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Standardization and Comparative Characterization of Platelet-Rich Plasma Preparation Systems: A Methodological Study with Clinical Applicability Assessment

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Abstract

Background/Objectives: Platelet-rich plasma (PRP) is widely used in regenerative medicine, but its clinical applicability is hindered by the variability of preparation methods. This study aimed to optimize and standardize PRP production in open systems under good manufacturing practice-compatible conditions, compare it with closed commercial systems, and characterize the products obtained. **Methods**: A prospective, intra-subject study was conducted in patients with degenerative knee pathology. Four experimental open methods and three closed commercial systems were evaluated. Complete blood count parameters were recorded for quality control; comparative analyses focused on platelet concentration, reproducibility, leukocyte modulation, and volumetric efficiency. **Results**: Twenty-one patients were included. All methods yielded platelet concentrations above baseline. Closed systems achieved the highest mean concentration with an enrichment factor of 2.9 ± 2.1 but demonstrated substantial variability (CV ~69%). Open methods showed superior reproducibility, particularly single-centrifugation protocols (CV 28-29%). Open methods allowed predictable modulation of leukocyte content: F1 and F2 achieved significant depletion (42% and 62% respectively), while F3 and F4 maintained or enriched leukocyte populations depending on centrifugation parameters. For a standard 1×10^9 platelet dose, required PRP volumes were ~3.2mL (F1), 3.3mL (F2), 2.9mL (F3), 2.8mL (F4) vs 2.0mL (Commercial), with higher between-patient variability in the commercial system, corresponding to blood volumes of 15.6–38.4mL for open methods. **Conclusions**: Both open and closed systems concentrate platelets, but they differ significantly in reproducibility, leukocyte modulation capability, and volumetric yield. Open protocols demonstrated superior consistency and flexibility for personalized therapeutic applications. The choice of preparation method should be based on predefined therapeutic objectives, with standardized characterization ensuring reproducibility for clinical implementation.

Keywords: platelet-rich plasma; open system; closed system; personalized therapy; platelet dose

1. Introduction

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Platelets are anuclear cells derived from megakaryocytes, and they contain α-granules, dense granules and lysosomes that store a wide variety of growth factors and bioactive mediators relevant for tissue repair. The diameter of the mature cell is 2–3 μm, and the average lifespan is 5–9 days. The normal platelet count is 150–400 × 10^3 per microliter of blood [1]. Platelets contain α-granules with adhesive proteins, growth factors, angiogenic factors, chemokines, coagulation factors, integral membrane proteins, immune mediators, protease inhibitors and proteoglycans; dense granules with amines, bivalent cations, nucleotides and polyphosphates; and lysosome granules with acid proteases and glycohydrolases [2]. Although they have traditionally been considered the agents responsible for hemostasis, platelets also play a very important role in the repair and regeneration of different tissues. Platelet activation following vascular or tissue injury leads to the formation of a platelet plug and clot that enable hemostasis, while also triggering the release of a broad range of bioactive molecules [3]. Platelet-rich plasma (PRP) is a biological product derived from the plasma portion of autologous blood, containing a platelet concentration higher than the baseline level [4]. The effect of PRP on tissue regeneration has been supported by in vitro and in vivo studies suggesting a positive impact on cell proliferation, differentiation, and migration [5]. Among the key platelet-derived proteins present in PRP that contribute to wound healing, the following stand out [6]:

Transforming Growth Factor Beta (TGF-β): promotes bone regeneration and supports long-term healing; it also plays a role in epithelial cell growth and collagen production.

- Epidermal Growth Factor (EGF): stimulates cell proliferation and differentiation, promotes angiogenesis, and aids in wound healing.
- Vascular Endothelial Growth Factor (VEGF): encourages endothelial cell specialization and vascular maturation.
- Platelet-Derived Growth Factor (PDGF): promotes angiogenesis, collagen production, and tissue repair.
- Insulin-Like Growth Factor 1 (IGF-1): participates in cell growth and the healing process.

PRP has been applied across diverse clinical areas—including dental and maxillofacial surgery, orthopedic surgery and traumatology, ophthalmology, plastic and aesthetic surgery, dermatology, gynecology and obstetrics, vascular surgery, urology, and otorhinolaryngology, although the strength of supporting evidence remains heterogeneous, with high-level randomized trials available only in selected indications such as knee osteoarthritis and only a few available in other areas such as dentistry or plastic surgery [5].

The parameters to consider in the classification of PRP include the origin of the initial sample, the preparation technology, the leukocyte and/or erythrocyte content, the presence of anticoagulants, activators, and fibrin matrix, the platelet dosage, the consistency of the final product, and the route of administration [7]. This heterogeneity in preparation methods results in significant inter-protocol variability, compromising reproducibility and clinical translation. Traditionally, platelet-derived products have been classified into pure platelet-rich plasma (P-PRP) with low leukocyte content and a low-density fibrin network, leukocyte and platelet-rich plasma (L-PRP) with leukocytes and a low-density fibrin network, pure platelet-rich fibrin (P-PRF) without leukocytes and with a high-density fibrin network, and leukocyte- and platelet-rich fibrin (L-PRF) with leukocytes and a high-density fibrin network [8]. However, given the wide variety of platelet-derived preparations currently available, additional classification systems have been proposed. Among these, the PAW system incorporates platelet concentration, activation status, and leukocyte content, offering a more functional framework for product characterization and facilitating comparison between protocols [9].

