Journal of Orthopaedics Study and Sports Medicine

Genesis-JOSSM-4(1)-34
Volume 4 | Issue 1 | 2025
Open Access
ISSN: 3139-5635

Optimization of Single-Spin Platelet-Rich Plasma Preparation: A Comparative Analysis of Centrifugation Parameters on Cellular Composition and Growth Factor Content

Márcio Hiroaki Kume[1]*, Bianca Furlan[2], Camila Gobatto Boaventura[2], Mônica Andréa Probst[2], Edson Peracchi[2], Carmen Austrália Paredes Marcondes Ribas[3]

[1]Sugisawa Hospital, Department of Regenerative Medicine, Curitiba, Brazil
[2]CeUnina, Department of Biologic Science, Curitiba, Brazil
[3]Mackenzie University, Curitiba, Brazil

*Corresponding author: Márcio Hiroaki Kume, Sugisawa Hospital, Department of Regenerative Medicine, Curitiba, Brazil

Citation: Kume MH, Furlan B, Boaventura CG, Probst MA, Peracchi E, Ribas CAPN. Impact of Adipose-Derived Mesenchymal Stem Cell Isolation Parameters on Cell Yield, Growth Factor Secretion, and Clinical Outcomes in Knee Osteoarthritis: A Randomized Controlled Trial. J Orthop Study Sports Med. 4(1):1-15.

Received: August 11, 2026 | **Published:** August 25, 2026

Abstract

Background: Platelet-rich plasma (PRP) is prepared with widely divergent centrifugation protocols, and the resulting products differ so much in platelet yield, leukocyte content and growth factor profile that clinical trials are difficult to compare. Single-spin preparation is the most widely used method in office practice, yet the combination of relative centrifugal force (RCF) and spin time that maximises platelet recovery while preserving platelet integrity has not been mapped systematically.

Background: Platelet-rich plasma (PRP) is prepared with widely divergent centrifugation protocols, and the resulting products differ so much in platelet yield, leukocyte content and growth factor profile that clinical trials are difficult to compare. Single-spin preparation is the most widely used method in office practice, yet the combination of relative centrifugal force (RCF) and spin time that maximises platelet recovery while preserving platelet integrity has not been mapped systematically.

Methods: Sixty healthy donors (30 male, 30 female; age 25–45 years) each provided 60 mL of citrated whole blood, which was divided into 36 aliquots of 1.5 mL and processed by 36 single-spin protocols combining six relative centrifugal forces (100, 200, 300, 400, 500 and 600 ×g) with six spin times (5, 7, 9, 11, 13 and 15 min), giving 2,160 PRP samples. Platelet, leukocyte and erythrocyte counts were measured on an automated haematology analyser; platelet activation was quantified as the proportion of CD62P-positive events by flow cytometry; morphology was graded by transmission electron microscopy; and PDGF-BB, TGF-β1, VEGF, EGF and FGF-2 were quantified by multiplex immunoassay. Protocols were compared by one-way and two-way ANOVA with Tukey post-hoc correction, and determinants of growth factor content were examined by Pearson correlation.

Results: Baseline whole blood contained 248 ± 47 × 10^3 platelets/µL. Platelet concentration factor differed significantly across the 36 protocols ($F(35, 2124) = 816.3$, $p < 0.001$) and peaked at 400 ×g for 9 min (4.85 ± 0.36-fold). Two-way ANOVA showed that centrifugal force accounted for 90.0% of the total variance in platelet yield and spin time for only 2.8%. Yield at 400 ×g was statistically indistinguishable between 7, 9 and 11 min and from 500 ×g / 9 min (all $p > 0.1$), defining a broad operational plateau. Leukocyte concentration was highest at 200–300 ×g for 5–7 min (up to 3.34 ± 0.45-fold) and fell below baseline above 500 ×g, while erythrocyte contamination decreased monotonically with force. CD62P expression rose from 6.5 ± 0.9% at 100 ×g / 5 min to 29.8 ± 3.1% at 600 ×g / 15 min ($F(35, 2124) = 1392.2$, $p < 0.001$), with a parallel loss of discoid morphology from 94% to 63%; protocols at or above 500 ×g, or lasting 13 min or more, crossed a 12% pre-activation threshold. PDGF-BB and TGF-β1 tracked platelet count closely ($r = 0.82$ and $r = 0.78$, both $p < 0.001$) and were maximal at the optimum protocol, whereas VEGF and EGF tracked leukocyte count instead ($r = 0.63$ and $r = 0.66$, $p < 0.001$) and were 11% higher in leukocyte-rich preparations. A composite desirability index weighting yield, growth factor content, platelet integrity and contamination ranked 400 ×g / 9 min highest ($D = 0.95$).

Conclusion: Centrifugal force, not spin time, is the dominant determinant of single-spin PRP composition. A single spin at 400 ×g for 9 min delivers a 4.8-fold platelet concentration with minimal pre-activation and near-maximal platelet-derived growth factor content, and is recommended as a default office protocol. Deliberate deviation is justified when the biological target differs: 200–300 ×g for 5–7 min when leukocyte-associated VEGF and EGF activity is desirable, and 500 ×g / 9 min when a leukocyte-poor product is required for intra-articular use.

