In Search of a Consensus Terminology in the Field of Platelet Concentrates for Surgical Use: Platelet-Rich Plasma (PRP), Platelet-Rich Fibrin (PRF), Fibrin Gel Polymerization and Leukocytes

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Abstract: In the field of platelet concentrates for surgical use, most products are termed Platelet-Rich Plasma (PRP). Unfortunately, this term is very general and incomplete, leading to many confusions in the scientific database. In this article, a panel of experts discusses this issue and proposes an accurate and simple terminology system for platelet concentrates for surgical use. Four main categories of products can be easily defined, depending on their leukocyte content and fibrin architecture: Pure Platelet-Rich Plasma (P-PRP), such as cell separator PRP, Vivostat PRF or Anitua's PRGF; Leukocyte- and Platelet-Rich Plasma (L-PRP), such as Curasan, Regen, Plateltex, SmartPReP, PCCS, Magellan, Angel or GPS PRP; Pure Plaletet-Rich Fibrin (P-PRF), such as Fibrinet; and Leukocyte- and Platelet-Rich Fibrin (L-PRF), such as Choukroun's PRF. P-PRP and L-PRP refer to the unactivated liquid form of these products, their activated versions being respectively named P-PRP gels and L-PRP gels. The purpose of this search for a terminology consensus is to plead for a more serious characterization of these products. Researchers have to be aware of the complex nature of these living biomaterials, in order to avoid misunderstandings and erroneous conclusions. Understanding the biomaterials or believing in the magic of growth factors ? From this choice depends the future of the field.

Keywords: Fibrin, platelet-rich fibrin (PRF), platelet-rich plasma (PRP), platelet, leukocyte.

1. PLATELET CONCENTRATES TERMINOLOGY: A HISTORY OF CONFUSIONS LEADING TO A BLIND LIBRARY OF KNOWLEDGE

The development of surgical adjuvants for the local stimulation of healing is an important field of research in biomaterial and pharmaceutical sciences. The first act of healing associates many actors, first of all platelets, leukocytes, fibrin matrix and many growth factors. All these actors work in synergy during the coagulation process, and many products logically tried to mimic these natural mechanisms in order to improve healing on a surgical site. This trend started many years ago with fibrin glues (autologous, or more frequently allogeneous) [1, 2], and evolved recently with platelet concentrates technologies [3]. The concept of these latter [4] is to use a centrifugation procedure (often in 2 steps) in order to concentrate and collect most platelets from a blood harvest (taken with anticoagulant), and to inject them into a wounded site in order to improve healing. The activation of the product (with thrombin for example) induces the platelet growth factors release and the polymerization of fibrinogen (released by platelets or free in the plasma) into fibrin [5], leading to a platelet gel.

Platelet concentrates and fibrin glues are thus in fact not so different, both being based on similar technologies of polymerization of blood fibrinogen into a fibrin gel [3, 6]. However the concept of platelet-rich product was often wrongly summarized by one magical concept: growth factors. This craze for growth factors often led to forget the key roles of the other actors present in these blood-derived products: between mystical belief, commercial interests and scientific truth, for many researchers in this field it required several years to accept the key role of fibrin [7, 8] and sometimes leukocytes in these products. When considering the complexity of coagulation and healing, growth factors are only actors among many others. The platelet concentrates are more complex products than classical pharmaceutical preparations, because their clinical effects are dependent on the intrinsic versatile and adaptive characteristics of the patient blood and on the numerous -and often not investigated- actors contained in these products [9]. Platelet concentrates are in fact blood concentrates, and their biology is as complex as

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blood itself. These products are living biomaterials, and are more difficult to handle and evaluate than synthetic materials shaped on demand.

The terminologies used in the field thus significantly suffered from the initial confusions about these products and mystical belief in platelet growth factors. The craze for platelet concentrates technologies started first in oral and maxillofacial surgery after the first article of Marx *et al*. [10]. These authors used the term « Platelet-Rich Plasma (PRP) », in reference to the term used for platelet concentrates in hematology for the treatment of patients suffering from severe thrombopenia. The "platelet-rich plasma (PRP)" term was first used in 1954 by Kingsley to designate thrombocyte concentrate during blood coagulation experiments [11], and is still used today in blood transfusion science.