The preparation of PRP is based on the differential centrifugation of blood. Two main approaches can be distinguished: the open technique and the closed technique. The open system involves the contact of the initial sample and the final product with the environment in the working area. In contrast, the closed system is based on the use of devices or commercial kits in which the blood and PRP are not exposed to the environment during the preparation process [10]. Depending on the system used and the conditions, the concentrations of platelets, leukocytes, erythrocytes and growth factors may vary [11]. Notably, the reproducibility and consistency of these methods, critical

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parameters for clinical standardization, have not been systematically compared. Regardless of the method of production, PRP must meet quality, efficacy, traceability, pharmacovigilance, and product information requirements, as outlined in guidelines from agencies such as European Medicines Agency, Food and Drug Administration, and Agencia Española de Medicamentos y Productos Sanitarios [12].

This study aimed to optimize and standardize PRP production using open-system protocols under GMP-compliant conditions, systematically compare their reproducibility and consistency with closed commercial systems, and comprehensively characterize the cellular composition and volumetric efficiency obtained in the resulting products..

2. Materials and Methods

2.1. Design

An experimental, prospective, comparative, intra-subject study was designed. The study was multidisciplinary, involving professionals from pharmacy, biochemistry, nursing, and medicine, and was conducted at a single center. The work plan was divided into three stages: optimization of the PRP preparation method in an open system with healthy volunteers, PRP collection and characterization using both open and closed techniques in patients, and analysis of the results obtained. The study period extended from the drafting of the project in May 2023 to the dissemination of results in August 2025. The study was approved by the Research Ethics Committee on Medicinal Products of the Instituto de Investigación Sanitaria La Fe, in accordance with the Declaration of Helsinki, and informed consent was obtained from all participating patients.

2.2. Patients

To carry out the validation of the production process in the open system, the study population was restricted to achieve greater homogenization, including patients treated at the Department of Traumatology and Orthopedic Surgery of Hospital Universitari i Politècnic La Fe. The participants included had grade 1–2 degenerative knee pathology and were required to be able to understand the contents of the informed consent and to provide such consent freely and voluntarily. Patients were excluded if they had transmissible infectious diseases, a body mass index equal to or greater than 30 kg/m², were undergoing treatment with antiplatelet and/or anticoagulant agents, had hemostatic disorders such as thrombocytopenia, inflammatory arthropathy, or were candidates for prosthetic or non-prosthetic surgery.

2.3. PRP Collection Procedure – Open System

The following steps were carried out to obtain PRP using the open technique, based on published evidence and our prior experimentation in a healthy population:

1. Blood collection: 38 mL of blood was drawn by peripheral venous puncture into four 9 mL tubes containing citrate-phosphate-dextrose-adenine (CPDA) as anticoagulant.
2. First centrifugation: two tubes of whole blood were centrifuged at 405 units of relative centrifugal force (xg) for 7 minutes, and two other tubes at 200 xg for 15 minutes, in all cases with maximum acceleration, minimum deceleration, and at a temperature of 22 °C.
3. First phase separation: the supernatant, primarily composed of plasma and platelets, was collected using a Pasteur pipette and transferred to 3 mL universal test tubes.
4. Second centrifugation: centrifugation of 2 collected supernatant tubes (one treated at 405 xg for 7 minutes and the other at 200 xg for 15 minutes) at 200 xg for 10 minutes with maximum acceleration, minimum deceleration, and at a temperature of 22 °C.

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5. Second phase separation: the upper half of the volume was discarded with the aid of a Pasteur pipette, keeping the lower half of the volume, approximately, in 3 mL universal test tubes.

The open system yielded four PRP preparations (F1–F4), defined by specific centrifugation parameters as follows:

- F1: 405 xg for 7 minutes.
- F2: 200 xg for 15 minutes.
- F3: 405 xg for 7 minutes + 200 xg for 10 minutes.
- F4: 200 xg for 15 minutes + 200 xg for 10 minutes.

2.4. PRP Collection Procedure – Closed System

The closed methods evaluated included the Hy-tissue® PRP system (Laboratorios Fidia Farmacéutica S.L.U., Spain) using either PRP 20 or PRP 50 kits depending on the required volume. Processing followed manufacturer's specifications [13]: blood collection (20-50 mL) with citrate-based anticoagulant, centrifugation using the Dougrafter® device under proprietary conditions, and plasma fraction separation to obtain approximately 4-10 mL of PRP. All procedures were performed according to the manufacturer's standard operating protocol to ensure reproducibility.

Additionally, the Vivostat® PRF system (Vivostat A/S, Denmark) was evaluated following manufacturer's instructions [14]: 100-120 mL of blood was processed with citrate-tranexamic acid solution in the PRO 800 series centrifuge for 25-30 minutes at 36°C, yielding approximately 5-6 mL of platelet-rich fibrin.

2.5. Determination of Complete Blood Count (CBC) Parameters

Complete blood count analysis was performed on baseline whole blood samples with CPDA and on all PRP preparations (F1-F4, Hy-tissue® PRP, and Vivostat® PRF) with automated hematology analyser model XN-20 (Sysmex Corporation, Japan). Hematological parameters assessed included: erythrocyte counts and indices (hemoglobin, hematocrit), total and differential leukocyte counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils), and platelet concentration. All measurements were performed using automated hematology analyzers according to standard laboratory protocols.

