

Platelet-Rich Plasma Differs According to Preparation Method and Human Variability

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Background: Varying concentrations of blood components in platelet-rich plasma preparations may contribute to the variable results seen in recently published clinical studies. The purposes of this investigation were (1) to quantify the level of platelets, growth factors, red blood cells, and white blood cells in so-called one-step (clinically used commercial devices) and two-step separation systems and (2) to determine the influence of three separate blood draws on the resulting components of platelet-rich plasma.

Methods: Three different platelet-rich plasma (PRP) separation methods (on blood samples from eight subjects with a mean age [and standard deviation] of 31.6 ± 10.9 years) were used: two single-spin processes (PRP_{LP} and PRP_{HP}) and a double-spin process (PRP_{DS}) were evaluated for concentrations of platelets, red and white blood cells, and growth factors. Additionally, the effect of three repetitive blood draws on platelet-rich plasma components was evaluated.

Results: The content and concentrations of platelets, white blood cells, and growth factors for each method of separation differed significantly. All separation techniques resulted in a significant increase in platelet concentration compared with native blood. Platelet and white blood-cell concentrations of the PRP_{HP} procedure were significantly higher than platelet and white blood-cell concentrations produced by the so-called single-step PRP_{LP} and the so-called two-step PRP_{DS} procedures, although significant differences between PRP_{LP} and PRP_{DS} were not observed. Comparing the results of the three blood draws with regard to the reliability of platelet number and cell counts, wide variations of intra-individual numbers were observed.

Conclusions: Single-step procedures are capable of producing sufficient amounts of platelets for clinical usage. Within the evaluated procedures, platelet numbers and numbers of white blood cells differ significantly. The intra-individual results of platelet-rich plasma separations showed wide variations in platelet and cell numbers as well as levels of growth factors regardless of separation method.

Clinical Relevance: The variability of components and its effects on dosage should be considered in single or consecutive treatments of platelet-rich plasma. Significant differences in components were observed in different separation methods and may have specific results on treated tissue.

Over the past decade, platelet-rich plasma has gained increased attention in orthopaedic sports medicine. Several investigators have advocated the use of platelet-rich plasma in the management of bone, muscle, tendon, and cartilage injury¹⁻³. Platelets contain growth factors in their alpha-granules, such as transforming growth factor-beta (TGF- β), fibroblast growth factor-2 (FGF-2), platelet-derived growth factors (PDGF-AB), insulin-like growth factor-1 (IGF-1), epi-

dermal growth factor (EGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF-A), which are thought to produce beneficial effects on the healing process. The ultimate goal of platelet-rich plasma treatment is to concentrate these growth factors and reintroduce them to a site of injury¹.

Despite the growing popularity of platelet-rich plasma treatment, little is known regarding the specifics of plasma preparations or the devices used in their production¹⁻³. Platelet-

Disclosure: One or more of the authors received payments or services, either directly or indirectly (i.e., via his or her institution), from a third party in support of an aspect of this work. In addition, one or more of the authors, or his or her institution, has had a financial relationship, in the thirty-six months prior to submission of this work, with an entity in the biomedical arena that could be perceived to influence or have the potential to influence what is written in this work. No author has had any other relationships, or has engaged in any other activities, that could be perceived to influence or have the potential to influence what is written in this work. The complete **Disclosures of Potential Conflicts of Interest** submitted by authors are always provided with the online version of the article.

rich plasma is a generic term referring to any sample of autologous plasma with platelet concentrations above baseline blood values². Defining platelet-rich plasma preparations according to platelet count can be difficult, as consensus on adequate concentration is lacking. Platelet concentrations of 200×10^3 platelets/ μL up to 1000×10^3 platelets/ μL are considered therapeutic for tissue-healing, whereas much higher counts appear to be biologically unfavorable^{1,4-6}. Describing platelet-rich plasma according to the method of production may be more appropriate as either one or two-step centrifugation processes are used to fraction whole blood and concentrate the platelets^{1,3,7}.

Currently, there are several commercial devices available for the preparation of platelet-rich plasma. While their common purpose is to fraction whole blood into its composite parts to allow isolation of plasma with elevated levels of platelets, each device functions differently. Specifically, these devices and their protocols differ in their method of isolation (one or two-step centrifugation), type and operation of the collecting tube, speed of the centrifuge, and other processes of production, which result in plasma preparations with varying volumes, platelet numbers, quantities of growth factors, and concentrations of residual white and red blood cells^{1,3,7,8}.

Variability in the cellular composition of platelet-rich plasma preparations can create methodological challenges for investigators^{1,3,7}. Results obtained from both in vitro and in vivo studies utilizing platelet-rich plasma can be difficult to interpret if cell type and quantity of the plasma preparations are inconsistent. Despite its importance, research to quantify differences in separation methods is limited. Recently, Castillo et al. compared cell concentrations in platelet-rich plasma preparations from three different single-step separation methods (MTF Cascade, Arteriocyte Magellan, and Biomet GPS III)⁹. While concentrations of platelets, red blood cells, fibrinogen levels, and active TGF- β 1 remained consistent, substantial differences in the amount of VEGF-A and PDGFs as well as the amount and concentration of white blood cells were observed⁹. Considering the concentration-dependent function of white blood cells to be either beneficial or harmful in platelet-rich plasma treatments, it is critical to define the differences in the cellular characteristics of platelet preparations¹⁰⁻¹².