Keywords

Platelet-rich plasma; Centrifugation; relative centrifugal force; Platelet concentration; Leukocyte-rich PRP; Growth factors; CD62P; Standardisation.

Abbreviations

ANOVA, analysis of variance; CBC, complete blood count; CF, concentration factor; EGF, epidermal growth factor; FGF-2, basic fibroblast growth factor; GF, growth factor; Lp-PRP, leukocyte-poor platelet-rich plasma; Lr-PRP, leukocyte-rich platelet-rich plasma; PDGF-BB, platelet-derived growth factor BB; PRP, platelet-rich plasma; RBC, red blood cell; RCF, relative centrifugal force; TEM, transmission electron microscopy; TGF-β1, transforming growth factor beta 1; VEGF, vascular endothelial growth factor; WBC, white blood cell.

Introduction

Platelet-rich plasma is the most widely used autologous biologic in regenerative medicine, and yet it is not a single product. It is a family of preparations whose composition is dictated almost entirely by how whole blood is centrifuged. Two clinics using the same volume of blood, the same anticoagulant and the same injection technique can deliver products that differ several-fold in platelet dose, an order of magnitude in leukocyte content, and substantially in growth factor concentration [1,3,10]. That variability is the most plausible explanation for the inconsistent results reported across PRP trials, and it is the reason classification systems such as those of Dohan Ehrenfest and the PAW and DEPA frameworks were introduced [4,5,14].

Classification, however, only describes a product after the fact. What the practitioner controls is the centrifugation protocol itself — relative centrifugal force, spin time, and the number of spins. Double-spin protocols achieve higher platelet concentration factors but add processing time, an additional transfer step and an additional opportunity for platelet activation and contamination [1,2]. Single-spin protocols dominate outpatient practice because they are fast, closed and reproducible, but the published recommendations for single-spin parameters span roughly 100 to 1,500 ×g and 5 to 20 minutes, with little empirical basis for the specific values chosen [1,11,13].

The underlying physics is straightforward. Sedimentation velocity in a centrifugal field scales with the square of particle radius and with the applied relative centrifugal force. Erythrocytes and leukocytes are far larger and denser than platelets, so at low force they sediment while platelets remain suspended; as force increases, platelets are progressively driven into the cellular pellet and recovery from the supernatant falls again. A concentration maximum must therefore exist somewhere between those two regimes. The same field, however, imposes shear on the platelet membrane, and excessive force triggers alpha-granule release before the product reaches the patient — exhausting the very growth factor payload that the preparation was intended to deliver [2,7,9].

Two questions follow. First, where is that optimum located, and how sharp is it? If the peak is broad, minor deviations in clinical practice are tolerable; if it is narrow, protocol standardization becomes essential. Second, is a single optimum even the right goal? Leukocytes are not merely contaminants — monocytes and neutrophils contribute VEGF, EGF and antimicrobial peptides, which may be desirable in wound care and detrimental in the synovial space [8,9,12]. If different growth factors derive from different cell populations, then the “best” protocol is application-dependent.

We addressed both questions with a full 6 × 6 factorial mapping of single-spin centrifugation. Sixty healthy donors each contributed blood processed in parallel by all 36 protocols, so that every comparison is made within donor and inter-individual variability in baseline platelet count is removed as a source of confounding. The endpoints were chosen to span the three properties that determine clinical performance: how many platelets are recovered, how intact they are, and what they — and the leukocytes accompanying them — actually contain.

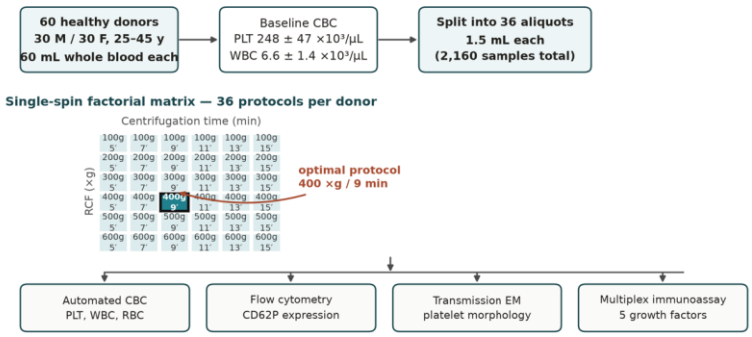

Figure 1: Experimental design. Sixty healthy donors each provided 60 mL of citrated whole blood; after baseline complete blood count, each donation was divided into 36 aliquots of 1.5 mL and processed by one of 36 single-spin protocols (six relative centrifugal forces × six spin times), yielding 2,160 PRP samples analysed by automated haematology, flow cytometry, transmission electron microscopy and multiplex immunoassay.