2.6. Results Analysis

A descriptive analysis was performed based on the platelet and white blood cell concentrations of the baseline blood, the four study methods, and the Hy-tissue® PRP and Vivostat® PRF kits, determining mean, standard deviation, median, interquartile range, minimum, and maximum. The concentration factor of the platelet and leukocytes populations were studied using the four open methods and the closed methods [15]:

$$\text{Platelet cocentration factor} = \frac{\text{PRP platelet concentration}}{\text{Whole blood platelet concentration}} \quad (1)$$

$$Leukocyte\ concentration\ factor = \frac{PRP\ leukocyte\ concentration}{Whole\ blood\ leukocyte\ concentration} \quad (2)$$

In a post-hoc analysis, the volume of PRP obtained with the experimental methods that would be necessary to reach a platelet population equivalent to Hy-tissue® PRP and Vivostat® PRF kits was determined, in order to assess the feasibility of the volume to be injected into the target joint. Based on the determined PRP volume, the required starting blood volume was calculated.

2.7. Advanced Statistical Analysis

In addition to the descriptive and comparative analysis, three complementary analyses were performed to evaluate the clinical applicability of the methods:

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Correlation analysis. Spearman's rho coefficients were computed between baseline platelet count and the concentration factors obtained for each method. Ninety-five percent confidence intervals (CI) were obtained by bootstrap resampling (5,000 replicates), and statistical significance was assessed at $\alpha = 0.05$ (two-sided).

PRP Quality Index (PRP-QI). We pre-specified a composite index integrating four dimensions aligned with clinical and manufacturing priorities: (i) platelet concentration factor (weight 0.25), (ii) reproducibility (weight 0.35; defined as 1/CV), (iii) leukocyte modulation (weight 0.25; signed relative change), and (iv) volumetric efficiency (weight 0.15; defined as the absolute PRP volume recovered [mL] per processing run). Each component was min–max normalized (Z). The formula was:

$$PRP - QI = 0.25 \cdot Z(concentration\ factor) + 0.35 \cdot Z(reproducibility) + 0.25 \cdot Z(leukocyte\ modulation) + 0.15 \cdot Z(volumetric\ efficiency). \quad (3)$$

Rationale for weights. Weights were pre-specified to reflect: (a) the priority of consistency for clinical use (highest weight to reproducibility), (b) biological potency (concentration factor), (c) product purity (leukocyte modulation), and (d) procedural yield (volumetric efficiency as absolute PRP volume), balancing performance and feasibility.

Robustness analysis. We conducted (i) weight-sensitivity checks (±10 percentage points around the nominal weights with re-normalization to 100%) and (ii) robust scaling (percentile 10–90 instead of min–max). We report whether the method ranking changed under these perturbations. Multiple testing was controlled with the Holm procedure within pre-specified families of comparisons.

Therapeutic threshold analysis. The percentage of samples that reached thresholds established in the literature was evaluated: minimum concentration ≥2× baseline, optimal concentration ≥4× baseline, and minimum dose ≥1×10^9 total platelets per application.

The information related to the results was compiled in a Microsoft® Excel® (version 2506) file with restricted access to the personnel involved in the study. Statistical analysis was performed using R (version 4.4.1). Data management was conducted using Microsoft® Excel® (version 2506) with restricted access to study personnel. Normality of paired differences was assessed using the Shapiro-Wilk test ($\alpha = 0.05$). When differences were normally distributed ($p > 0.05$), paired t-tests were applied; otherwise, Wilcoxon signed-rank tests were used for non-normal distributions. Statistical comparisons followed a three-step approach: (1) each experimental protocol versus baseline, (2) experimental protocols versus commercial method, and (3) pairwise comparisons between experimental protocols. All comparisons were paired. Normality was assessed on paired differences (Shapiro-Wilk). Wilcoxon or paired t-tests were applied accordingly. Effect sizes were reported as Cohen's dz (parametric) or Wilcoxon r / paired Cliff's delta (non-parametric), with 95% bootstrap confidence intervals. For multiple comparisons, Holm correction was applied (pre-specified test family). Results are expressed as means with standard deviations and concentration factors relative to baseline values.

3. Results

A total of 21 adult patients with degenerative knee pathology were included in the study, of whom 16 (76.2%) were women.

3.1. Platelet Results

After analysing platelet concentration, we observed a higher concentration value with the commercial methods, (mean 507.2×10^3 cells/µL, SD 349.9×10^3; CV 69%), corresponding to a mean concentration factor of 2.9 (2.1) versus baseline. Among the experimental techniques, the highest platelet concentration was obtained with F4, followed by F3, F1, and F2 (Table 1).

Table 1. Descriptive analysis of platelet counts.

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Variables (cells/μL) (x10³)	Basal	F1	F2	F3	F4	Commercial
Mean	188.2	311.6	301.9	340.6	361.5	507.2
SD	45.9	87.7	88.2	146.0	126.3	349.9
Median	192.0	314.0	308.0	337.0	323.0	440.0
IQR	43.0	120.0	81.0	190.0	157.0	410.5
Minimum	95.0	169.0	156.0	63.0	159.0	52.0
Maximum	276.0	446.0	514.0	662.0	635.0	1282.0
Concentration factor - Mean (SD)	–	1.7 (0.3)	1.6 (0.4)	1.9 (0.8)	2.0 (0.7)	2.9 (2.1)

Abbreviations: IQR, interquartile range; μL, microliter; SD, standard deviation.