To elucidate differences between methods of procurement of platelet-rich plasma, laboratory investigation conducted in a clinically applicable manner is needed. Historically, basic-science investigation has utilized automatic cell separation to produce platelet-rich plasma¹. While valid, this method does not reflect a clinical environment in which centrifuge devices are used and platelet-rich plasma treatment is sometimes administered in repeated doses over a given period of time. These conditions raise questions over the consistency of platelet preparations with regard to methods of separation and within-individual consistency of preparations with repeated blood draws¹.

Our hypotheses were that (1) despite variability in specific methods, so-called one-step separation methods will result in comparable preparations of platelet-rich plasma with regard to platelet, growth factor, and red and white blood-cell concentrations, and (2) inconsistent blood cell counts and

concentrations will be seen with each system following repeated blood draws. Two major study goals developed for these hypotheses were (1) to quantify the level of platelets, growth factors, red blood cells, and white blood cells in one-step (clinically used commercial devices) and two-step separation systems (literature-based platelet-rich plasma method) and (2) to determine the influence of three separate blood draws in terms of within-subject differences on the resulting components of platelet-rich plasma.

Materials and Methods

Subjects

Blood samples were obtained from eight healthy subjects (two female and six male subjects with a mean age [and standard deviation] of 31.6 ± 10.9 years) as part of an investigative study examining various properties of platelet-rich plasma. Institutional review board approval was obtained. Inclusion criteria included healthy subjects between the ages of eighteen and sixty-five years without known blood dyscrasia. Exclusion criteria included a medical history of blood-derived illness or any medication known to affect platelet or bone marrow function or concentration for a minimum of two weeks prior to testing.

Platelet-Rich Plasma Preparation

Approximately 125 mL of peripheral blood was drawn from each subject at three different time points (zero, fourteen, and thirty days) to allow sufficient platelet recovery. A 60-mL syringe prefilled with 5 mL of acid citrate dextrose (ACD-A) was used for the standardized blood draw. ACD-A binds calcium and prevents blood clotting with no known interference to platelet function. Blood was then transferred directly to each of the three different separation systems. As separation methods, a single-spin method that would be expected to result in platelet-rich plasma (PRP) with a lower platelet and white blood-cell number (PRP_{LP}), an alternative system expected to result in a high amount of platelets and high number of white blood cells (PRP_{HP}), and a double-spin method (PRP_{DS}) were chosen to represent an overall survey of the techniques clinically available.

PRP_{LP}

The Arthrex ACP Double Syringe (Arthrex, Naples, Florida) was used for production of autologous conditioned plasma. Ten milliliters of blood was filled into the double syringe to produce 3 mL of PRP_{LP}. Syringes were centrifuged at 1500 rpm for five minutes. This separated the erythrocytes from the remaining plasma components. The top portion of plasma was drawn up with use of the inner syringe without disruption of the erythrocyte layer.

PRP_{HP}

The GPS III Platelet Concentrate System (Biomet, Warsaw, Indiana) was used to produce approximately 3 mL of PRP_{HP} out of 27 mL of blood. The tubes were centrifuged for fifteen minutes at 3200 rpm according to the manufacturer's protocol. With the specific construction of the tubes, it was possible to draw the portion of platelet-rich plasma into a 3-mL syringe according to the manufacturer's instructions.

PRP_{DS}

A literature-based double-spin method was utilized to fractionate whole blood¹³. After a first centrifugation of 1500 rpm for five minutes, the top layer of plasma was separated and centrifuged a second time (twenty minutes at 6300 rpm). Finally, half of the superficial plasma layer was removed, and the platelet pellet was suspended in the remaining half of the plasma volume.

Platelet Concentration and Number of Blood Cells

A 1-mL sample of each platelet-rich plasma preparation and each native blood specimen were analyzed by the clinical core laboratory at the University of Connecticut Health Center. The platelet concentration, number of red blood cells,

TABLE I Concentration of White Blood Cells*

	Lymphocytes ($10^3/\mu\text{L}$)	Neutrophils ($10^3/\mu\text{L}$)	Eosinophils ($10^3/\mu\text{L}$)	Basophils ($10^3/\mu\text{L}$)	Monocytes ($10^3/\mu\text{L}$)
Blood	1.77 ± 0.55	3.19 ± 1.2	0.14 ± 0.07	0.02 ± 0.01	0.35 ± 0.08
PRP _{LP}	0.5 ± 0.3	0.06 ± 0.03	0.01 ± 0.00	0.01 ± 0.0	0.06 ± 0.04
PRP _{HP}	8.29 ± 3.54	8.71 ± 3.86	0.15 ± 0.18	0.90 ± 2.94	1.66 ± 0.97
PRP _{DS}	0.48 ± 0.26	0.06 ± 0.03	0.00 ± 0.00	0.01 ± 0.01	0.05 ± 0.04

*The values are given as the mean and the standard deviation. PRP_{LP} = platelet-rich plasma prepared with single-spin method resulting in lower number of white blood cells and platelets, PRP_{HP} = alternative method resulting in a high amount of white blood cells and platelets, and PRP_{DS} = double-spin method.

and white blood-cell differentiation was determined by blood cell count (Gen-S System 2 Hematology Analyzer; Coulter, Miami, Florida)¹⁴. Linearity is $10 - 1000 \times 10^3/\mu\text{L}$ for platelet count, $0.3 - 7.0 \times 10^6/\mu\text{L}$ for red blood-cell count, and $0.1 - 100 \times 10^3/\mu\text{L}$ for white blood-cell count, respectively (data provided by the University of Connecticut Health Center blood laboratory).