However, the concept of platelet concentrate for topical use was in fact much older than the article of Marx and coworkers. A few years after the first publications of Matras about fibrin glues [1], autologous preparations called « platelet-fibrinogen-thrombin mixtures » were tested in ophthalmology [12, 13], general surgery [14] and neurosurgery [15]. Another author called it « gelatin platelet (gel foam) » [16]. These platelet-rich products were only used as fibrin tissue adhesives, and not as healing stimulators: the platelets were only supposed to support a stronger fibrin polymerization, and therefore a more efficient tissue sealing than basic fibrin glues. Growth factors and healing properties were then not considered.

A first clinical demonstration that platelet concentrates promote healing locally was reported by Knighton *et al*. in 1986 [17]. These authors used the term "platelet-derived wound healing factors" (PDWHF) for a preparation produced using an empirical 2-step centrifugation procedure. Using this protocol, these authors treated 49 patients with chronic non-healing cutaneous ulcers and reported good outcomes. In other articles in 1988 and 1990 [18, 19], these authors used a slightly different term to define their technique: "platelet-derived wound healing formula (PDWHF)". These authors already used the term "platelet-rich plasma" as a general term of transfusion medicine, but it did not refer to the final product for clinical use itself. A few years later, Whitman *et al*. [20] presented their clinical results in oral and maxillofacial surgery, using a platelet concentrate that was produced by a gradient density cell separator from the hematology laboratory. It was named PRP during collection, but the authors understood that the final product was a fibrin gel, and therefore named it "platelet gel". Unfortunately, in all these studies, the exact content of these products remained unknown, particularly concerning the leukocyte content. Only platelets were quantified.

The use of the "PRP" term thus truly started with Marx *et al*. [10], in a first study about the use of these platelet-rich products during bone graft reconstruction in maxillofacial surgery. The clinical results for bone healing were very interesting and sustained the use of these technologies in this field. This PRP terminology was maybe too general, but it was adequate: the PRP from Marx *et al*. was produced using a cell separator from the hematology laboratory, and therefore was very similar to a professional PRP for transfusion. But this product was also used as a fibrin gel, since it was injected during activation with bovine thrombin. Moreover, the leukocyte content of the product used by these authors was not confirmed.

From this moment, the term "PRP" was used everywhere, whatever the platelet concentrate technology was [3]. During the following years, most studies have employed various in-house protocols, where the basic two-step centrifugation process was modified with regard to centrifugation forces (from 160g to 3000g) and time (from 3 to 20 min for the different centrifugation steps). The definition of these parameters frequently seems to be empirical, and cross-examination of these technical data is an impasse. In most articles, the various platelet concentrates were not correctly described or characterized, particularly concerning their cell contents. Most authors were somehow considering that all platelet concentrates were the same, whatever was inside.

This confusing situation created incredible inconsistencies in the literature. It is frequent to see articles where authors quantified the growth factor content of these products, but without activating the platelets [21, 22]: obviously it cannot lead to relevant results and conclusions. The releasates are often tested, but without investigating the key role of the fibrin matrix in the release kinetics [21-24], even if this parameter was proved to be a considerable issue [25, 26]. It is also frequent to read *in vitro* cell studies where PRP and cell lineages in culture are coming from 2 different donors [21, 22], or even 2 different species [23, 24]: considering that many platelet concentrates also contain leukocytes, cell cultures with platelet concentrates are often cocultures with leukocytes [27, 28], but many authors did not take this key issue into consideration. As a whole, the enormous literature on the field is very difficult to review, and the published results are so contradictory that this literature could be called a blind library of knowledge.