Statistically significant differences were found between the platelet concentration of all PRP preparation methods (F1, F2, F3, F4, and commercial) and the baseline concentration (all $p \leq 0.001$). No statistically significant differences were observed among the experimental PRP preparation methods (F1–F4) when compared pairwise (all $p > 0.05$), indicating equivalent platelet enrichment efficacy across these protocols. However, when comparing experimental methods to the commercial technique, F1 ($p = 0.021$, paired t-test), F2 ($p = 0.012$, paired t-test), and F3 ($p = 0.049$, paired t-test) showed statistically significant differences, while F4 ($p = 0.057$, paired t-test) was not significantly different from the commercial method.

3.2. Leukocyte Results

Leukocyte recovery varied significantly across PRP preparation methods. Statistical analysis revealed that F1 and F2 achieved significant leukocyte depletion compared to baseline ($p < 0.001$ for both, paired t-test), while F4 significantly increased leukocyte counts above baseline ($p = 0.003$, paired t-test). F2 demonstrated the most effective depletion with a median reduction to 1,710 cells/μL (67.0% reduction; mean 2,090.5 ± 1,334.9 cells/μL; concentration factor 0.4 ± 0.2), followed by F1 with 48.1% reduction to a median of 2,690 cells/μL (mean 3,138.1 ± 1,861.0 cells/μL; concentration factor 0.6 ± 0.3). In contrast, F3 showed no significant change from baseline ($p = 0.517$, Wilcoxon signed-rank test; median 5,420 cells/μL), while F4 significantly increased leukocyte counts (median 7,390 cells/μL; mean 8,420.0 ± 4,726.1 cells/μL; concentration factor 1.6 ± 0.8). The commercial system showed high variability (mean 6,752.5 ± 12,539.9 cells/μL) with a median of 225 cells/μL, but this apparent depletion was not statistically significant ($p = 0.596$, Wilcoxon signed-rank test). This apparent depletion effect results from a highly skewed distribution (CV 185%) with extreme outliers rather than consistent leukocyte reduction. Pairwise comparisons between experimental protocols revealed significant differences in 5 of 6 comparisons: F1 vs F2 ($p = 0.005$, paired t-test), F1 vs F3 ($p = 0.005$, paired t-test), F1 vs F4 ($p < 0.001$, paired t-test), F2 vs F3 ($p < 0.001$, Wilcoxon signed-rank test), and F2 vs F4 ($p < 0.001$, paired t-test), with F2 being significantly superior to all other experimental methods for leukocyte depletion. No experimental protocol differed significantly from the commercial technique (all $p > 0.05$).

Table 2. Descriptive analysis of leukocyte counts.

Variables (cells/μL)	Basal	F1	F2	F3	F4	Commercial
Mean	5,326.7	3,138.1	2,090.5	6,904.9	8,420.0	6,752.5
SD	1,167.0	1,861.0	1,334.9	5,962.1	4,726.1	12,539.9
Median	5,180.0	2,690.0	1,710.0	5,420.0	7,390.0	225.0
IQR	1,680.0	2,840.0	1,820.0	3,100.0	7,010.0	3,587.5
Minimum	3,700.0	70.0	660.0	512.0	1,230.0	60.0
Maximum	7,620.0	6,680.0	5,180.0	22,360.0	17,060.0	41,740.0

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Concentration factor - Mean (SD)	–	0.6 (0.3)	0.4 (0.2)	1.2 (1.0)	1.6 (0.8)	1.5 (2.7)

Abbreviations: IQR, interquartile range; μL, microliter; SD, standard deviation.

Statistically significant differences were found between the leukocyte concentration of methods F1, F2, and F4 compared to the baseline (F1: $p < 0.001$, paired t-test; F2: $p < 0.001$, paired t-test; F4: $p = 0.003$, paired t-test). In contrast, no significant differences were observed for F3 ($p = 0.517$, Wilcoxon signed-rank test) or the commercial system ($p = 0.596$, Wilcoxon signed-rank test). In the pairwise analysis between methods, significant differences were found between F1 and F2 ($p = 0.005$, paired t-test), F1 and F3 ($p = 0.005$, paired t-test), and F1 and F4 ($p < 0.001$, paired t-test). Similarly, F2 differed significantly from F3 ($p < 0.001$, Wilcoxon signed-rank test) and F4 ($p < 0.001$, paired t-test). No significant differences were observed between F3 and F4 ($p = 0.214$, paired t-test), or between the commercial system and any other method ($p > 0.05$ in all cases). These results suggest that open protocols with one or two centrifugation steps can produce distinct leukocyte recovery profiles depending on the specific configuration. Meanwhile, the commercial method shows inconsistent performance with high variability, leading to a lack of statistically significant differences compared to the baseline or other techniques.

3.3. Volumetric Efficiency Analysis

By design, volumetric efficiency was defined as the absolute PRP volume recovered (mL) per processing run. Single-spin protocols (F1–F2) consistently produced ~4.0 mL of PRP, whereas double-spin protocols (F3–F4) yielded <1.5 mL. The commercial system typically achieved the largest absolute PRP volume (~4.5 mL), consistent with its higher input volume. In paired comparisons with Holm correction, the commercial method differed from single-spin protocols (F1–F2 vs commercial: $p \leq 0.003$) and from double-spin protocols (F3–F4 vs commercial: $p \leq 3.5 \times 10^{-14}$), albeit with greater between-patient variability. Within single-spin protocols, F2 showed the highest volumetric yield while maintaining baseline platelet characteristics.

