Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i18.3557

COMPARISON OF THE CELLULAR COMPOSITION OF VARIOUS PLATELET-RICH PLASMA PREPARATIONS

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Abstract:
Purpose of the study is to evaluate variations in formulations used to prepare platelet-rich plasmas (PRPs) result in differences in the cellular composition and biomolecular characteristics. Study design was controlled laboratory study. In this study five preparation procedures were performed for 14 healthy subjects, including 2 manual procedures (single-spin at 900*g* for 5 minutes; double-spin at 900*g* for 5 minutes and then 1500*g* for 15 minutes) and 3 methods with commercial kits (Arthrex ACP, Biomet GPS, and Prodizen Prosys). Results of the study showed that the DS PRP had a higher concentration of platelets and leukocytes than did the SS PRP. Every PRP preparation exhibited an increase in PDGF, TGF, VEGF, and FGF release when compared with whole blood samples. In short word the DS method generally led to a higher concentration of platelet relative to the SS method.

Key words: platelet-rich plasma, preparations, cell composition

Introduction:

Platelet-rich plasma

Platelet-rich plasma PRP is broadly defined as an autologous formulation from whole blood that is centrifuged to extract a solution with a platelet concentration of at least 1000 × 103 platelets/μl or a three-to-five fold increase in growth factors compared with normal plasma.[1] Platelet-rich plasma (PRP) is gaining popularity in pain medicine as a potentially safe and effective alternative to standard treatments. However, due to its autologous nature, PRP injectate may vary based on the unique manufacturer and methodology. However, the definition of PRP remains ambiguous, with no universal agreement. Originally developed as an adjunct to supplement bone-grafting procedures, PRP has become increasingly utilized in the clinical setting for knee OA due to the recent investigative and technological developments.[2-4] In the 1970s, haematologists coined the term PRP to describe plasma with a higher platelet count than peripheral blood, 6 which was used as a transfusion product for thrombocytopenic patients. Since then, it has been utilised in numerous medical specialties, including plastic surgery, paediatric surgery, cardiac surgery, gynaecology,

urology, and ophthalmology. [5,6] However, it is within the musculoskeletal field where there has been a surge of PRP use for multiple pathologies, largely due to widespread commercial interest following PRP use in professional sport. PRP has been shown to modulate the inflammatory process by interacting with endogenous cells within the joint, contributing to tissue maintenance and repair. [7,8] More recently, a growing interest in preparations comprising HA-PRP combinations as emerged due to its potential synergistic effect.[9] In general, PRP is regarded as a "minimally manipulated" biologic, and its use is governed by various governing bodies in each country and medical system. Numerous studies with highly variable formulations and control subjects have been conducted to evaluate its efficacy as an injectable, resulting in significant heterogeneity in reported outcomes and necessitating improved formula standardisation and nomenclature due to its widespread use. [10] Multiple variables compose the formulations of PRP, with platelet concentration and white blood cell (WBC) concentration being the predominant categories.[11] Despite the use of multiple preparation systems, few classification schemes have been developed to standardise PRP administration and research. In 2012, DeLong et al. proposed the PAW (Platelet, Activation, and WBC) classification system based on platelet concentration, mechanism of platelet activation, and WBC content. [1,12] Magalon et al. added a modified DEPA classification (dose of injected platelets, efficacy of production, purity of the PRP, and activation of the PRP). [13-15] Unfortunately, efforts to promote standardisation were largely devised after several research had already begun, and this has led to their not being universally used in the succeeding literature. Future collaborative work towards development of an international consensus for standardising PRP treatment is needed, as the lack of standardisation in PRP components hinders the ability to aggregate available data, preventing the development of concrete, evidence-based treatment algorithms for the use of PRP. [16] Preparation procedure has a significant impact on the efficacy of PRP. The centrifugal conditions used to prepare purified platelet-rich plasma (P-PRP) influence the cellular composition of the PRP obtained. In this study, we compared single and double centrifugation protocols to obtain an optimal platelet count and yield with minimal alteration to platelets' physiology in order to establish a simple and effective method of PRP preparation in settings with limited resources. [1]

METHODS

The Project was approved by Ethics Review Committee (Institutional Review Board) of Ziauddin University and Hospital wide letter No. (manuscript no: 1434/SAHS dated 06/12/2021). Subjects were enrolled after fulfilling the inclusion criteria and a written informed consent. The inclusion criteria included healthy subjects without any known blood dyscrasia, and the exclusion criteria included medical history of any blood-derived illness or medication known to affect platelet or bone marrow function for a minimum of 2 weeks before testing.