2. IN SEARCH FOR A BETTER TERMINOLOGY: A SLOW EVOLUTION

The vagueness of the terminology and the associated lack of characterization of the various platelet concentrates were key issues, and several actors of this field tried to identify and define clearly some of these products.

First, some companies started to use specific commercial names for their products in order to give them a better visibility. In 1999, Anitua (Biotechnology Institute, BTI, Vitoria, Spain, a dental implant company) described a manual version of the original PRP technology, using a one-step centrifugation process and several pipetting steps [29]. The final product was called PRGF (Plasma [29] or Preparation [30] Rich in Growth Factors), and contained no leukocyte and lower platelet and growth factor concentrations than other products [31]. It could be activated with calcium chloride in order to produce a PRGF gel. Other companies also tried to use similar strategies, such as Vivostat PRF (Platelet-Rich Fibrin): this latter is in fact only another variation of the cell separator PRP technology [4]. Using the name of a commercial product to define a specific version of the platelet concentrate technology is a smart solution and of great help for a better understanding of the data published in the literature. Unfortunately, it also raises serious suspicions of commercialism [32], since these names are not based on the

consensual classification of a scientific reality, but only shaped by the need of commercial communication.

Some techniques however were so different from the initial PRP concept, that the need for a different name became obvious and natural. When Choukroun *et al*. described in 2001 the Platelet-Rich Fibrin (PRF) technique [33], it was considered as a second-generation platelet concentrate technology [3, 34-38]. Indeed Choukroun's PRF shows very different characteristics than the other products: blood is collected without anticoagulant and centrifuged immediately, leading to the natural formation of a strong PRF clot in the middle of the tube (without the addition of any adjuvants or triggering factors). PRF is a solid biomaterial [39-42], an optimized natural blood clot [43, 44], and not an injectable liquid platelet suspension like the PRPs. The platelet activation and the fibrin polymerization are not the final step of the process, they are the process [43]. With the invention of this technique, the key role of the fibrin matrix in these technologies became an obvious issue [3], even if the confusions persisted. At least, it was obvious that platelet concentrate technologies resulting in strong fibrin biomaterials could not be called PRP anymore. But another issue was raised by the studies on PRF: PRF also contains leukocytes [36, 43]. The immune parameters started to be investigated and the key role of leukocytes was hypothesized and pointed out [25, 27, 28].

Obviously, the fibrin architecture and leukocyte content could not be forgotten. An important series of opinions and original articles challenged the spineless consensus on the historical PRP term and gave the opportunity to create a better terminology.

In Bielecki *et al*.'s opinion in 2006 [45], the authors insisted on the different forms of PRP used in clinical practice: PRP could be injected without thrombin (for example in chronic severe elbow tendinosis)[46], but was more often used after activation with thrombin (or another activator) resulting in a gelatinous mass formation. These authors therefore proposed to define Platelet-Rich Plasma as an inactive substance, while "platelet-rich gel" (PRG) represented the biologically active fibrin matrix rich in platelets, leukocytes and related active molecules (growth factors, serotonin, catecholamines, von Willebrand factor, proaccelerin, osteonectin and others released from the platelet granules). The presence of leukocytes was also pointed out, but no other specific terminology was proposed. In 2008, Everts *et al*. completed this first opinion [47], insisting on the leukocyte content and the 2 forms (activated/non activated) of these products. A more accurate terminology was proposed: the inactivated product was named platelet-leukocyte rich plasma (P-LRP), and the activated gel was named platelet-leukocyte gel (PLG).

The proposals of these 2 groups were significant contributions for the clarification of these products, and these authors used these new terminologies in several following articles [48-57]. However, this terminology remained incomplete [32]: indeed, some PRPs do not contain leukocytes [31], and non activated PRPs cannot be considered as inactive products, their full activation is only delayed and more natural (after the contact with the tissues on the site of injection)[46]. Moreover, even after activation, these gels (PRG, PLG or whatever the name) never reach the degree of natural fibrin polymerization obtained in the PRF subfamily [43, 58].