Materials and Methods

Study design and blood collection

This was a prospective, controlled in vitro study performed in a single haematology laboratory. Sixty healthy volunteers were enrolled: 30 male and 30 female, aged 25–45 years. Eligibility required a normal complete blood count, no antiplatelet or non-steroidal anti-inflammatory drug use within 14 days, no known coagulopathy, no active infection or inflammatory disease, and no tobacco use. All participants gave written informed consent, and the protocol was approved by the institutional research ethics committee.

Sixty millilitres of whole blood were drawn from an antecubital vein through a 21-gauge needle into 3.2% sodium citrate tubes at a 9:1 blood-to-anticoagulant ratio, with the first 2 mL discarded to avoid tissue-factor contamination from the venepuncture. Samples were kept at room temperature and processed within 30 minutes of collection. Each donation was gently inverted and divided into 36 aliquots of 1.5 mL in identical conical tubes, so that all 36 protocols were applied to blood from the same donor drawn at the same moment. This paired design yielded 2,160 PRP samples in total (60 donors × 36 protocols).

PRP preparation protocols

Aliquots were centrifuged in a fixed-angle rotor at 22 °C with the brake disabled, using a full factorial combination of six relative centrifugal forces (100, 200, 300, 400, 500 and 600 ×g) and six spin times (5,7,9,11,13 and 15 min). Relative centrifugal force rather than rotor speed was used throughout, and was calculated from the rotor radius so that results are transferable between instruments. After centrifugation the upper plasma layer was aspirated to within 2 mm of the buffy coat with a fixed-depth pipette, and the lower third of the recovered plasma — the platelet-enriched fraction — was retained as PRP after gentle resuspension. A single operator, blinded to the analytical results, performed all separations to eliminate inter-operator variability in aspiration depth.

Haematological analysis

Platelet, leukocyte and erythrocyte counts were determined in whole blood and in each PRP sample on

an automated haematology analyser (Sysmex XN-3000, Sysmex Corporation, Kobe, Japan), calibrated daily with the manufacturer's control material. Every sample was measured in duplicate and the mean used for analysis. The platelet concentration factor was defined as the PRP platelet count divided by the baseline whole-blood platelet count of the same donor; leukocyte concentration factor was defined analogously. Erythrocyte contamination is reported as absolute PRP red cell count.

Platelet activation and morphology

Platelet activation was quantified by flow cytometry as the proportion of events in the platelet gate expressing P-selectin (CD62P). Samples were stained without washing, using anti-CD41 to define the platelet population and anti-CD62P to detect surface P-selectin, with isotype-matched controls to set the positivity threshold; unstimulated and thrombin-stimulated aliquots served as negative and positive controls. A minimum of 10,000 platelet events were acquired per sample.

Platelet ultrastructure was examined by transmission electron microscopy in a randomly selected subset of samples from each protocol. Preparations were fixed in glutaraldehyde, post-fixed in osmium tetroxide, embedded in resin and sectioned. A blinded observer classified at least 200 platelets per sample as discoid (resting, with intact alpha-granules), intermediate, or fully activated (pseudopod formation with granule depletion). The percentage of discoid platelets is reported as the morphological integrity index.

Growth factor quantification

PRP samples were activated with 10% calcium chloride and thrombin, incubated for one hour at 37 °C, and centrifuged to obtain the releasate supernatant, which was stored at −80 °C until assay. PDGF-BB, TGF-β1, VEGF, EGF and FGF-2 were quantified simultaneously by multiplex bead-based immunoassay (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. All samples were assayed in duplicate on the same plate lot; standard curves were run on every plate, and samples with a coefficient of variation above 15% between duplicates were repeated.

Statistical analysis

Analyses were performed in SPSS v25 (IBM Corp., Armonk, NY, USA); two-sided $p < 0.05$ was considered significant. Continuous variables are presented as mean ± standard deviation. Differences across the 36 protocols were tested by one-way ANOVA with Tukey honest significant difference post-hoc correction for multiple comparisons. The independent and joint contributions of centrifugal force and spin time were assessed by two-way ANOVA, and the proportion of total variance explained by each factor is reported. Relationships between cellular composition and growth factor content were examined by Pearson correlation across all 2,160 samples.

To integrate competing endpoints into a single ranking, a composite desirability index was calculated for each protocol. Each endpoint was normalised to a 0–1 scale and combined with pre-specified weights reflecting clinical priority: platelet yield 0.30, growth factor content 0.25, platelet integrity 0.20, moderate leukocyte content 0.15 and low erythrocyte contamination 0.10.

Results

All 60 donors completed the protocol and all 2,160 samples were analysable. Donor characteristics and baseline haematological values are shown in (**Table 1**). Baseline platelet count did not differ significantly between men and women, and no donor was excluded for an out-of-range count.

