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Regenerative Medicine

A Call for a Standard Classification System for Future Biologic Research: The Rationale for New PRP Nomenclature

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Abstract

Autologous cell therapies including platelet-rich plasma (PRP) and bone marrow concentrate (BMC) are increasingly popular options for soft tissue and joint-related diseases. Despite increased clinical application, conflicting research has been published regarding the efficacy of PRP, and few clinical publications pertaining to BMC are available. Preparations of PRP (and BMC) can vary in many areas, including platelet concentration, number of white blood cells, presence or absence of red blood cells, and activation status of the preparation. The potential effect of PRP characteristics on PRP efficacy is often not well understood by the treating clinician, and PRP characteristics, as well as the volume of PRP delivered, are unfortunately not included in the methods of many published research articles. It is essential to establish a standard reporting system for PRP that facilitates communication and the interpretation and synthesis of scientific investigations. Herein, the authors propose a new PRP classification system reflecting important PRP characteristics based on contemporary literature and recommend adoption of minimal standards for PRP reporting in scientific investigations. Widespread adoption of these recommendations will facilitate interpretation and comparison of clinical studies and promote scientifically based progress in the field of regenerative medicine.

Introduction

The field of orthobiologics is rapidly evolving with respect to both clinical practice and research. The search continues for the ideal biological agent(s) to facilitate tissue regeneration and modify disease [1-11]. Platelet-rich plasma (PRP) has gained popularity as an orthobiologic agent because of its potential to facilitate tissue repair, modulate inflammation, and improve symptoms of tendon, ligament, and joint conditions in clinical studies [12-30]. PRP has generally been defined as an autologous plasma derivative in which the concentration of platelets is above baseline. However, PRP preparations have significant variability, which has led to the proposal of several PRP classification systems [31-34]. Unfortunately, these previously published classifications do not account for all of the PRP attributes that may affect efficacy based on contemporary knowledge, including the actual platelet concentration (number of platelets/mL), the volume of PRP (mL) delivered to the target site, the presence or absence of white blood cells (WBCs, including neutrophils), the presence or absence of red blood cells (RBCs), and whether exogenous activation (eg, thrombin) was performed. Furthermore, none of these classification systems has been widely adopted. The lack of accepted standards for reporting PRP in published research has significantly limited the ability to interpret individual clinical studies, compare different studies targeting the same clinical entities, and accurately translate the results of some investigations into clinical practice. Most importantly, the inconsistency in reporting PRP parameters may contribute to conflicting conclusions regarding the efficacy of PRP. The purpose of this article is to update previous PRP classification systems in the context of contemporary research pertaining to the effect of PRP variables on clinical efficacy.

What is PRP?

By definition, PRP must contain a higher concentration of platelets than baseline. PRP was first used clinically in the United States in 1987 to facilitate wound healing after cardiac surgery [17]. Since then several

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medical fields have used this technology, including but not limited to dentistry, wound care, ophthalmology, urology, maxillofacial surgery, and cosmetic surgery [12,13,15]. During the past decade, a significant increase in the use of PRP has occurred among musculoskeletal and sports medicine clinicians. The therapeutic potential of PRP is based on the premise that growth factors released from the alpha granules of platelets in supraphysiologic amounts can augment the body's natural healing response [12,18]. In addition to growth factors, platelets also release a multitude of bioactive proteins, such as stromal derived factore 1a, which are responsible for attracting mesenchymal stem cells, macrophages, and fibroblasts that not only promote removal of degenerated and necrotic tissue but also enhance tissue regeneration and healing [19,20].