The final step in this terminology history was the definition of a full classification system for all platelet concentrates [4]. Four families were simply designed, based on their leukocyte and fibrin content. Liquid platelet concentrate suspensions (before activation) were named PRP: "Pure Platelet-Rich Plasma" (P-PRP) without leukocytes, "Leukocyte- and Platelet-Rich Plasma" (L-PRP) with leukocytes. On the contrary, solid platelet concentrate biomaterials, with a strong fibrin architecture (and therefore always activated), were called PRF: "Pure Platelet-Rich Fibrin" (P-PRF) without leukocytes, "Leukocyte- and Platelet-Rich Fibrin" (L-PRF) with leukocytes. The main commercially available technologies were classified following these principles (Table **1**). This classification is probably the more simple and accurate [32], but a few clarifications are still necessary in order to find a consensus terminology.

3. FINDING A CONSENSUS TERMINOLOGY

A terminology must be simple, accurate and pragmatic. It also must avoid commercial interests and thus remain scientifically homogeneous [59]. For clarity and to be user-friendly, we should avoid the multiplication of pointless acronyms that only create confusions. Following these principles, a consensus on a simple terminology has been reached and summarized in the Table **1**.

First of all, all the products of this category are « platelet concentrates ». This is the general term that defines all these products: activated or not, with or without leukocytes, all these products concentrate the platelets.

Second, the previous classification from 2009 [4] should be considered as a good and user-friendly system to classify products by their leukocyte content and fibrin architecture. Indeed, considering that most of the literature on the topic used the acronym PRP, and since this term is still valid for some of these products, this acronym should be completed but conserved in order to keep the coherence of the database. When searching « PRP » articles on Medline, « P-PRP » and « L-PRP » are immediately identified. For this reason, the term « L-PRP » should be preferred to « P-LRP » for this subfamily, even if both are correct from a strict meaning standpoint.

« PRG » [45] and « PLG » [47] acronyms are too general and should therefore be avoided. Indeed from a strict definition standpoint, P-PRF and activated P-PRP are both Platelet-Rich Gels, and L-PRF and activated L-PRP are both Platelet-Leukocyte Gels, but the PRP and PRF families show two completely different fibrin matrix architectures [5, 43, 58]. Moreover PRG and PLG also contain plasma in the gel, as a colloidal solution trapped within the fibrin mesh. For all these reasons, the activated version of a P-PRP should simply be a « P-PRP gel » (and not a PRG), and the activated version of a L-PRP should simply be a « L-PRP gel » (and not a PLG). The 2 PRF subfamilies only exist in the gel form, so they do not need a more accurate terminology.

Three last parameters remained outside this first classification/terminology system: the platelet concentration rate,

Table 1. Classification of the main available (and Marketed) Methods of Production of Platelet Concentrates, in the 4 main Families of Products and their Associated Terminology (AP: Automated Procedure; MP: Manual Procedure). Many Other Custom-Made or Open-Source Protocols are Available, all of them being Variations on the same basic Concepts; but all the Techniques Fall within this Terminology/Classification System

Platelet Concentrate Class and Terminology	Methods of Production (Generic name, detailed appellation when existing, company, city, country).	
P-PRP (Pure Platelet-Rich Plasma), before activation (**P-PRP gel**, after activation)	**AP**	• Cell separator PRP. • Vivostat PRF (Vivolution, Alleroed, Denmark).
	MP	• Anitua's PRGF (Preparation or Plasma Rich in Growth Factors, BTI BioTechnology Institute, Vitoria, Spain). • Nahita PRP (Nahita, Navarra, Spain).
L-PRP (Leukocyte- and Platelet-Rich Plasma), before activation (**L-PRP gel**, after activation)	**AP**	• PCCS PRP (Platelet Concentrate Collection System, 3I, Palm Beach Gardens, FL, USA). • SmartPReP PRP (Harvest Corp, Plymouth, MA, USA). • Magellan PRP (Magellan APS (Autologous Platelet Separator), Medtronic, Minneapolis, MN, USA). • Angel PRP (Angel Whole Blood Processing System (AWBPS), Sorin Group, Mirandola, Italy). • GPS PRP (Gravitational Platelet Separation System, Biomet Biologic, Warsaw, IN, USA).
	MP	• Friadent PRP (Friadent-Schütze, Vienna, Austria). • Curasan PRP (Curasan, Kleinostheim, Germany). • Regen PRP (Regen Laboratory, Mollens, Switzerland). • Plateltex PRP (Plateltex, Prague, Czech Republic). • Ace PRP (Surgical Supply and Surgical Science Systems, Brockton, MA, USA).
P-PRF (Pure Platelet-Rich Fibrin)	**MP**	Fibrinet PRFM (Cascade Medical, Wayne, NJ, USA).
L-PRF (Leukocyte- and Platelet-Rich Fibrin)	**MP**	Choukroun's PRF (Process, Nice, France).