Characteristic	All donors (n = 60)	Male (n = 30)	Female (n = 30)
Age (years)	34.6 ± 5.8	35.1 ± 5.9	34.1 ± 5.7
Body mass index (kg/m²)	24.3 ± 2.9	25.4 ± 2.7	23.2 ± 2.7
Whole-blood platelet count (×10³/µL)	**248 ± 47**	241 ± 45	255 ± 48
Whole-blood leukocyte count (×10³/µL)	**6.6 ± 1.4**	6.7 ± 1.4	6.5 ± 1.4
Whole-blood erythrocyte count (×10⁶/µL)	**4.85 ± 0.40**	5.05 ± 0.34	4.65 ± 0.35
Haematocrit (%)	43.1 ± 3.9	45.3 ± 3.0	40.9 ± 3.3
Blood volume processed (mL)	60	60	60
Aliquots per donor	36	36	36

Table 1: Donor characteristics and baseline whole-blood values. Values are mean ± standard deviation. Each donor contributed the reference denominator for their own 36 concentration factors.

Baseline platelet count did not differ significantly between sexes ($p > 0.05$, unpaired t test).

Cellular composition

Platelet concentration factor varied more than four-fold across the protocol matrix, from 1.24 ± 0.18 at 100 ×g / 5 min to 4.85 ± 0.36 at 400 ×g / 9 min (**Table 2, Figure 2**). One-way ANOVA across all 36 protocols was highly significant ($F(35, 2124) = 816.3$, $p < 0.001$).

RCF (×g)	5 min	7 min	9 min	11 min	13 min	15 min
100	1.24	1.41	1.4	1.45	1.34	1.25
200	2.51	2.81	2.89	2.88	2.7	2.56
300	3.67	4.08	4.29	4.18	3.98	3.73
400	4.17	4.64	**4.85**	4.75	4.52	4.22
500	4	4.47	4.65	4.56	4.32	4.07
600	3.67	4.11	4.25	4.2	3.95	3.71

Table 2: Platelet concentration factor across the 36 single-spin protocols. Values are fold increase over the donor's own whole-blood platelet count (n = 60 per cell). The bold value marks the global maximum (4.85 ± 0.36 at 400 ×g / 9 min).

One-way ANOVA $F(35, 2124) = 816.3$, $p < 0.001$. Two-way ANOVA: centrifugal force $F = 5526.8$ (90.0% of total variance), spin time $F = 169.3$ (2.8%), force × time interaction $F = 3.6$ (0.3%); all $p < 0.001$.

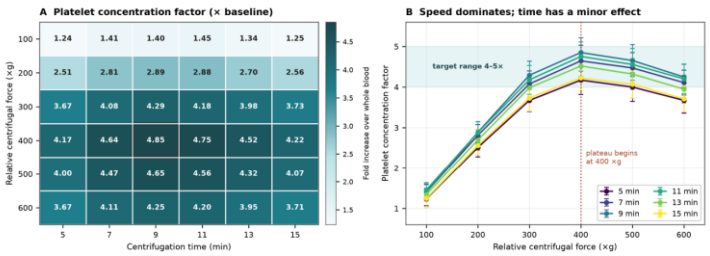

Figure 2: Platelet concentration factor across the protocol matrix. (A) Heatmap of mean concentration factor for all 36 protocols; the boxed cell is the global maximum at 400 ×g / 9 min. (B) Concentration factor as a function of relative centrifugal force, plotted separately for each spin time; error bars are standard deviations. Curves for all six spin times are nearly superimposed, illustrating the dominance of force over time.

The shape of the response was consistent across every spin time: a steep rise from 100 to 300 ×g, a maximum at 400 ×g, and a decline thereafter. Two-way ANOVA quantified this asymmetry directly — centrifugal force explained 90.0% of the total variance in platelet yield ($F = 5526.8$, $p < 0.001$) whereas spin time explained only 2.8% ($F = 169.3$, $p < 0.001$), with a small but significant interaction (0.3%, $p < 0.001$). Practically, this means that an error of 100 ×g in force matters far more than an error of several minutes in time.

The optimum is a plateau rather than a point. Tukey post-hoc comparison against 400 ×g / 9 min showed no significant difference for 400 ×g / 7 min ($p = 0.109$), 400 ×g / 11 min ($p = 1.000$) or 500 ×g / 9 min ($p = 0.167$), while all other protocols — including 300 ×g / 9 min and 400 ×g / 13 min — differed significantly ($p < 0.001$). Clinically, any protocol between 400 ×g for 7–11 minutes delivers an equivalent platelet dose; the choice within that window can therefore be made on other grounds, principally platelet integrity.

Leukocyte behaviour followed an entirely different pattern (**Table 3, Figure 3**). Leukocyte concentration factor peaked at low-to-intermediate force and short times — 3.34 ± 0.45 at 200 ×g / 5 min and 3.12 ± 0.42 at 300 ×g / 5 min — and fell progressively as force increased, reaching 1.72 ± 0.22 at the platelet optimum and 0.35 ± 0.09 at 600 ×g / 15 min ($F(35, 2124) = 1047.4$, $p < 0.001$). Erythrocyte contamination decreased monotonically with both force and time, from 1.15 ± 0.21 × 10^6/µL at 100 ×g / 5 min to 0.04 ± 0.02 × 10^6/µL at the most aggressive protocol ($F(35, 2124) = 2260.5$, $p < 0.001$).