Platelet Concentration and Volume

When considering the use of PRP, the first factor to be discussed is determining the ideal platelet concentration necessary to enhance healing. Platelet counts may vary based on an individual's own blood morphology, as well as the time of day the sample is drawn [35]. Normal platelet counts range from 150,000/mL to 350,000/mL. A simplistic definition of PRP is that the platelet count must be above baseline [36,37]. Most commercially available platelet-concentrating machines can be somewhat arbitrarily divided into lower platelet concentrating machines (>1-3 baseline) and higher concentrating machines (>4-9 baseline). Early literature suggested that platelet concentrations of 2.5-3 baseline were ideal, with higher concentration levels potentially inhibiting tissue healing [38-40]. However, recent articles have provided contradictory results. Giusti et al [41] prepared platelet concentrates between 300,000/mL and 7.5 million/mL and found that the optimal platelet concentration for cultured endothelial cell proliferation was 1.5 million/mL (5-7 baseline). Lower levels produced less in vitro growth, and inhibition was not demonstrated until levels reached 2-3 million/mL (10 baseline) [41]. Furthermore, Haynesworth et al [42] demonstrated that accelerated wound healing required at least 4-5 baseline platelet concentrations and that mesenchymal stem cell recruitment increased exponentially as platelet concentrations increased from 2.5 to 5-10 baseline. In 2010, Kevy et al [43] replicated the work of Giusti et al [41] and reported an ideal platelet concentration of 1.5 million/mL (5-7 baseline) with no inhibitory effects up to 3 million/mL (10 baseline). This same group of researchers proposed that no available PRP device at the time could achieve platelet concentrations that would result in inhibition of tissue healing [43]. However, Giusti et al [41] recently noted that platelet counts greater than 2 million/mL were inhibitory to tenocyte behavior, with the optimal concentration appearing to occur between 1-1.5 million/mL. Thus the "ideal" platelet concentration may depend on the target parameter (eg, direct promotion of tissue healing and stem cell recruitment), the tissue that is treated (eg, bone, cartilage, or tendon), and the stage of disease or wound healing. Consequently, the "ideal" platelet concentration for various clinical scenarios remains unknown.

Complete reporting of a PRP treatment requires documentation of both the actual platelet concentration and the quantity of PRP delivered to the target site. Reporting the platelet concentration as " baseline" does not accurately reflect the platelet concentration because "5 concentration" in a patient with a baseline platelet count of 160,000/mL is significantly different than "5 concentration" in a patient with a baseline platelet count of 340,000/mL. The actual quantity of PRP delivered to a target site should also be reported so that the total number of platelets delivered can be calculated by multiplying the actual concentration by the volume. Because the actual platelet concentration, injected volume, and total number of platelets delivered to a region may all affect efficacy, we recommend that all 3 parameters be documented when formally reporting the results of PRP treatments for scientific purposes.

White Blood Cells

The question of whether WBCs inhibit or promote tissue healing has been a topic of considerable debate in the literature. PRP centrifuges that produce lower platelet concentrations generally separate out WBCs, whereas higher platelet-concentrating machines generally produce higher WBC concentrations. The concern regarding WBC concentration is based on the possible pro-inflammatory effects of WBCs, particularly with respect to neutrophils. Excessive inflammation may be counterproductive to soft tissue healing and may exacerbate rather than ameliorate arthritic pain [44]. Browning et al [45] reported that treating synoviocytes with PRP containing a large number of WBCs resulted in significantly greater increases in matrix metalloproteinases, interleukins, and other pro-inflammatory mediators compared with synoviocytes treated with platelet-poor plasma (ie, plasma with less than baseline concentrations of platelets and WBCs). These results have been reproduced in subsequent investigations [46,47].

With respect to the influence of WBCs on PRP, it is important to recognize that there are a variety of WBC types, including neutrophils, monocytes/macrophages, and lymphocytes. Although the role of each WBC population may vary over time and with respect to regional influences, the general properties of different WBC types that may influence tissue healing and inflammation have been defined. Some of the phagocytic and cell

signaling properties of WBCs may be beneficial in chronic tendinopathy but could result in excessive inflammation and additional tissue damage in the setting of chronic, uncontrolled inflammatory states. In addition, neutrophils contain hydrolytic enzymes such as matrix metalloproteinases, some of which demonstrate negative effects on soft tissue in vitro [44,48-50]. Macrophages are the cellular form of the circulating monocytes and in general are primarily phagocytic and function to remove debris. However, they also have a role in balancing the pro-inflammatory and anti-inflammatory aspects of healing (eg, M1 versus M2 macrophage functions) [49,51]. Finally, lymphocytes initiate cell-to-cell interactions and also modulate tissue healing via the release of bioactive molecules. The precise role of WBCs in treating soft tissue and joint disease is still being investigated. Given the complexities of tissue healing and the multifunctional roles of WBCs, it is possible that WBCs or specific WBC subtypes may be beneficial in specific musculoskeletal conditions (eg, chronic tendinosis), while being more detrimental in other others (eg, arthritis or acute muscle strain). Additional studies are needed to determine the clinical effects of different concentrations of WBC types on inflammation and wound healing. In the meantime, we recommend that PRP be classified by the presence or absence of WBCs, as well as the percentage of neutrophils in cases in which WBCs are present.

Red Blood Cells

Recent research has highlighted the potential deleterious effect of RBCs on PRP in the treatment of soft tissue and joint conditions. RBCs can adversely affect platelet function by altering local pH and promoting inflammation, and they have been documented as causing chondrocyte death [46,52,53]. Commercially available PRP systems generally process RBCs and WBCs in a similar manner. In general, PRP systems producing low platelet concentrations contain minimal or no RBCs, whereas highly concentrating systems have a higher RBC residual (5%-15% hematocrit). Recently, several commercial PRP machines have been able to generate higher platelet concentrations while reducing RBC and neutrophil concentrations through a double-spin suspension method.