Five commercially available kits were selected, that were being utilized by most of the PRP Clinics in Karachi Pakistan. The Kits were coded as PRP-1, PRP-2, PRP-3, PRP-4 and PRP-5. These five kits were compared with PRP X-cell Kit of Surecell. For each of the commercially available selected kits, the method was used as per instructions of the manufacturer/ inventor of that kit. However, for Surecell method, Double-spin was performed; first spin at 1500g for 17 minutes and then for second spin at 900g for 5 minutes. 60 ml of venous blood samples were obtained from each individual at day 1 and the same amount of blood after 120 days. Complete Blood Count (CBC) was performed for each individual before selection as volunteer for this study to ensure platelets within normal range (150-450 $\times 10^3/\mu l$) and the count was recorded. CBC was also performed for each of the PRP kits and for X-cell kit to analyze the platelet count and concentration/yield, residual RBCs and WBCs in platelet-rich plasma (PRP) samples prepared from all the six selected kits. All measures were performed using automated SYSMEX (XP-100) hematology analyzer in triplicate and mean values were taken into analysis. Platelet yield or platelet concentration (%) measurement was derived from the study by Tamimi et al and Nagata MJ et al.10,11 It was calculated by using the formula as given:

$$\text{Platelet Yield or platelet concentration (\%)} = \frac{\text{PRP Platelet count}}{2a\text{Whole blood Platelet count of the given sample}} X\ 100$$

The SPSS version 23 was used to analyze the data. The mean and standard deviation for platelet, RBCs and WBCs counts and platelet yield was determined using Independent Samples T-Test and p-value <0.01 was taken as significant.

Comparison with 5 Commercially Available Kits

Five commercially available kits were used according to the manufacturer's instructions. None of these 5 commercial kits were exogenously activated by calcium or thrombin. The details of spin and duration are mentioned in Table 1.

Preparation	Centrifugation		Isolation	Final vol/WB vol
	First Spin	Second Spin		
PRP-1	900g, 5 min		Plasma layer	3.0 mL/6 mL
PRP-2	900g, 5 min	1500g15 min	Plasma layer	3.3 mL/6 mL
PRP-3	1012.5g,5 min		Plasma layer	3.0 mL/6 mL
PRP-4	2011g, 15 min		Buffy coat layer	2.8 mL/6 mL
PRP-5	1660g,3 min or 1446g, 3 min	2008g,3min	Plasma layer	3.0 mL/6 mL
Surecell PRP	1500g, 17 min	900g, 5min	Plasma layer	3.5 mL/8 mL

Table 1: Comparison of Rotational Force and Duration of Spin for Different PRP Kits with Surecell PRP

RESULTS

Cellular Concentrations and Compositions:

The mean platelet concentration of the whole blood (control) samples was 131±36.5 x10^3 cells/µL. Mean whole blood characteristics from 07 healthy donors, which were all within the ranges of normal biological value, are demonstrated in Table 2. [1,17] The Leukocyte concentration was also within normal range for all the 07 healthy volunteers and were 5.92±2.5 x10^3 cells/µL. Erythrocyte concentration was 3982±465 x10^3 cells/µL and Hematocrit was 45%..

Table 2: Mean Whole Blood Characteristics from 07 Healthy Enrolled Subjects

Characteristics	Platelet Concentration x 103 cells / µL	Leukocyte Concentration x 103 cells / µL	Erythrocyte Concentration x 103 cells / µL	Hematocrit (%)
Subject-1	142.5	6.1	4012	46
Subject-2	137.3	6.1	4032	43
Subject-3	128.8	6.2	4123	42
Subject-4	148.1	5.6	3978	44
Subject-5	127.7	5.5	3990	47
Subject-6	126.8	6.1	4023	46
Subject-7	127.3	5.7	4168	43
Mean ± SD	131±36.5	5.92±2.5	3982±465	44.3±1.5

Table 3: Comparison of Platelet Count and Yield of five Marketed PRP Kits with Surecell PRP

Preparations	Platelet Countx103/µL	Platelet Yield%	p-value
PRP-1	594.6±127.4	125.92 ± 15.8%	<0.01
PRP-2	623.06±167.5	176.38±12.5%	<0.01
PRP-3	696.6±147.4	145.51 ± 45.8%	<0.01
PRP-4	773.06±117.8	166.34±11.7%	<0.01
PRP-5	794.6±177.2	155.15 ± 55.8%	<0.01
X-cell PRP	923.06±127.58	276.70±12.7%	<0.01

Statistical Analysis

The measured data are presented here as the arithmetic mean and the standard deviation (SD).