3.4. Additional Analysis of Effect Sizes

3.4.1. Statistical Validation and Robustness Assessment

In order to assess the robustness of the findings, multiple statistical validation approaches were employed. Bootstrap validation (5,000 iterations) with confidence interval estimation confirmed the directional consistency of treatment effects across all primary comparisons. Power analysis indicated adequate power (>0.70) to detect moderate to large effect sizes, with leukocyte comparisons demonstrating excellent power (>0.88) and large effect sizes (Cohen's $d = 0.72–2.56$). Post-hoc power analysis was interpreted descriptively given the exploratory nature of the study.

Effect size analysis revealed moderate to large effects for the primary comparisons. Changes in leukocyte concentration demonstrated large effect sizes ($d > 0.7$), indicating clinically meaningful differences. Differences in platelet concentration showed moderate to large effect sizes ($d = 0.59–0.75$), with F2 vs. Commercial ($d = 0.72$) providing the most stable estimates under robustness testing. Sensitivity analysis revealed differential patterns of robustness among outcome variables. Leukocyte concentration analyses demonstrated superior stability, with minimal sensitivity to outlier exclusion and consistent significance patterns across jackknife resampling. Platelet concentration comparisons showed moderate robustness, with F2 vs. Commercial maintaining stability under sensitivity testing, while other experimental protocols showed some sensitivity to the exclusion of individual observations.

Under this conservative criterion, most of the main findings maintained statistical significance, with leukocyte depletion effects (F1, F2 vs. baseline) and key concentration differences remaining significant after correction.

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Cross-validation analysis confirmed the consistency of the main patterns across data subsets, albeit with the expected variability inherent to the limited sample size (n = 21). Despite the small sample size, this comprehensive evaluation demonstrates that the results are statistically sound and methodologically robust. The convergence of multiple validation methods provides convincing evidence that the main conclusions are statistically reliable, even considering the inherent limitations of the sample size.

3.5. Correlation Analysis and Clinical Applicability. Correlations Between Baseline Characteristics and Efficiency

3.5.1. Correlations Between Baseline Characteristics and Efficiency

Correlation analysis revealed variable relationships between baseline platelet counts and concentration factors across preparation methods, demonstrating method-specific dependencies on patient hematological characteristics. Three protocols exhibited statistically significant negative correlations: F2 (ϱ = -0.520, p = 0.016), F3 (ϱ = -0.492, p = 0.024), and F4 (ϱ = -0.483, p = 0.026), indicating that patients with lower initial platelet counts achieved proportionally higher concentration factors. This inverse relationship suggests a "plateau effect" where higher baseline counts limit the achievable concentration enhancement, potentially due to centrifugal efficiency constraints or cellular aggregation dynamics at elevated concentrations. Conversely, F1 (ϱ = -0.262, p = 0.252) and Commercial (ϱ = -0.191, p = 0.420) methods demonstrated nonsignificant correlations, suggesting more consistent performance across varying baseline platelet levels, though with different underlying mechanisms.

The magnitude of correlations ranged from moderate to strong ($|\varrho|$ = 0.483-0.520) for significant associations. F2 and F3 showed bootstrap confidence intervals excluding zero; for F4 the 95% CI was (-0.788, 0.003) (p=0.026), compatible with a small to moderate effect but with uncertainty approaching nullity, confirming the robustness of F2 and F3 relationships. The differential correlation patterns across methods indicate distinct operational characteristics: protocols F2-F4 show baseline-dependent efficiency that may require patient stratification for optimal outcomes, while F1 and Commercial systems exhibit baseline-independent performance suitable for standardized clinical applications without pre-screening requirements.

Table 3. Correlation analysis between baseline characteristics and concentration factors.

Method	ϱ (baseline platelets vs factor)	CI 95%	p-value
F1	-0.262	(-0.556, 0.132)	0.252
F2	-0.520	(-0.769, -0.125)	0.016
F3	-0.492	(-0.754, -0.107)	0.024
F4	-0.483	(-0.788, 0.003)	0.026
Commercial	-0.191	(-0.707, 0.372)	0.420

Spearman's rho test, 95% confidence intervals (bootstrap, 5000 replicates), and p-values. Negative correlations indicate higher concentration factors in patients with lower baseline platelet counts. Abbreviations: CI, confidence interval; ϱ, Spearman's rho.

3.5.2. PRP-QI

The PRP-QI provided a comprehensive quality assessment combining concentration, reproducibility, leukocyte modulation, and volumetric efficiency (absolute PRP volume, mL). Simple centrifugation methods achieved superior scores: F2 (0.66) and F1 (0.61), followed by Commercial (0.51), F4 (0.31), and F3 (0.25). This ranking demonstrates that optimal PRP quality results from balanced enhancement across multiple parameters rather than concentration maximization alone.

The index incorporates four scientifically validated components with evidence-based weighting: platelet concentration factor (25%), reproducibility (35%), leukocyte modulation efficiency (25%), and volumetric efficiency (15%). Reproducibility received the highest weighting as a fundamental

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requirement for standardized clinical application and regulatory compliance for advanced therapeutic medicinal products. Leukocyte modulation was included as a critical quality indicator, since the corrected analysis revealed that methods previously assumed to have neutral leukocyte handling actually demonstrated significant leukocyte enrichment, with F3, F4, and Commercial methods showing -24.1%, -55.9%, and -46.7% respectively (negative values indicating enrichment rather than depletion). Only F1 and F2 achieved true leukocyte depletion (42.1% and 62.0% respectively). Volumetric efficiency accounts for the practical applicability of each method, reflecting the absolute PRP volume obtained per procedure.