Growth Factor Concentration

Enzyme-linked immunosorbent assay (ELISA) in duplicate aliquots with the Quantikine Human Immunoassay kits (R&D Systems, Minneapolis, Minnesota) were used to quantify the growth factor concentration of each

platelet-rich plasma preparation and native blood sample. Samples for each were immediately frozen (-20°) to preserve growth factor integrity, stored for less than twenty-eight days, and thawed on ice (one freeze-thaw cycle) before the ELISA assays were performed. Due to the high costs of the multiple ELISA assays, only quantification for all products of all subjects at the time point of the first blood draw was feasible. All assays had in common the employment of the quantitative sandwich enzyme immunoassay technique.

The growth factors EGF, FGF-2, HGF, IGF-1, PDGF-AB, TGF- β 1, and VEGF-A were chosen for analysis because of their specific roles in tissue-healing

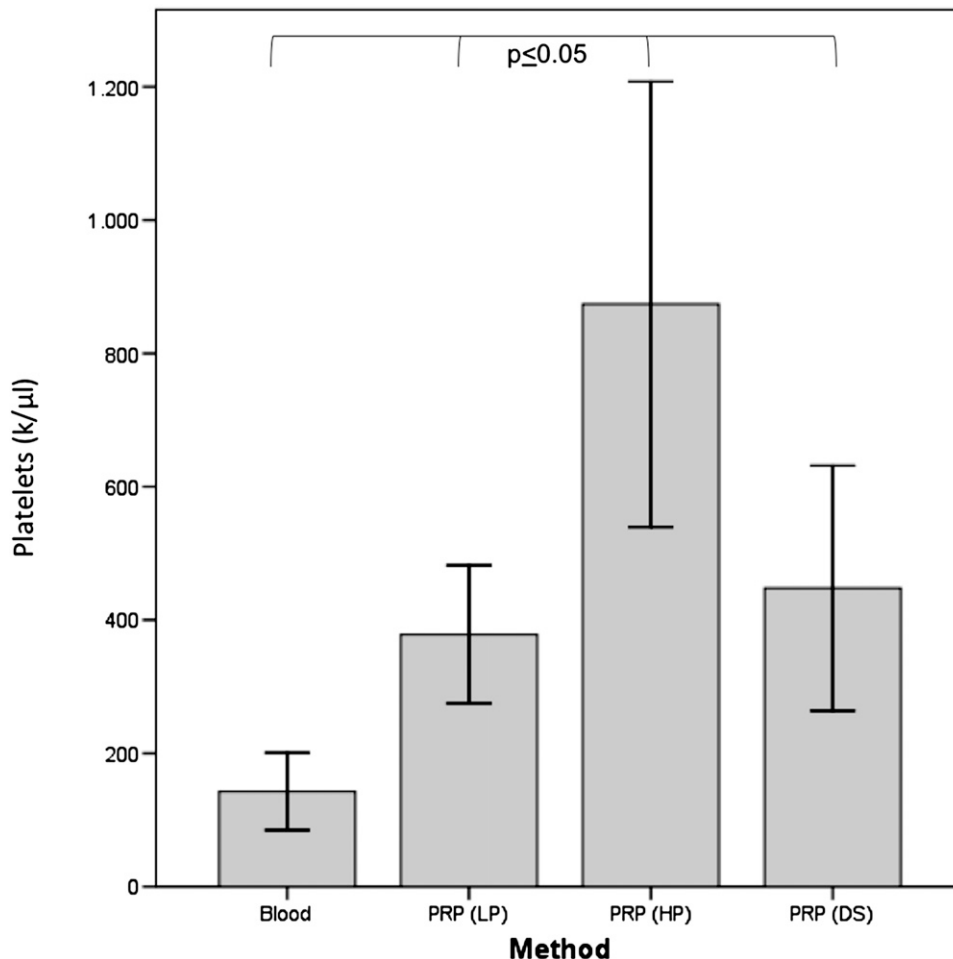


Fig. 1
Platelet concentration according to separation method. The values are given as the mean and the standard deviation. PRP(LP) = platelet-rich plasma prepared with single-spin method resulting in lower number of white blood cells and platelets, PRP(HP) = alternative method resulting in a high amount of white blood cells and platelets, and PRP(DS) = double-spin method.

TABLE II Reliability for Repetitive Blood Draws

Sex, Age of Subjects (yr)	Blood* ($10^3/\mu\text{L}$)	PRP _{LP} * ($10^3/\mu\text{L}$)	PRP _{HP} * ($10^3/\mu\text{L}$)	PRP _{DS} * ($10^3/\mu\text{L}$)
F, 22	77.0 ± 20.55	265.0 ± 34.60	649.3 ± 340.74	403.7 ± 52.77
M, 22	99.4 ± 28.14	275.7 ± 41.97	578.3 ± 137.25	397.7 ± 209.55
M, 23	133.8 ± 40.00	432.3 ± 31.47	1004.3 ± 319.06	328.0 ± 260.86
M, 24	144.8 ± 34.12	439.7 ± 20.31	607.0 ± 64.09	474.0 ± 165.95
M, 30	128.7 ± 53.40	296.0 ± 95.57	733.3 ± 59.68	380.7 ± 116.10
M, 33	193.6 ± 89.31	376.0 ± 142.79	1077.7 ± 531.14	472.7 ± 290.15
M, 48	178.6 ± 16.91	500.7 ± 43.66	1283.7 ± 138.16	580.7 ± 172.15
F, 50	185.5 ± 72.78	441.3 ± 58.77	1056.7 ± 72.45	544.3 ± 202.46
Overall	142.68 ± 44.40	378.3 ± 58.64	873.8 ± 207.82	447.7 ± 183.75
α	0.75	0.44	-0.84	0.10