Discussion

The biomolecular characteristics of PRP are important to determine the method of local application as well as to evaluate the effectiveness of the procedure. This study evaluated the different qualities of bioactive molecules in PRP according to different preparation protocols. The commercial preparations exhibited wide variations in their ability to concentrate platelets and leukocytes. Accordingly, proper PRP components should be selected by considering their biomolecular characteristics and patient indications.[18] The optimal platelet concentration of PRP for tissue healing and regeneration is believed to be 3 to 5 times higher than that of whole blood, and 10003 10^3 cells/mL is widely considered to be an effective platelet concentration to induce an efficient local cellular response.[19-21] On the other hand, a platelet concentration of 6 times higher than that of whole blood has been reported to have an inhibitory effect on healing.[1] Conversely, the effect of highly concentrated WBCs in PRP preparation has been widely debated.[11] Leukocytes in PRP may play a valuable antimicrobial role.[22] Leukocyte-rich preparations do not seem to be exclusively good or bad. For some specific indications such as chronic tendinopathy, leukocyte-rich PRP has been shown to be superior to leukocyte-poor PRP in clinical trials. In this study, the DS method generally produced higher concentrations of platelets and leukocytes, and similar outcomes were observed when using commercial kits.[1] More specifically, Prodizen Prosys produced higher concentrations of platelets and leukocytes than did Arthrex ACP. It is noteworthy that Biomet GPS showed the highest concentrations of platelet and leukocytes, which may be due to direct PRP extraction from the buffy coat layer after a single round of centrifugation.

Future Prospect and Conclusions

Technological advances in PRP devices and preparation methodologies show promising patient outcome results, although clarity in different PRP bioformulations and the related biological properties of the final product is still not conclusive. Moreover, the full potential of PRP indications and applications has yet to be determined. Until recently, PRP has been commercially marketed as an autologous blood-derived product potentially offering physicians the ability to use autologous platelet growth factor technologies in specific indicated pathologies and disorders. Originally, the frequently cited sole criterion for successful PRP applications was a prepared specimen with a platelet concentration above the whole blood value.

REFERENCES:

1.Collins, T., Alexander, D., & Barkatali, B. (2021). Platelet-rich plasma: a narrative review. *EFORT Open Reviews*, *6*(4), 225.

2.Lin KY, Yang CC, Hsu CJ, et al. Intra-articular injection of platelet-rich plasma is superior to hyaluronic acid or saline solution in the treatment of mild to moderate knee osteoarthritis: a randomized, double-blind, triple-parallel, placebo-controlled clinical trial. Arthroscopy. 2019;35(1):106–117. doi:10.1016/j. arthro. 2018.06.035

3.Guillibert C, Charpin C, Raffray M, et al. Single injection of high volume of autologous pure PRP provides a significant improvement in knee osteoarthritis: a prospective routine care study. *Int J Mol Sci*. 2019;20:6. doi:10.3390/ijms20061327

4.Phillips, M., Bhandari, M., Grant, J., Bedi, A., Trojian, T., Johnson, A., & Schemitsch, E. (2021). A systematic review of current clinical practice guidelines on intra-articular hyaluronic acid, corticosteroid, and platelet-rich plasma injection for knee osteoarthritis: an international perspective. *Orthopaedic Journal of Sports Medicine*, *9*(8), 23259671211030272.

5.Sheth, U., Dwyer, T., Smith, I., Wasserstein, D., Theodoropoulos, J., Takhar, S., & Chahal, J. (2018). Does platelet-rich plasma lead to earlier return to sport when compared with conservative treatment in acute muscle injuries? A systematic review and meta-analysis. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, *34*(1), 281-288.