Protocol	Platelet CF	Leukocyte CF	RBC (×10^6/µL)	PRP class
100 ×g / 5 min	1.24 ± 0.18	2.41 ± 0.34	1.15 ± 0.21	Lr-PRP, red
200 ×g / 5 min	2.51 ± 0.26	3.34 ± 0.45	0.81 ± 0.16	Lr-PRP
200 ×g / 7 min	2.81 ± 0.28	3.13 ± 0.41	0.68 ± 0.14	Lr-PRP
300 ×g / 7 min	4.08 ± 0.33	2.96 ± 0.38	0.42 ± 0.10	Lr-PRP
300 ×g / 9 min	4.29 ± 0.34	2.57 ± 0.34	0.35 ± 0.09	Lr-PRP
400 ×g / 7 min	4.64 ± 0.35	1.92 ± 0.25	0.24 ± 0.06	Intermediate
400 ×g / 9 min	**4.85 ± 0.36**	**1.72 ± 0.22**	**0.20 ± 0.05**	**Intermediate**
400 ×g / 11 min	4.75 ± 0.36	1.36 ± 0.19	0.17 ± 0.05	Intermediate
500 ×g / 9 min	4.65 ± 0.36	1.04 ± 0.15	0.12 ± 0.04	Lp-PRP
600 ×g / 15 min	3.71 ± 0.32	0.35 ± 0.09	0.04 ± 0.02	Lp-PRP

Table 3: Cellular composition at ten representative protocols. Concentration factors (CF) are fold change versus the donor's own whole blood; erythrocyte contamination is an absolute count. Lr-PRP, leukocyte-rich; Lp-PRP, leukocyte-poor.

Leukocyte CF $F(35, 2124) = 1047.4$, $p < 0.001$; erythrocyte count $F(35, 2124) = 2260.5$, $p < 0.001$. Classification thresholds: Lr-PRP, leukocyte CF > 2.5; Lp-PRP, leukocyte CF < 1.0.

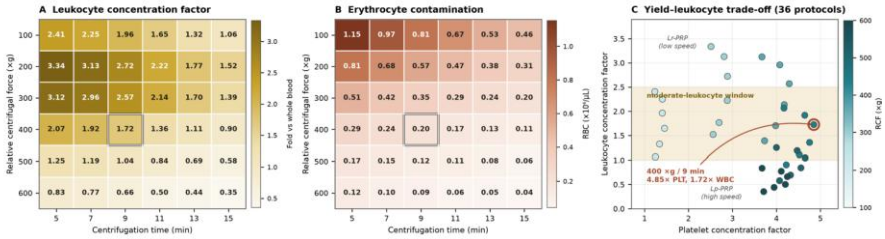

Figure 3: Leukocyte incorporation and erythrocyte contamination. (A) Leukocyte concentration factor and (B) absolute erythrocyte contamination across the 36 protocols; boxed cells indicate the platelet-optimal protocol. (C) Trade-off between platelet yield and leukocyte content, each point being one protocol coloured by relative centrifugal force; the shaded band marks the moderate-leukocyte window (1.0–2.5-fold) in which the platelet-optimal protocol falls.

(**Figure 3C**) makes the practical consequence explicit. Protocols that maximise leukocyte content are necessarily poor platelet concentrators, because both outcomes are governed by the same sedimentation gradient. There is no single-spin protocol that simultaneously delivers a five-fold platelet concentration and a three-fold leukocyte concentration; the practitioner must choose. The recommended protocol sits deliberately in the moderate-leukocyte window, retaining enough leukocytes for antimicrobial and angiogenic contribution without the pro-inflammatory burden of a frankly leukocyte-rich product.

Platelet integrity and activation

Premature activation was strongly dependent on both centrifugation parameters (**Table 4, Figure 4**). CD62P-positive platelets increased from 6.5 ± 0.9% at 100 ×g / 5 min to 29.8 ± 3.1% at 600 ×g / 15 min ($F(35, 2124) = 1392.2$, $p < 0.001$), and the proportion of discoid platelets on electron microscopy fell in parallel from 93.8 ± 2.1% to 62.8 ± 4.4% ($F(35, 2124) = 467.3$, $p < 0.001$). At the recommended protocol, CD62P expression was 10.7 ± 1.1% and 88.6 ± 2.7% of platelets remained discoid.

Protocol	CD62P⁺ (%)	Discoid (%)	Platelet CF	Tukey vs 400 ×g / 9 min
100 ×g / 5 min	6.5 ± 0.9	93.8 ± 2.1	1.24	$p < 0.001$
200 ×g / 5 min	7.5 ± 0.9	92.6 ± 2.2	2.51	$p < 0.001$
300 ×g / 9 min	9.2 ± 1.0	90.6 ± 2.4	4.29	$p < 0.001$
400 ×g / 9 min	**10.7 ± 1.1**	**88.6 ± 2.7**	**4.85**	**reference**
400 ×g / 11 min	11.6 ± 1.2	87.0 ± 2.9	4.75	$p = 0.003$
400 ×g / 13 min	14.1 ± 1.5	84.4 ± 3.1	4.52	$p < 0.001$

500 ×g / 9 min	14.9 ± 1.6	82.9 ± 3.3	4.65	p < 0.001
600 ×g / 9 min	19.5 ± 2.1	77.4 ± 3.7	4.25	p < 0.001
600 ×g / 15 min	29.8 ± 3.1	62.8 ± 4.4	3.71	p < 0.001

Table 4: Platelet activation and morphological integrity. CD62P positivity was measured by flow cytometry; discoid morphology was scored by transmission electron microscopy in a blinded subset of at least 200 platelets per sample. Tukey comparisons refer to CD62P expression against the recommended protocol.