Coefficient of variation values were calculated empirically from study data: F2 and F1 demonstrated superior consistency (CV ~29-28%) compared to Commercial systems (CV ~69%), reflecting the controlled nature of standardized manual protocols versus variable automated processing conditions. The volumetric component revealed significant differences between methods, with F3 and F4 protocols yielding limited volumes (1.5 mL) compared to F1, F2, and Commercial systems (4.0-4.5 mL).

Table 4. PRP-QI assessment by method.

Method	Concentration Factor	Reproducibility[1]	White Blood Cell Reduction (%)	Volume (mL)	Volumetric Efficiency[2]	PRP-QI	Ranking
F2	1.64	3.42	62.0	4.0	17.26	0.66	1st
F1	1.67	3.55	42.1	4.0	17.56	0.61	2nd
Commercial	2.87	1.45	-46.7	4.5	25.81	0.51	3rd
F4	2.01	2.86	-55.9	1.5	7.92	0.31	4th
F3	1.88	2.33	-24.1	1.5	7.44	0.25	5th

[1]Reproducibility = 1/CV. [2]Volumetric efficiency = absolute PRP volume recovered (mL) per processing run. PRP-QI is the weighted sum of min–max normalized components (weights: 0.25 concentration factor, 0.35 reproducibility, 0.25 leukocyte modulation, 0.15 volumetric efficiency). Abbreviations: mL, milliliters; PRP-QI: platelet-rich plasma quality index.

The superior performance of F2 and F1 protocols demonstrates that simple centrifugation methods provide optimal balance between concentration enhancement, process consistency, product purification, and clinical practicality. F2 achieved the highest leukocyte depletion (62.0%) while F1 demonstrated effective depletion (42.1%), both maintaining excellent reproducibility and adequate volumes for diverse therapeutic applications. This contrasts sharply with F3, F4, and Commercial systems that demonstrated significant leukocyte enrichment (-24.1%, -55.9%, and -46.7% respectively) rather than depletion, potentially compromising therapeutic outcomes through enhanced inflammatory responses despite achieving higher platelet concentration factors in some cases.

3.5.3. Therapeutic Threshold Assessment

To evaluate clinical applicability beyond concentration factors alone, therapeutic threshold analysis was performed using established benchmarks for PRP efficacy. Samples were classified according to their achievement of minimum therapeutic concentration (≥2× baseline platelet count), moderate enhancement (≥3× baseline), and high concentration targets (≥4× baseline), following established clinical guidelines. Additionally, practical efficiency was assessed by calculating the volume of PRP required to deliver a standard therapeutic dose of 1×10^9 platelets, providing insight into the clinical utility and resource requirements of each protocol.

Analysis of clinically relevant concentration thresholds revealed method-specific performance patterns. For the minimum therapeutic threshold (≥2× baseline), F4 achieved this target in 57% of

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samples, F3 in 52%, and Commercial in 55%, while F1 and F2 showed limited performance (14% each). However, the commercial system demonstrated significant inter-sample variability, with concentration values ranging from 52,000 to 1,282,000 platelets/μL (25-fold variation), indicating unpredictable performance despite equivalent threshold achievement rates.

For optimal concentration delivery, analysis of volume requirements for standard platelet doses (1×10^9 platelets) demonstrated apparent Commercial advantage (1.97 mL) driven by extreme observations; however, given that 50% of samples yielded concentrations below 366,000/μL, this benefit is inconsistent and complicates dose planning. In contrast, F3 and F4 protocols provided more predictable volume requirements, reducing the risk of therapeutic failure due to insufficient concentration.

Table 5. Therapeutic Threshold Assessment by method.

	≥2× Baseline	≥3× Baseline	≥4× Baseline	Volume for 1×10^9 platelets (mL)
F1	14%	0%	0%	3.21
F2	14%	0%	0%	3.31
F3	52%	5%	0%	2.94
F4	57%	5%	0%	2.77
Commercial	55%	45%	35%	1.97*

*Commercial efficiency based on mean values; actual performance highly variable (range: 52,000-1,282,000 platelets/μL). Proportion of samples reaching therapeutic thresholds and volume requirements for standard platelet doses, calculated from empirically verified data. Abbreviations: mL, milliliters.

4. Discussion

The biological rationale of PRP is based on the supraphysiological release of growth factors and cytokines contained in platelets [16]. In recent years, the number of indications and publications on the use of PRP in regenerative medicine has increased considerably. However, consistent scientific evidence is needed to support its use in various indications [17]. The practical application of PRP research in clinical settings is hindered by considerable variability in preparation methods, resulting in significant differences in products with respect to variables such as volume, platelet and leukocyte concentration or growth factors levels [8]. The lack of standardized preparation protocols compromises the reliability and effectiveness of PRP treatments and may account for the wide variety of reported outcomes [18].

Interpreting the results according to platelet concentration, all methods demonstrated statistically significant platelet enrichment compared to baseline means (all $p \leq 0.001$), with median concentration factors ranging from 1.6x to 2.3x, despite individual sample variability. The commercial methods achieved variable enrichment, with a median of 440×10^3 cells/μL and a concentration factor of approximately 2.3 times baseline (median of 192×10^3 cells/μL). While allowing for higher cellular concentration in some cases, the commercial methods showed substantial variability in results, with concentration factors ranging widely and a coefficient of variation of ~70%. This extreme variability manifested as a 25-fold difference between minimum and maximum concentrations (52,000 to 1,282,000 platelets/μL), indicating unpredictable performance. In the experimental techniques, we observed modest differences between methods, with medians of 314×10^3, 308×10^3, 337×10^3, and 323×10^3 cells/μL for F1, F2, F3, and F4, respectively. The experimental techniques demonstrated superior reproducibility with lower coefficients of variation, particularly the single-spin methods: F1 (28.1%) and F2 (29.2%), compared to double-spin methods F3 (42.9%) and F4 (34.9%).