*The values are given as the mean platelet concentration (and standard deviation) of three repetitive blood draws (zero, fourteen, and thirty days) for each subject and overall (n = 8). Overall intrasubject variability was calculated with Cronbach α for standardized items for each product. PRP_{LP} = platelet-rich plasma prepared with single-spin method resulting in lower number of white blood cells and platelets, PRP_{HP} = alternative method resulting in a high amount of white blood cells and platelets, and PRP_{DS} = double-spin method.

and regeneration^{1,7}. Human EGF was measured with a 3.5 to 4.5-hour solid phase ELISA containing *Escherichia coli*-derived recombinant human EGF and antibodies raised against the recombinant factor. The FGF basic (FGF-2) kit

contained recombinant human FGF-2 and antibodies raised against the recombinant factor (4.5-hour solid phase). The 4.25 to 4.5-hour HGF ELISA contains Sf 21-expressed recombinant human pro-HGF and antibodies raised

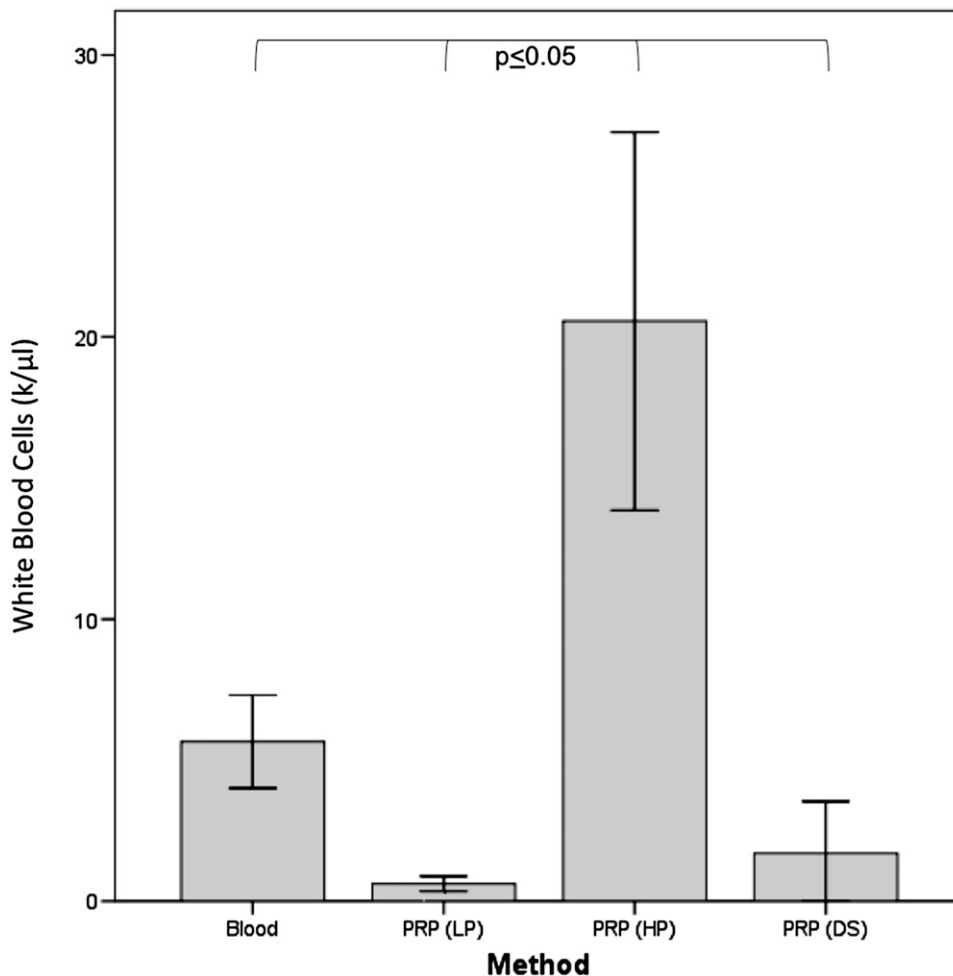


Fig. 2
White blood-cell concentration according to separation method. The values are given as the mean and the standard deviation. PRP(LP) = platelet-rich plasma prepared with single-spin method resulting in lower number of white blood cells and platelets, PRP(HP) = alternative method resulting in a high amount of white blood cells and platelets, and PRP(DS) = double-spin method.