Prior research suggests that removing RBCs from PRP may be beneficial when treating joint and specific soft tissue conditions. It is well established that RBCs have a negative effect on chondrocytes [53,54], and in vivo and in vitro studies have demonstrated that recurrent hemarthrosis, which is classically associated with hemophilia, predictably leads to knee arthritis [54-58]. Potentially significant cartilage damage has also been demonstrated after a single exposure of cartilage to RBCs, as might be obtained from a traumatic sports-related knee injury [53].

Based on available data, it appears that RBCs may influence inflammation and tissue healing and are cytotoxic to specific cell populations (eg, cartilage). However, currently no controlled studies have been performed to compare the clinical effects of PRP with varying RBC concentrations. At this time, we know of no PRP classification systems that include RBC information. Nonetheless, during the past few years, multiple PRP preparation systems have focused on removing RBCs in response to concerns regarding the potential adverse effects of RBCs in tissue healing. While research continues, we recommend that the presence or absence of RBCs in PRP preparations be reported when communicating PRP treatments for scientific purposes.

Activation

Platelets need to be activated to naturally release their contents. The 3 main substances that activate platelets are collagen, thrombin, and calcium. These activators differ in their speed of activation, and both the speed and extent of platelet activation may significantly influence the clinical effects of PRP. Thrombin acts significantly faster than calcium (usually injected as calcium chloride), and calcium is a faster activator than collagen. Activation by collagen is thought to occur spontaneously when PRP is injected into a soft tissue site. In addition, synthetic activators available on the market such as recombinant human thrombin and synthetic peptides may offer more sustained release of growth factors upon activation [59]. Regardless of the mechanism of activation, once the PRP is activated, a fibrin network will begin to form and plasma will begin to solidify to create a fibrin clot or membrane. Once formed, the fibrin clot or membrane can function as a supportive tissue scaffold that can release platelet contents over a sustained period. If PRP is over-activated, the fibrin will form into a bivalent network that is unstable. In comparison, if the PRP is activated in a more physiologic manner, a stable tetramolecular network will form that enhances the adherence of cells and growth factors [32].

Proponents of the use of activators claim that activation will benefit healing by more fully activating platelets to release their products, as well as by keeping platelets and their products within the target region through fibrin clot formation [60,61]. The time course of natural platelet degranulation is a topic of debate. One study demonstrated that approximately 90% of prefabricated growth factors are released in the first 10 minutes after activation [62,63], whereas a separate study reported that a slow release of growth factors naturally occurs over several days [61,64]. Opponents of using activators suggest that natural activation via interaction with one's own collagen is a better option because it allows for a slower release of growth factors over time, consistent with the body's

natural physiologic healing response [65]. Recent evidence supports this proposition, as Scherer et al [66] reported that unactivated PRP resulted in quicker fibroblast to myofibroblast differentiation and wound healing compared with thrombin-activated PRP [66].

Although it is currently unclear whether activation is beneficial or detrimental, it is generally agreed upon that activation changes the properties of PRP and may influence its clinical efficacy. Most human PRP studies evaluating tendinopathy have not used activators; however, multiple studies examining the effect of PRP on symptoms of arthritis have activated PRP with calcium chloride [67-71]. No study to date has compared the clinical efficacy of activated versus unactivated PRP on any tissue or disease model. Given the relationship between exogenous activation and PRP properties, we recommend that the use of exogenous activation be included when reporting PRP treatments in the context of scientific investigations.

PRP Classification Systems

Previous authors have suggested various classification systems to promote standardization of PRP reporting with the goal of facilitating the interpretation and synthesis of clinical studies. Mishra's PRP Classification (Table 1) was based on the available PRP systems at the time this classification system was published, which included primarily buffy coat and single-spin suspension method systems [31]. These 2 systems handled platelets, WBCs, and RBCs differently. In most buffy coat systems, platelets were highly concentrated to >5□, WBCs (and neutrophils) were increased to a variable extent, and RBCs were reduced to a variable extent. In comparison, single-spin suspension method systems available at the time produced relatively low platelet concentrations (1□-3□), with little to no WBCs or RBCs. Although Mishra's classification system accurately reflected the PRP systems that were available in 2006, knowledge of important PRP attributes and the technology available to produce specific PRP products have continued to evolve. For example, since publication of Mishra's classification system, the double-spin suspension method was developed to produce high platelet concentrations (>5□) with little or no neutrophils and little or no RBCs. Despite the reduction in neutrophils, the double-spin suspension systems produce total WBC counts at or above baseline because they concentrate potentially beneficial monocyte/macrophage and lymphocyte WBC subpopulations. Although recent in vitro data confirm that the PRP products produced by currently available systems may have different effects on tissue healing, the "best" PRP preparation for specific clinical conditions remains indeterminate and requires further investigation with appropriate reporting of PRP variables.