Statistical validation revealed important insights into the comparative effectiveness of methods. Effect size analysis demonstrated that while F4 vs Commercial showed a moderate effect size ($r = 0.40$), this comparison did not achieve statistical significance ($p = 0.073$). Similarly, F3 vs Commercial demonstrated a comparable pattern ($p = 0.057$) without reaching conventional significance thresholds. These findings indicate that experimental methods are statistically equivalent to

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commercial systems in terms of platelet recovery, while offering distinct advantages in reproducibility and consistency.

Despite the relatively small sample size (n=21), this study demonstrated sufficient statistical power to detect meaningful differences between preparation methods. Bootstrap validation with 5000 iterations confirmed that the observed patterns represent genuine characteristics rather than statistical artifacts, particularly the superior reproducibility of single-centrifugation methods. The study design proved robust enough to identify clinically relevant advantages across multiple parameters, including reproducibility and leukocyte depletion capabilities.

4.1. Implications for Protocol Personalization

The correlation findings suggest potential for baseline-guided protocol selection. Three protocols (F2, F3, F4) demonstrated significant negative correlations between baseline platelet counts and concentration factors (ρ = -0.520, -0.492, -0.483 respectively, all $p < 0.05$), indicating particular utility in patients with lower initial counts through a "plateau effect" mechanism. However, F1 and Commercial systems showed baseline-independent performance, suggesting consistent applicability across varying patient populations without pre-screening requirements.

4.2. Tools for Standardization

The developed PRP-QI provided an objective framework for protocol comparison, integrating concentration, reproducibility, leukocyte reduction, and volumetric efficiency metrics. Simple centrifugation methods achieved superior scores: F2 (0.66) and F1 (0.61), followed by Commercial (0.51), F4 (0.31), and F3 (0.25). This hierarchy reflects the superior reproducibility and leukocyte depletion capabilities of single-spin methods, which compensates for their modest concentration factors. The commercial system's intermediate ranking resulted from high concentration potential offset by poor reproducibility and absence of leukocyte reduction. Allowance rationale and robustness. The PRP-QI weights were pre-specified to mirror clinical priorities reproducibility (consistency under routine conditions), platelet enrichment (biological potency), leukocyte modulation (product purity), and volumetric efficiency (procedural yield, defined strictly as absolute PRP volume [mL] per run). This scheme avoids data-driven overfitting and was challenged in pre-planned sensitivity analyses: varying each weight by ±10 points (with re-normalization) and adopting robust scaling (p10–p90) did not alter the top-ranked methods (rank changes ≤1 position). Therefore, conclusions are robust to reasonable changes in the composite specification.

4.3. Identified Therapeutic Limitations and Advantages

Threshold analysis revealed distinct performance patterns across methods. For the minimum therapeutic threshold (≥2× baseline), F4 achieved this target in 57% of samples, F3 in 52%, and Commercial in 55%, while F1 and F2 showed limited performance (14% each). However, analysis of volume requirements for standard therapeutic doses (1×10^9 platelets) revealed that apparent Commercial superiority (1.97 mL) was driven by occasional extremely high concentrations, while 50% of samples yielded concentrations below 366,000/μL. In contrast, F3 and F4 protocols provided more predictable volume requirements (2.94 and 2.77 mL respectively), reducing the risk of therapeutic failure due to insufficient concentration. F1 and F2, while requiring larger volumes (3.21 and 3.31 mL), offered superior consistency and predictability for applications where volume availability is not limiting.

4.4. Clinical Selection Algorithm

Based on comprehensive analysis of multiple validated parameters, an evidence-based selection algorithm is proposed: (1) For routine applications requiring optimal reproducibility and leukocyte depletion, F2 remains the preferred method, demonstrating effective leukocyte depletion (62.0% reduction); (2) For patients requiring moderate concentration enhancement with predictable

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outcomes, F3 and F4 emerge as optimal choices, particularly in patients with lower baseline platelet counts; (3) For applications requiring maximum consistency across diverse patient populations, F1 provides baseline-independent performance with excellent reproducibility; and (4) For cases requiring maximum concentration potential with tolerance for significant variability, commercial systems maintain utility, though with unpredictable performance that may require backup protocols.

Regarding leukocytes, analysis revealed distinct depletion patterns across methods. F1 and F2 achieved significant leukocyte reduction compared to baseline (42.1% and 62.0% reduction respectively, both $p < 0.001$), effectively producing leukocyte-poor PRP suitable for applications where inflammatory response minimization is desired. F3 and F4 showed no significant leukocyte reduction, maintaining baseline levels that may be appropriate for indications requiring leukocyte presence. The commercial system demonstrated inconsistent leukocyte handling with high variability that prevented reliable classification as either leukocyte-poor or leukocyte-rich, limiting its predictability for applications where leukocyte content is therapeutically relevant.

Robustness validation confirmed the reliability of these findings using multiple analytical approaches. Multiple comparisons were controlled with the Holm procedure; under this conservative criterion, the main results remained significant, including, leukocyte depletion (F1 and F2 vs baseline) and key concentration differences. Bootstrap resampling further supported the reproducibility advantages of single-spin protocols, indicating that these reflect genuine methodological characteristics rather than statistical artifacts.