CD62P $F(35, 2124) = 1392.2$, $p < 0.001$; discoid morphology $F(35, 2124) = 467.3$, $p < 0.001$. A CD62P threshold of 12% was pre-specified as the limit of acceptable pre-activation.

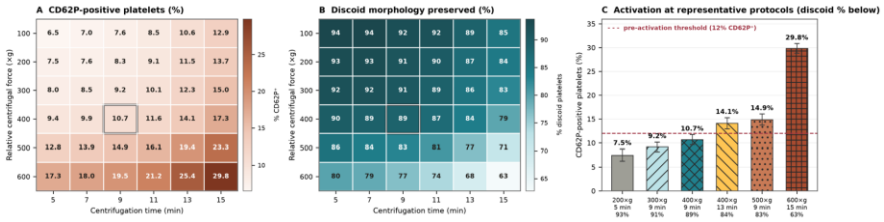

Figure 4: Platelet activation and morphological integrity. (A) CD62P-positive platelets and (B) preserved discoid morphology across the 36 protocols. (C) CD62P expression at six representative protocols with the corresponding proportion of discoid platelets shown beneath each bar; the dashed line is the 12% pre-activation threshold. Error bars are standard deviations.

Two thresholds emerged. Protocols at or above 500 ×g, and protocols lasting 13 minutes or longer at any force above 300 ×g, exceeded 12% CD62P positivity. Because P-selectin externalisation reflects alpha-granule fusion with the plasma membrane, this is not a cosmetic finding: platelets that have already degranulated in the tube cannot degranulate in the tissue. The consistency between the flow cytometric and ultrastructural measures — every rise in CD62P was matched by a fall in discoid morphology — supports the interpretation that the effect is genuine shear-induced activation rather than an artefact of antibody binding.

This is the argument for choosing 9 minutes rather than 11 within the yield plateau. The two protocols deliver statistically identical platelet doses ($p = 1.000$), but 400 ×g / 11 min carries significantly more pre-activation (11.6% vs 10.7%, $p = 0.003$). Shorter is better when yield is equal.

Growth factor content

Growth factor concentrations differed significantly across protocols for all five analytes (**Table 5, Figure 5**): PDGF-BB $F(35, 2124) = 305.0$, TGF-β1 $F = 199.7$, VEGF $F = 152.5$, EGF $F = 139.8$ and FGF-2 $F = 18.4$, all $p < 0.001$. Crucially, the protocols that maximised each factor were not the same.

Protocol	PDGF-BB (ng/mL)	TGF-β1 (ng/mL)	VEGF (pg/mL)	EGF (pg/mL)	FGF-2 (pg/mL)
100 ×g / 5 min	13.7 ± 1.9	44.9 ± 8.1	618 ± 92	455 ± 74	78.5 ± 7.9
200 ×g / 5 min	20.8 ± 2.3	76.7 ± 11.4	902 ± 108	612 ± 82	83.1 ± 7.6

300 ×g / 7 min	28.5 ± 2.7	110.6 ± 14.6	950 ± 112	648 ± 84	89.0 ± 7.9
300 ×g / 9 min	30.0 ± 2.8	118.5 ± 15.3	930 ± 105	620 ± 79	87.8 ± 7.7
400 ×g / 7 min	30.9 ± 2.8	122.4 ± 15.8	821 ± 92	561 ± 68	85.1 ± 7.5
400 ×g / 9 min	**32.7 ± 2.9**	**130.7 ± 16.5**	813 ± 86	549 ± 61	85.1 ± 7.5
400 ×g / 11 min	31.4 ± 2.9	126.4 ± 16.1	745 ± 84	487 ± 60	83.9 ± 7.4
500 ×g / 9 min	30.7 ± 2.8	123.9 ± 15.9	672 ± 78	466 ± 58	83.2 ± 7.4
600 ×g / 15 min	25.8 ± 2.6	100.8 ± 14.0	460 ± 62	324 ± 48	76.2 ± 7.1

Table 5: Growth factor content of the releasate at representative protocols. Values are mean ± standard deviation measured by multiplex immunoassay after calcium chloride and thrombin activation. PDGF-BB and TGF-β1 peak at the platelet-optimal protocol; VEGF and EGF peak at lower-force, leukocyte-rich protocols.