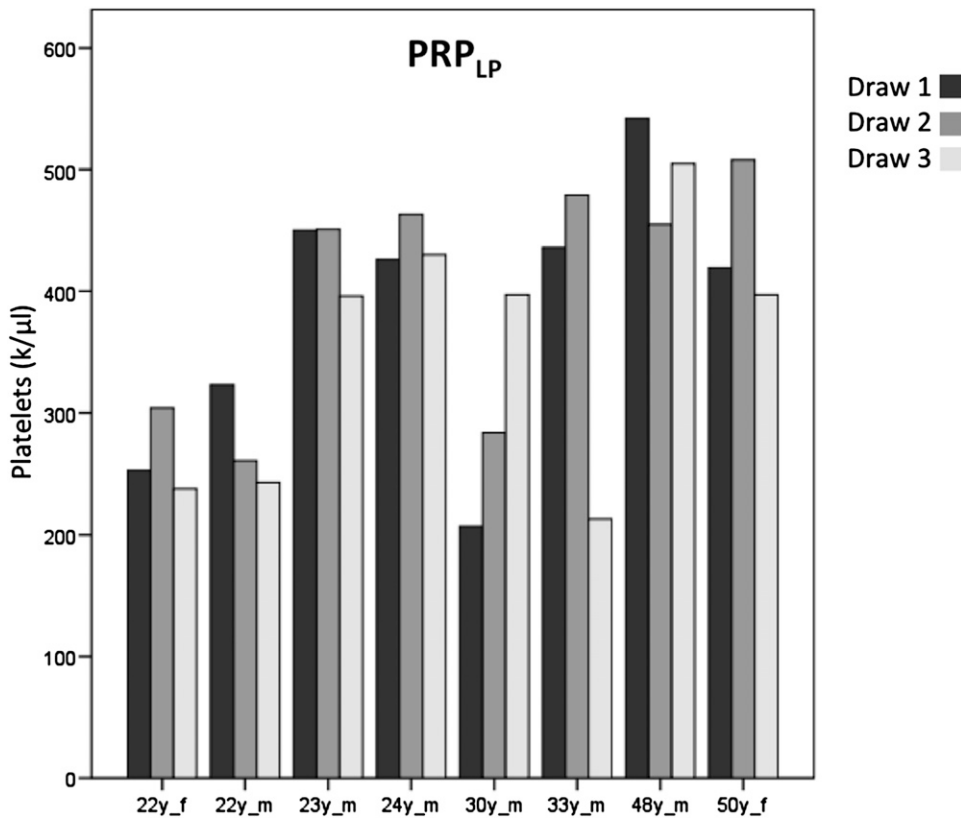


Fig. 3-A

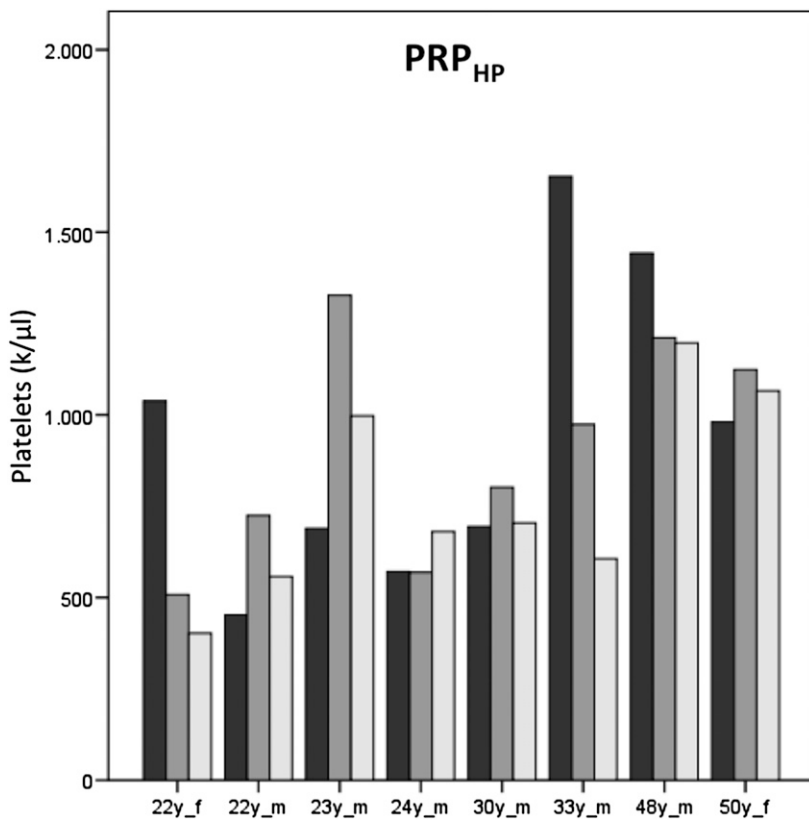


Fig. 3-B

Draw 1 ■
 Draw 2 ■
 Draw 3 ■

The concentration of platelets after repetitive blood draws according to subject and group, including PRP_{LP} (Fig. 3-A), PRP_{HP} (Fig. 3-B), and PRP_{DS} (Fig. 3-C). PRP_{LP} = platelet-rich plasma prepared with single-spin method resulting in lower number of white blood cells and platelets, PRP_{HP} = alternative method resulting in a high amount of white blood cells and platelets, and PRP_{DS} = double-spin method.