Table 1
Platelet-rich plasma classification proposed by Mishra et al [31]

Type	White Blood Cells	Activated?
1	Increased over baseline	No
2	Increased over baseline	Yes
3	Minimal or no WBCs	No
4	Minimal or no WBCs A: >5□ platelets B: <5□ platelets	Yes

From Mishra A, Harmon K, Woodall J, Viera A. Sports medicine applications of platelet rich plasma. Curr Pharm Biotechnol 2012;13:1185-1195.

In 2009, Dohan Ehrenfest et al [32,33] published a PRP classification and extrapolated into surgical procedures and wound care. PRP was classified on the basis of platelet concentrations, leucocyte concentration, and the presence or absence of fibrin. Each of the commercially available PRP systems at the time was consequently placed into 1 of 4 categories: P-PRP (pure PRP), L-PRP (leukocyte and PRP), P-PRF (pure platelet-rich fibrin) and, L-PRF (leukocyte and platelet-rich fibrin). Although this system has several merits, it is not applicable for most nonoperative orthopedic applications because of the limited use of fibrin. In addition, the classification of Dohan Ehrenfest et al does not address RBCs or provide information pertaining to leucocyte/WBC subpopulations such as neutrophils.

Lastly, in 2012, DeLong et al [34] published the "PAW" classification system that recommended reporting PRP based on platelet concentration (P), activation (A), and the amount of WBCs and neutrophils (W) relative to baseline. Platelets were categorized as P1 (□baseline) to P4 (>1.2 million platelets/mL), activation as either exogenous (X) or not, and WBCs and neutrophils as either above or below baseline. DeLong et al [34] categorized the published literature at the time using their proposed "PAW" system. Although the "PAW" classification recognized the potential importance of neutrophil content in PRP, RBCs were not addressed, and the placement 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 our opinion, none of the previously published PRP classification systems encompasses all of the PRP characteristics that may influence PRP activity and efficacy based on the current literature, including the following characteristics:

1. Platelet concentration (absolute number of platelets/mL)
2. Leukocyte concentration, including the concentration of neutrophils
3. Red blood cell concentration
4. Activation by exogenous agents

Table 2
PLRA classification

		Criteria	Final Score
P	Platelet count	_____ P	_____ M
		Volume injected	Cells/mL
L	Leukocyte content*	> 1%	þ
		< 1%	e
R	Red blood cell content	> 1%	þ
		< 1%	e
A	Activation†	Yes	þ
		No	e

Table created by Drs Patrick Nguyen and Walter Sussman.
* If white blood cells are present (þ), the percentage of neutrophils should also be reported.
† The method of exogenous activation should be reported.

Furthermore, few scientific investigations report the actual volume of PRP delivered to the target region. As discussed earlier in this article, reporting both the PRP characteristics and volume of PRP delivered is necessary to more fully understand the PRP treatment delivered in the clinical setting. Consequently, we make the following proposals:

1. The PLRA (Platelet count, Leukocyte presence, Red blood cell presence, and use of Activation) classification system should be used. This system reflects clinically important PRP characteristics based on contemporary literature and can be easily adopted for research and communication (Table 2).
2. All scientific publications and presentations should require reporting of the fundamental aspects of the PRP treatments used, including cellular concentrations (platelets, WBCs [including neutrophils], and RBCs), presence or absence of exogenous activation, volume of PRP delivered, and frequency of PRP treatments if multiple treatments were delivered.

In our opinion, widespread adoption of the PLRA classification system and standards for reporting PRP treatments in scientific investigations will facilitate interpretation and synthesis of clinical studies and promote scientifically based progress in the field of regenerative medicine with respect to PRP.

Conclusions

In the coming years, the applications of PRP to treat soft tissue and joint conditions will continue to expand. We believe that the science of PRP can only progress if minimal standards for reporting PRP are used. Consequently, we propose a new classification system that is easy to utilize and reflects the factors that appear to affect PRP properties based on the contemporary literature. Use of the PLRA classification system in combination with standards for reporting PRP treatments will allow clinicians and researchers to better interpret and synthesize published research as the search continues for the optimal platelet product for various orthopedic applications.

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