Beyond statistical considerations, experimental methods offer significant clinical advantages that enhance treatment feasibility and patient adherence. The ability to prepare multiple therapeutic doses from a single blood extraction eliminates repeated venipunctures, substantially improving patient comfort and treatment compliance. This operational advantage, combined with superior batch homogeneity, reduces intersession variability and contributes to more predictable clinical outcomes.

Volumetric efficiency analysis revealed that single-spin methods achieved superior PRP recovery relative to blood volume requirements compared to double-spin methods and commercial systems. The commercial methods, despite occasional high concentrations, demonstrated substantial preparation-to-preparation variability that complicated dose planning and resource allocation. Cost-effectiveness analysis strongly favors experimental protocols, with preparation costs significantly lower than commercial systems while achieving equivalent or superior reproducibility.

The commercial system maintains clinical utility for specific applications requiring maximum platelet concentration potential, occasionally achieving ≥4× baseline in 35% of cases compared to 0% for experimental methods. However, this advantage comes at the cost of substantial variability (25-fold concentration range) and reduced predictability of outcomes, limiting its applicability for routine therapeutic protocols where consistent performance is prioritized over maximum potential concentration.

In the various indications where regenerative therapy with PRP is used, multiple administrations are often required [26,27]. This generally implies the need for venipuncture to obtain the initial blood sample and to prepare the PRP prior to each administration. Several studies have evaluated the stability of PRP obtained through both open and closed systems under different freezing conditions by measuring bioactive molecules in the PRP [28,29]. In the next phase of the project, the aim is to assess the stability of PRP obtained through an open system in order to provide the different doses a patient may require from a single extraction.

In addition to the quantitative and qualitative findings, the open methods evaluated offer clear operational advantages in clinical settings where repeated PRP administrations are required. Preparing all doses needed for a therapeutic cycle from a single extraction avoids multiple venipunctures, improving patient comfort and adherence to treatment. Moreover, obtaining all aliquots from the same sample ensures batch homogeneity, reducing intersession variability and contributing to more consistent clinical responses.

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Although this study did not assess post-thaw microbiological and functional stability, previous reports support the feasibility of storing PRP under controlled conditions while preserving safety and bioactivity. This strategy, easily adaptable to routine practice, allows standardized preparation without relying exclusively on commercial kits, whose performance variability and elevated costs may limit widespread use. Within this framework, open methods, particularly single-spin protocols for reproducibility-critical applications and optimized double-spin protocols for concentration-dependent indications, emerge as reproducible, efficient, and GMP-compatible approaches, providing a sustainable and customizable alternative for PRP preparation in regenerative medicine.

This study has several limitations that should be considered. The relatively small sample size reduces statistical power for some comparisons, though bootstrap validation confirmed the robustness of key findings. The study population was restricted to adult patients with degenerative knee pathology, potentially limiting extrapolation to other clinical conditions or anatomical sites. The single-center design also constrains external validity. In addition, only one commercial PRP kit was evaluated, which prevents generalization of the conclusions to other commercially available systems, though the observed variability patterns may be representative of automated processing challenges. Future multi-center studies with larger and more diverse populations, and including clinical outcome measures, will be required to confirm and expand these findings, particularly regarding the clinical significance of the reproducibility advantages and therapeutic threshold achievements observed with experimental methods.

5. Conclusions

This study demonstrates that standardized open-system PRP protocols, performed under GMP-compatible conditions, achieve therapeutic platelet concentrations with superior reproducibility compared to commercial systems evaluated. While commercial methods reached higher mean platelet concentrations, they exhibited substantial variability (CV ~70%), whereas open protocols, particularly single-centrifugation methods, demonstrated more consistent performance (CV 28-29%). Both approaches successfully concentrate platelets above baseline levels, offering distinct advantages for different clinical scenarios. The developed PRP Quality Index provides an objective framework for method comparison, revealing that single-centrifugation open methods (F1-F2) achieved favorable scores through their combination of reproducibility, leukocyte depletion capability, and adequate volumetric yield. Open systems also demonstrated the ability to predictably modulate leukocyte counts, either depleting or maintaining levels based on centrifugation parameters offering flexibility for diverse therapeutic applications. These findings support the implementation of open-system protocols as a valid alternative for PRP preparation in clinical practice. The demonstrated feasibility of these GMP-compatible methods provides institutions with additional options for PRP therapy, enabling method selection based on specific therapeutic objectives, resource availability, and the relative importance of concentration versus consistency for each clinical application. Both open and commercial systems have their place in the therapeutic arsenal, with the choice depending on institutional capabilities and patient-specific requirements.

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Abbreviations

The following abbreviations are used in this manuscript:

CBC	complete blood count
CI	confidence intervals
CPDA	citrate-phosphate-dextrose-adenine
CV	coefficient of variation
EGF	epidermal growth factor
GMP	good manufacturing practice
IGF-1	Insulin-like growth factor 1
IQR	interquartile range
L-PRF	leukocyte- and platelet-rich fibrin
L-PRP	leukocyte- and platelet-rich plasma
PDGF	platelet-derived growth factor
P-PRF	pure platelet-rich fibrin
P-PRP	pure platelet-rich plasma
PRF	platelet-rich fibrin
PRP	platelet-rich plasma
PRP-QI	PRP quality index
SD	standard deviation
TGF-β	transforming growth factor beta
VEGF	vascular endothelial growth factor
xg	units of relative centrifugal force
ϱ	Spearman's rho

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