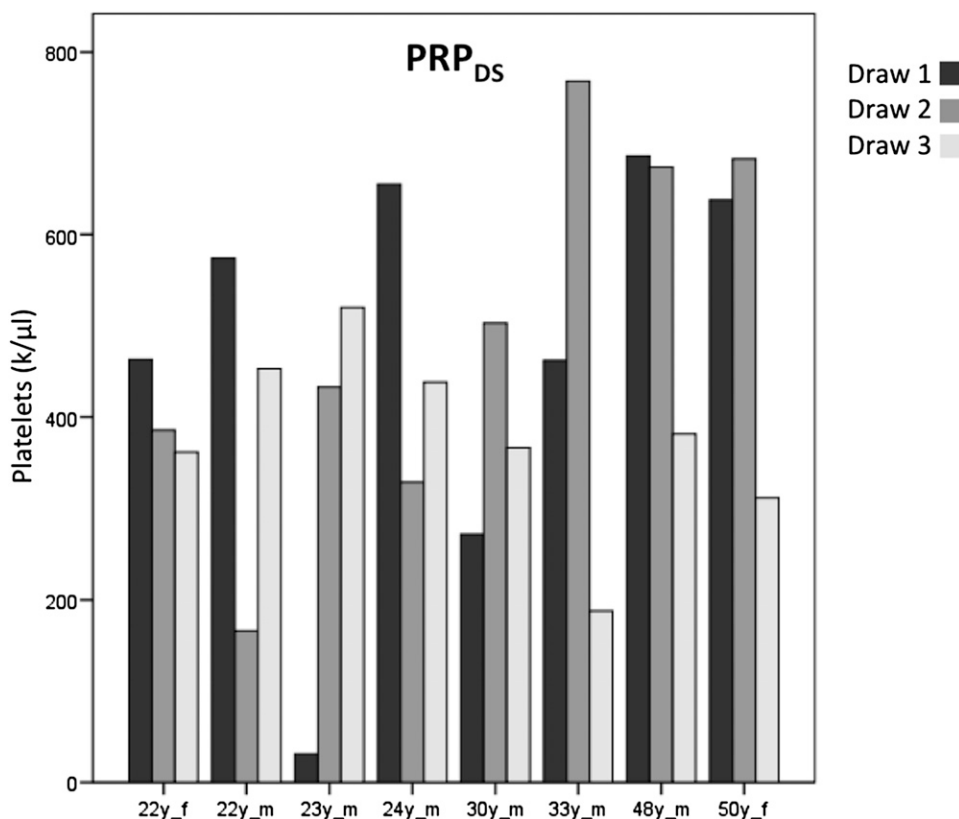


Fig. 3-C

against the recombinant factor. The Quantikine Human IGF-1 Immunoassay is a 3.5-hour solid-phase ELISA containing *Escherichia coli*-expressed recombinant human IGF-1. PDGF-AB was determined by a 4.5-hour solid-phase ELISA containing *Escherichia coli*-expressed recombinant human PDGF-AB. The TGF-β1 assay contained recombinant human TGF-β1 expressed by CHO (Chinese hamster ovary) cells and used a 4.5-hour solid-phase ELISA. Finally, the human VEGF kit contained Sf21-expressed recombinant human VEGF₁₆₅ and antibodies raised against the recombinant protein to measure VEGF₁₆₅ in a 4.5-hour solid-phase ELISA.

Statistical Analysis

Data were analyzed with SPSS software (version 15.0; SPSS, Chicago, Illinois). The Kolmogorov-Smirnov and Shapiro-Wilk tests were performed for each variable to identify non-normal distributions. Since none of the variables showed normative distribution ($p \leq 0.05$), the nonparametric Kruskal-Wallis test was used to compare group means. Comparisons with a significant difference in means were followed by post hoc tests (Tamhane T2). A p value of ≤ 0.05 was used to determine significance. For comparison of the variability of repetitive blood draws, means and standard deviations were determined for each subject and overall. Additionally, Cronbach α was calculated as a measure of reliability with the intraclass correlation measurement for two-way random average measurements ($\alpha \geq 0.7$ was regarded as reliable). This was used as a measure of the internal consistency and/or reliability of a repetitive measurement in multiple subjects.

Source of Funding

The University of Connecticut Health Center-New England Musculoskeletal Institute has received direct funding and material support for this study by Arthrex (Naples, Florida). The company had no influence on study design, data collection, or interpretation of the results.

Results

Platelet Concentration

With regard to the total number of platelets, all separation systems produced a significantly increased platelet number compared with native blood ($142.7 \pm 44.40 \times 10^3/\mu\text{L}$). The PRP_{HP} ($873.8 \pm 207.82 \times 10^3/\mu\text{L}$) also showed a significantly higher number of platelets compared with PRP_{LP} ($378.3 \pm 58.64 \times 10^3/\mu\text{L}$) or PRP_{DS} ($447.7 \pm 183.7 \times 10^3/\mu\text{L}$) ($p \leq 0.05$). No significant difference in platelet number was seen when PRP_{LP} was compared with PRP_{DS} ($p = 0.52$) (Fig. 1).

Red Blood-Cell Concentration

Overall, the highest level of red blood cells was in native blood ($4.1 \pm 0.4 \times 10^6/\mu\text{L}$). This was significantly different from all of the other separations. The PRP_{LP} ($0.2 \pm 0.1 \times 10^6/\mu\text{L}$) and the PRP_{DS} ($0.02 \pm 0.04 \times 10^6/\mu\text{L}$) were not significantly different compared with each other. However, both the PRP_{LP} and the PRP_{DS} group had significantly fewer red blood cells compared with the PRP_{HP} group ($1.0 \pm 1.4 \times 10^6/\mu\text{L}$).