6.Zhao, J., Huang, H., Liang, G., Zeng, L. F., Yang, W., & Liu, J. (2020). Effects and safety of the combination of platelet-rich plasma (PRP) and hyaluronic acid (HA) in the treatment of knee osteoarthritis: a systematic review and meta-analysis. *BMC Musculoskeletal Disorders*, *21*(1),
7.Meheux, C. J., McCulloch, P. C., Lintner, D. M., Varner, K. E., & Harris, J. D. (2016). Efficacy of intra-articular platelet-rich plasma injections in knee osteoarthritis: a systematic review. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, *32*(3), 495-505.
8.Knightly, N., Lee, C., O'Brien, L., Qayyum, T., Hurley, C., & Kelly, J. (2023). Role for platelet rich plasma as an adjuvant therapy in wound healing and burns. *European Journal of Plastic Surgery*, 1-10.
9.Hegab, A. F., Abd Al Hameed, H. I., Hassaneen, A. M., & Hyder, A. (2023). Synergistic effect of platelet rich plasma with hyaluronic acid injection following arthrocentesis to reduce pain and improve function in TMJ osteoarthritis. *Journal of Stomatology, Oral and Maxillofacial Surgery*, *124*(1), 101340.
10.Fadadu, P. P., Mazzola, A. J., Hunter, C. W., & Davis, T. T. (2019). Review of concentration yields in commercially available platelet-rich plasma (PRP) systems: a call for PRP standardization. *Regional Anesthesia & Pain Medicine*, *44*(6), 652-659.
11.Zhang, C., Cai, Y. Z., & Wang, Y. (2022). Injection of leukocyte-poor platelet-rich plasma for moderate-to-large rotator cuff tears does not improve clinical outcomes but reduces retear rates and fatty infiltration: a prospective, single-blinded randomized study. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, *38*(8), 2381-2388.
12.Saqlain, N., Mazher, N., Fateen, T., & Siddique, A. (2023). Comparison of single and double centrifugation methods for preparation of Platelet-Rich Plasma (PRP). *Pakistan Journal of Medical Sciences*, *39*(3), 634.
13.Magalon, J., Chateau, A. L., Bertrand, B., Louis, M. L., Silvestre, A., Giraudo, L., ... & Sabatier, F. (2016). DEPA classification: a proposal for standardising PRP use and a retrospective application of available devices. *BMJ open sport & exercise medicine*, *2*(1), e000060.
14.Lana, J. F. S. D., Purita, J., Paulus, C., Huber, S. C., Rodrigues, B. L., Rodrigues, A. A., ... & Annichino-Bizzacchi, J. M. (2017). Contributions for classification of platelet rich plasma–proposal of a new classification: MARSPILL. *Regenerative Medicine*, *12*(5), 565-574.
15.Rossi, L. A., Murray, I. R., Chu, C. R., Muschler, G. F., Rodeo, S. A., & Piuzzi, N. S. (2019). Classification systems for platelet-rich plasma. *The bone & joint journal*, *101*(8), 891-896.
16.Ryan, J., Imbergamo, C., Sudah, S., Kirchner, G., Greenberg, P., Monica, J., & Gatt, C. (2021). Platelet-rich product supplementation in rotator cuff repair reduces retear rates and improves clinical outcomes: a meta-analysis of randomized controlled trials. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, *37*(8), 2608-2624.
17.Oh, J. H., Kim, W. O. O., Park, K. U., & Roh, Y. H. (2015). Comparison of the cellular composition and cytokine-release kinetics of various platelet-rich plasma preparations. *The American journal of sports medicine*, *43*(12), 3062-3070.
18.Beitia, M., Delgado, D., Mercader, J., Sánchez, P., López de Dicastillo, L., & Sánchez, M. (2023). Action of platelet-rich plasma on in vitro cellular bioactivity: more than platelets. *International Journal of Molecular Sciences*, *24*(6), 5367.
19.Gentile, P., & Garcovich, S. (2020). Systematic review—The potential implications of different platelet-rich plasma (Prp) concentrations in regenerative medicine for tissue repair. *International journal of molecular sciences*, *21*(16), 5702.
20.Hersant, B., Sid-Ahmed, M., Braud, L., Jourdan, M., Baba-Amer, Y., Meningaud, J. P., & Rodriguez, A. M. (2019). Platelet-rich plasma improves the wound healing potential of mesenchymal stem cells through paracrine and metabolism alterations. *Stem cells international*, *2019*.
21.Everts, P., Onishi, K., Jayaram, P., Lana, J. F., & Mautner, K. (2020). Platelet-rich plasma: new performance understandings and therapeutic considerations in 2020. *International journal of molecular sciences*, *21*(20), 7794.

22. Chalidis, B., Givissis, P., Papadopoulos, P., & Pitsilos, C. (2023). Molecular and Biologic Effects of Platelet-Rich Plasma (PRP) in Ligament and Tendon Healing and Regeneration: A Systematic Review. *International Journal of Molecular Sciences*, *24*(3), 2744.