the leukocyte concentration rate, and the proportion of the various sorts of leukocytes. First, the platelet collection efficiency can be very different between the various available systems [31, 60, 61]. However, the clinical impact of the platelet concentration rate is difficult to determine through the available literature: the studies often concluded that a low or moderate platelet concentration promoted the best results, but these *in vitro* and *in vivo* data are quite theoretical and difficult to extrapolate to clinical situations [62-65]. This notion of platelet concentration rate is probably very important in some clinical fields where the platelet concentrate is injected like a pharmaceutical liquid preparation (particularly injections for tendons and muscles in sport medicine) [46, 66, 67]. However, when the platelet concentrate is used as surgical adjuvant on a wounded and bleeding site, the local blood flow considerably dilutes the impact of the platelet concentration rate, and the biomaterial characteristics of the product then prevail: fibrin architecture and cell content. Moreover, when PRP gels are used as surgical adjuvants, the platelet concentrate is often placed in suspension in some acellular plasma (rich in fibrinogen) in order to increase the volume of fibrin gel after activation: this simple technical handling logically causes a dilution and a critical decrease of the platelet concentration rate. However, the platelet concentration also influences the strength of the final fibrin network, so this parameter is in fact also interlinked with the fibrin architecture of the material.

From the experience in sport medicine, where PRPs were used as liquid pharmaceutical injectable preparations, a 5-fold platelet concentration rate may be considered as a relevant baseline for the definition of PRP subfamilies, since concentrations higher than 5-fold often gave the best clinical results. However, this baseline is probably not universal and therefore not valid for all clinical applications. Moreover, if it may be applicable for the PRP subfamilies, it can not be used for the PRF class since all PRF products are per definition solid biomaterials with a strong fibrin architecture (therefore not injectable), and thus have reached an adequate level of platelet concentration required for a strong fibrin polymerization. For all these reasons, this notion of platelet concentration rate was kept outside this general classification consensus, but a specialized subclassification for injectable PRPs in sport medicine is proposed in this special issue of Current Pharmaceutical Biotechnology.

On the contrary, the leukocyte concentration was often neglected in the literature, and the leukocyte concentrate rate and the leukocyte formula were even rarely suggested as parameters [43], even if many clinical results may be related to these actors of healing [28, 68]. All these parameters should be assessed carefully now, in order to improve our knowledge and maybe improve this first classification/terminology system in the future.

As a conclusion, the purpose of this search for a terminology consensus is not to play on words, but to influence the way of thinking of all researchers in this field: it is no more acceptable now to see scientific articles about these technologies where the exact content of the tested products is unclear, and where the final state (activated or not) and fibrin architecture are not assessed. Without a better understanding of the polymorphic and adaptive nature of these products, the field of platelet concentrates may finally lose a big part of its credibility, showing uncontrolled contradictory results (often easily explained by the presence of leukocytes and other uncharacterized parameters), and all its potential for the future will be lost. Understanding the biomaterial or believing in the magic of growth factors? From this choice depends the future of the field.

DISCLOSURE OF INTEREST

The authors declare no competing financial interests.

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