White Blood-Cell Concentration

There were significantly different amounts of white blood cells in all four separations compared with each other ($p \leq 0.05$). The PRP_{HP} ($20.5 \pm 6.7 \times 10^3/\mu\text{L}$) showed the highest amount, whereas the PRP_{LP} ($0.6 \pm 0.3 \times 10^3/\mu\text{L}$) system showed the fewest amounts of white blood cells. The PRP_{DS} contained

TABLE III Growth Factor Concentration Compared Between Separation Methods

Growth Factor*	PRP _{LP} † (pg/mL)	PRP _{HP} † (pg/mL)	PRP _{DS} † (pg/mL)
EGF	659.8 ± 35.9	2639.5 ± 197.7	670.7 ± 185.1
FGF-2	15.6 ± 2.4	75.2 ± 21.4	15.2 ± 3.4
HGF	645.2 ± 72.1	4277.3 ± 1508.2	581.7 ± 43.2
IGF	64.8 ± 55.4	672.9 ± 378.4	45.1 ± 60.7
PDGF	16,668.1 ± 5512.3	42,273.9 ± 2902.4	12,263.7 ± 3632.7
TGF-β	66,246.2 ± 7620.4	141,286.9 ± 12,576.1	83,011.7 ± 14,129.8
VEGF	138.7 ± 11.2	142.9 ± 12.5	138.7 ± 9.1

*EGF = epidermal growth factor, FGF-2 = fibroblast growth factor, HGF = hepatocyte growth factor, IGF = insulin-like growth factor, TGF-β = transforming growth factor-beta, and VEGF = vascular endothelial growth factor. †The values are given as the mean and the standard deviation. PRP_{LP} = platelet-rich plasma prepared with single-spin method resulting in lower number of white blood cells and platelets, PRP_{HP} = alternative method resulting in a high amount of white blood cells and platelets, and PRP_{DS} = double-spin method.

fewer white blood cells ($1.7 \pm 1.8 \times 10^3/\mu\text{L}$) than native blood ($5.6 \pm 1.7 \times 10^3/\mu\text{L}$) (Fig. 2).

White Blood-Cell Distribution

The distribution of the white blood cells according to separation methods is shown in Table I. All cell types showed significant differences compared with each other ($p \leq 0.05$), except for PRP_{LP} and PRP_{DS}, which showed no significant difference between any cell types. No significant differences were observed when numbers of eosinophils isolated from the PRP_{HP} were compared with whole blood.

Influence of Draw Repetition on Platelet Count

Table II and Figures 3-A, 3-B, and 3-C show the intrasubject and intersubject variability of platelet concentrations according to different blood draws.

Growth Factors

Results for each growth factor are shown in Table III. The single-spin method (PRP_{LP}) had a significantly higher concentration of the factors HGF, IGF-1 and PDGF-AB in comparison with the double-spin method (PRP_{DS}), whereas the PRP_{HP} separation produced significantly more growth factors compared with the other separations, with the exception of VEGF-A ($p \leq 0.05$). The PRP_{LP} separation method released significantly more HGF, IGF-1, and PDGF-AB compared with the PRP_{DS} separation ($p \leq 0.05$).

Discussion

This study was designed to explore differences between methods of procurement and administration for platelet-rich plasma. We observed differing amounts of platelets, white blood cells, and growth factors with each method of separation as well as wide intrasubject variation in cell type and count with repetitive blood draws. However, as anticipated, all separation techniques resulted in a significant increase in platelet concentration compared with native blood¹². Platelet concentrations in the PRP_{HP} procedure were significantly higher than platelet concentrations in the single-step PRP_{LP} and the two-step PRP_{DS}

procedures; however, significant differences between PRP_{LP} and PRP_{DS} were not observed. While these data assist in determining the extent to which devices and repetitive blood draws influence the type and number of cells in platelet-rich plasma preparation, it does not provide proof of clinical efficacy. In this regard, the results observed in the present study between methods of procurement and repetitive blood draws are discussed with respect to previously published laboratory research on plasma preparations.

Previous investigators have defined platelet-rich plasma according to platelet concentration. Marx required plasma preparations to have a platelet concentration of $1000 \times 10^3/\mu\text{L}$ to be considered therapeutic platelet-rich plasma⁴. In the present study, the PRP_{HP} resulted in the highest amounts of platelets ($873.8 \pm 207.82 \times 10^3/\mu\text{L}$), which approximately meets this criterion. Other investigators have reported lower platelet concentrations for therapeutic platelet-rich plasma. On the basis of the current use of centrifuges and plasma isolation procedures and the undetermined and unclear effect of platelets on targeted tissue, Mazzucco et al., in 2009, defined platelet numbers of $>200 \times 10^3/\mu\text{L}$ as sufficient for a therapeutic effect⁶. This definition seems reasonable, as platelet concentrations of approximately 2.5 times greater than native blood ($142.7 \pm 57.9 \times 10^3/\mu\text{L}$) have positive effects on osteoblasts and fibroblasts in vitro. Additionally, adverse events have been observed at higher dosages (>3.5 times platelet concentration of native blood)¹⁵. While the other evaluated methods, PRP_{LP} ($378.3 \pm 58.64 \times 10^3/\mu\text{L}$) and PRP_{DS} ($447.7 \pm 183.7 \times 10^3/\mu\text{L}$), resulted in significantly lower platelet numbers than the PRP_{HP}, all methods exceeded concentrations of $>300 \times 10^3/\mu\text{L}$, thereby satisfying this criterion of $>200 \times 10^3/\mu\text{L}$.