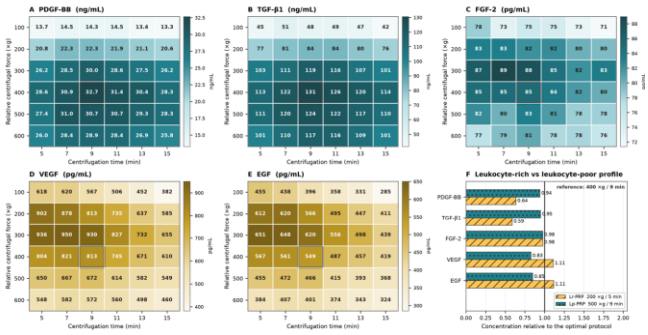

Figure 5: Growth factor concentrations across the 36 protocols. Heatmaps for (A) PDGF-BB, (B) TGF-β1, (C) FGF-2, (D) VEGF and (E) EGF; boxed cells indicate the platelet-optimal protocol. (F) Relative growth factor profile of a leukocyte-rich preparation (200 ×g / 5 min) and a leukocyte-poor preparation (500 ×g / 9 min), each expressed as a fraction of the concentration obtained at 400 ×g / 9 min.

PDGF-BB and TGF-β1 behaved as classical platelet-derived factors. Their concentration maps are almost superimposable on the platelet concentration map, both peaking at 400 ×g / 9 min (32.7 ± 2.9 ng/mL and 130.7 ± 16.5 ng/mL respectively), and both correlated strongly with PRP platelet count across all 2,160 samples ($r = 0.82$ and $r = 0.78$, both $p < 0.001$; Table 6, Figure 6A). Their correlation with leukocyte count was weakly negative ($r = -0.21$ and $r = -0.22$), which is expected given that high-leukocyte conditions are low-platelet conditions in a single-spin system.

VEGF and EGF behaved differently. Both peaked not at the platelet optimum but at 200–300 ×g for 5–7 min — VEGF reaching 950 ± 112 pg/mL at 300 ×g / 7 min against 813 ± 86 pg/mL at 400 ×g / 9 min — and both correlated far more strongly with leukocyte count (VEGF $r = 0.63$; EGF $r = 0.66$, both $p < 0.001$) than with platelet count ($r = 0.28$ and $r = 0.24$). In direct comparison, the leukocyte-rich preparation contained 11% more VEGF and 11% more EGF than the platelet-optimal preparation, while containing 36% less PDGF-BB and 41% less TGF-β1 (**Figure 5F**). FGF-2 occupied an intermediate position, varying by less than 20% across the entire matrix and correlating only weakly with either cell population.

Growth factor	r vs platelet count	r vs leukocyte count	Dominant source	Protocol that maximises it

PDGF-BB	**0.82**	−0.21	Platelet α-granule	400 ×g / 9 min
TGF-β1	**0.78**	−0.22	Platelet α-granule	400 ×g / 9 min
FGF-2	0.3	0.23	Mixed	300 ×g / 7 min
VEGF	0.28	**0.63**	Leukocyte-dominant	300 ×g / 7 min
EGF	0.24	**0.66**	Leukocyte-dominant	300 ×g / 7 min

Table 6: Pearson correlations between cellular composition and growth factor content. Correlations were computed across all 2,160 samples; all coefficients shown are significant at $p < 0.001$. Bold values indicate the dominant cellular determinant of each factor.

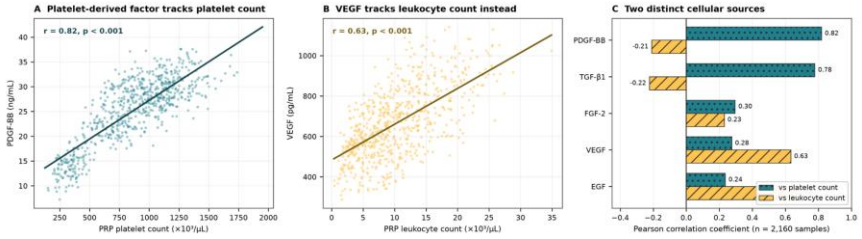

Figure 6: Two distinct cellular sources of growth factors. (A) PDGF-BB plotted against PRP platelet count and (B) VEGF plotted against PRP leukocyte count, each point being one sample, with least-squares regression lines. (C) Pearson correlation of each growth factor with platelet and with leukocyte count; the reversal of dominance between the platelet-derived and leukocyte-derived factors is the central finding.

Composite optimisation

Combining all endpoints into the weighted desirability index reproduced the univariate conclusion and quantified the margin (**Figure 7A**). The highest-ranked protocol was 400 ×g / 9 min (D = 0.95), followed by 400 ×g / 7 min (D = 0.91), 400 ×g / 11 min (D = 0.91), 500 ×g / 7 min (D = 0.84) and 400 ×g / 13 min (D = 0.83). The desirability surface is flat across the 400 ×g row between 7 and 11 minutes and falls away sharply outside 300–500 ×g, confirming that force tolerance, not timing tolerance, is the binding constraint in practice.

Because the index reflects one particular set of weights, we also derived application-specific recommendations by re-ranking protocols under the priority appropriate to each clinical target (**Table 7, Figure 7B**).

