reported higher GF concentrations in PRP at very early time points (15–60 minutes of incubation), whereas PRF protocols, particularly A-PRF, showed a more gradual release over a period of up to 10 days.

Miron et al. analyzed the properties of L-PRF and provided a more detailed characterization of platelet and leukocyte concentrations (56). By sequentially pipetting 1-mL layers from L-PRF preparations, they identified a marked accumulation of platelets and leukocytes directly above the RBC fraction. This 0.5–0.3-mL buffy-coat fraction was defined as concentrated PRF (C-PRF). After harvesting and processing, this PRF derivative showed higher concentrations of platelets and leukocytes/monocytes, greater GF release, and an enhanced cellular response compared with other PRF derivatives, such as i-PRF (57).

PLATELET LYSATE

Platelet lysate (PL) is a cell-free, GF- and protein-rich biomaterial that can be produced from platelet apheresis products or platelet concentrates (58). One of its earliest applications was the *ex vivo* expansion of mesenchymal stem/progenitor cells (MSCs) as an alternative to fetal bovine serum (FBS) (59). Subsequently, its therapeutic and regenerative properties have been investigated in tissue regeneration (60).

Several methods can be used to produce PL. Platelet lysis can be induced by ultrasonication or repeated freeze–thaw cycles. The platelet membranes are then removed by centrifugation, and the supernatant containing GFs and cytokines is collected. The resulting solution contains high concentrations of GFs and cytokines but no intact cellular components.

There is no consensus regarding the most effective lysis method or temperature protocol. However, all procedures appear to be more effective at concentrating GFs than PRP (61). The possibility of cryopreservation and subsequent reuse (58) makes PL an interesting alternative to PRP. However, an association has been observed between storage time, storage conditions, and the resulting GF concentrations (62), highlighting the complexity of these biomaterials and the need for standardized protocols to ensure consistent characterization of the final products and to determine the most appropriate procedures for GF release and handling.

DISCUSSION

NTHs represent a complex class of living biomaterials. At present, there is no consensus on optimal preparation methods, and a clear and comprehensive categorization of these products is still lacking in the literature (61, 62). Important parameters, including initial blood volume, type of anticoagulant, equipment and machine settings, type of processing system (43), number of centrifugation steps, centrifugation time, rotor size and angle, RCF, RPM, and platelet activation method, are closely interconnected and influence GF and cytokine release.

Even small changes in these parameters can alter platelet composition, leukocyte distribution, and three-dimensional fibrin architecture, thereby producing a substantially different biomaterial (63–64).

As research progresses, improved categorization of these living biomaterials will be essential to establish a scientific consensus not driven by commercial considerations.

CONCLUSIONS

The complex nature of NTHs remains an important issue in tissue healing. A better understanding of their biology and composition is also essential for the development of new techniques, such as C-PRF. Further research is needed to improve our understanding of these products and to establish more consistent preparation and characterization methods.

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