Investigators have reported differences between one and two-step procedures¹⁶. In the present study, comparison of the two-step procedure (PRP_{DS}) did not result in significantly higher levels of platelet separation than the one-step procedures (PRP_{HP} and PRP_{LP}). These results support the effectiveness of a one-step procedure to produce comparable amounts of platelets for therapeutic application. These data may be useful to clinicians as the

increase in time required for two-step procedures may not be needed to produce therapeutic platelet-rich plasma preparations¹⁶.

Our findings differ from the recent work of Castillo et al. who reported no significant difference regarding platelet concentration among the methods evaluated (MTF Cascade, Arteriocyte Magellan, and Biomet GPS III)⁹. Mean values of platelet concentrations for the Biomet GPS III (PRP_{HP}) in our study ($873.8 \pm 207.82 \times 10^3/\mu\text{L}$) were much higher than the results reported by Castillo et al. ($566.2 \pm 292.6 \times 10^3/\mu\text{L}$)⁹. We utilized three repeated measurements to establish mean values, which resulted in concentrations closely matching the manufacturer's information (platelet numbers that were nine times that of native blood) and other published data³. Interestingly, both studies demonstrated high standard deviations, indicating significant variability in the data. While each used subjects with comparable ages, the difference in results may be due to variability within individuals in each study as well as to the number of subjects.

The quantity of white blood cells in plasma preparations is of particular interest. Amounts of white blood cells and their effect in platelet-rich plasma application have been widely discussed. Some investigators have recommended avoiding tissue exposure to white blood cells as an inflammatory reaction may occur^{3,17}. Other investigators have reported a beneficial effect of white blood cells in terms of increased antibacterial and immunological resistance^{18,19}. Additionally, white blood cells have been reported to correlate with increased growth factor release²⁰. Our results demonstrate significant differences in white blood cells by the production method, which is consistent with other investigators who have reported similar differences between production methods⁹.

The differentiation of white blood-cell types may permit estimation of their respective effects. We evaluated the type of white blood cells present in each plasma preparation. As expected, the overall number of each type of white blood cells was significantly higher with the PRP_{HP} separation method. Quantities of lymphocytes were more than quadrupled and neutrophils were more than doubled compared with native blood with this technique. While these cell types have been reported to produce negative effects on tissue-healing because of their capacity to promote inflammatory effects^{11,19}, there is no clinical evidence to support their effect on cells. Rather, these data are intended to provide clinicians with data to assist in choosing a separation method to match the intended use of platelet-rich plasma.

Growth factor analysis was also performed in the present study for all platelet-rich plasma preparations in all subjects at the time point of the first blood draw. The single-spin method (PRP_{LP}) had a significantly higher concentration of the factors HGF, IGF-1, and PDGF-AB in comparison with the double-spin method (PRP_{DS}), whereas the PRP_{HP} separation produced significantly more growth factors compared with the other separations, with the exception of VEGF-A. This increase may be due in part to higher amounts of white blood cells and/or platelets, which correlate with variable levels of growth factors in different methods of platelet-rich plasma preparations^{12,21}. Considering the results of the variability in platelet concentrations observed

for repetitive blood draws, high variability in growth factor content might also be present for platelet-rich plasma preparations separated from repetitive blood draws. Additionally, quantifying growth factors can be performed through the activation of platelets with the application of calcium chloride or thrombin to stimulate complete release, which has been shown to substantially increase the locally available growth factor content²². In the present study, platelet activation was not performed to evaluate the physiologic growth factor levels.

Comparison of our results with the data of Castillo et al. revealed that different levels of growth factors were observed for the same separation systems⁹. Considering their results were higher than other reported data, contributing factors, including mechanical forces induced during the handling process, were discussed as potential causes of increased growth factors released⁹. According to the manufacturer's data (Quantikine ELISA kits; R&D Systems), incomplete removal of platelets and blood cells may cause variable results for assays of factors contained in platelets, although data within studies are comparable. Because of red blood-cell concentration, ELISA data should only be compared within studies, and comparison with other studies can only be performed with limitations.

We observed significant variation in platelets and white blood cells with repetitive blood draws from the same individual at different times for all methods of platelet-rich plasma separation. In an effort to quantify the degree to which blood draws produced consistent amounts of blood cells, Cronbach α was calculated for all individuals. The cell counts in the native blood of all included subjects were consistent between the three blood draws (zero, fourteen, and thirty days). However, the platelet concentration of all three methods showed a high variability within the subjects for the three time points. Considering platelet-rich plasma application methods tend toward a repetitive treatment, the lack of consistency at the level of the individual is important, as reliable platelet-rich plasma dosages may be needed to produce consistent results¹.

There are several limitations of this study. The sample size (eight subjects), although consistent with other platelet-rich plasma-related reports, may have impacted the study results^{1,3,7}. Variability due to different subjects and possible alterations in production methods impact the ability to compare our results with previously published data. We attempted to evaluate representative separation methods; however, since there are multiple methods commercially available, a study comparing all of the different available platelet-rich plasma kits would be financially prohibitive. As a result, we examined the higher (PRP_{HP}) and lower (PRP_{LP}) spectrum of routine platelet-rich plasma separation methods. ■

NOTE: The authors thank Steven Delaronde, MPH, MSW, for his statistical advice.

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