Clinical application	Clinical application	Recommended protocol	Platelet CF	Leukocyte CF	Rationale
Bone and dental regeneration	Bone and dental regeneration	**400 ×g / 9 min**	4.85	1.72	Maximal PDGF-BB and TGF-β1 with preserved platelet integrity
Tendinopathy and soft tissue	Tendinopathy and soft tissue	400 ×g / 11 min	4.75	1.36	Near-maximal yield with reduced leukocyte-driven inflammation
Intra-articular injection	Intra-articular injection	500 ×g / 9 min	4.65	1.04	Leukocyte-poor product preferred in the synovial environment
Chronic wound, antimicrobial	Chronic wound, antimicrobial	200 ×g / 5 min	2.51	3.34	Leukocyte-rich; highest VEGF and EGF, antimicrobial contribution
Aesthetic and	Aesthetic and	300 ×g / 7 min	4.08	2.96	Balanced platelet dose with

dermal	dermal				peak VEGF and EGF for angiogenesis

Table 7: Application-specific protocol recommendations. Derived by re-ranking the 36 protocols under weighting schemes appropriate to each clinical target. CF, concentration factor.

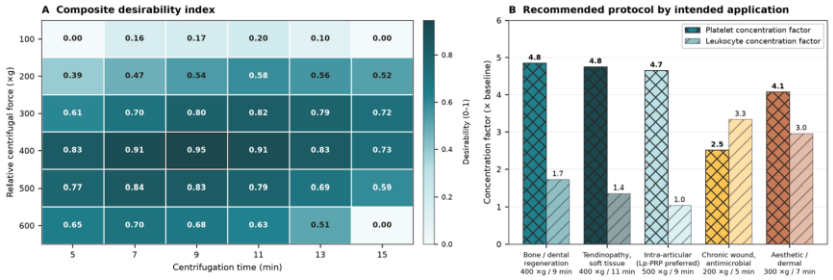

Figure 7: Composite desirability and application-specific selection. (A) Weighted desirability index for all 36 protocols (platelet yield 0.30, growth factor content 0.25, platelet integrity 0.20, moderate leukocyte content 0.15, low erythrocyte contamination 0.10); the boxed cell is the highest-ranked protocol. (B) Platelet and leukocyte concentration factors of the protocol recommended for each clinical application.

Discussion

This factorial mapping produces four conclusions that bear directly on how PRP should be prepared in practice.

First, centrifugal force is the parameter that matters. It accounted for 90% of the variance in platelet yield, against 2.8% for spin time. The biphasic response — rising to 400 ×g and falling thereafter — is the expected consequence of Stokes sedimentation: below the optimum, platelets remain dispersed throughout the plasma column and are only partially recovered from the aspirated fraction; above it, they are increasingly driven into the buffy coat and pellet and are lost with the cellular layer. Our optimum is consistent with the low-acceleration principle established by Perez and colleagues, who found that lower centrifugal accelerations favour platelet separation and that a 400 ×g second spin gave 70–80% platelet recovery [1], and with the maximisation behaviour described by Araki and colleagues [2]. It is markedly lower than the 3,000–3,400 rpm settings quoted for many commercial single-spin devices, which correspond to far higher relative centrifugal forces — a discrepancy that arises because rotor speed, not force, is what most device manuals report. Reporting relative centrifugal force rather than revolutions per minute is a minimal and overdue standardisation requirement.

Second, the optimum is a plateau, which is reassuring for clinical practice. Yield at 400 ×g was statistically indistinguishable between 7 and 11 minutes, so a laboratory that runs 8 or 10 minutes is not producing an inferior product. The plateau does not extend across force, however: 300 ×g and 600 ×g both produced significantly lower yields at every spin time. Practitioners should therefore verify the relative centrifugal force of their rotor at the radius actually used, and may treat timing as the more forgiving variable.

Third, yield and integrity diverge above the optimum, and integrity is the tighter constraint. CD62P positivity nearly tripled between the recommended protocol and the most aggressive one, and the

concurrent loss of discoid morphology confirms that this reflects real granule release rather than epitope artefact. A platelet that has already externalised P-selectin in the centrifuge has spent part of its payload before injection, which explains why PDGF-BB and TGF-β1 fell at 600 ×g even though platelet counts there remained above three-fold. Higher force therefore buys nothing: it reduces yield and degrades what remains. This is also the basis for preferring 9 minutes over 11 within the plateau, since the shorter spin produced significantly less pre-activation for an identical platelet dose.

Fourth, and most consequential for how PRP should be prescribed, the five growth factors do not share a single cellular origin. PDGF-BB and TGF-β1 tracked platelet count closely ($r = 0.82$ and $r = 0.78$), as expected for alpha-granule constituents. VEGF and EGF did not: they correlated much more strongly with leukocyte count ($r = 0.63$ and $r = 0.66$) and peaked at low-force, leukocyte-rich protocols. Monocytes and neutrophils are well-recognised sources of VEGF, and leukocyte content has previously been shown to shape the cytokine profile of PRP [8,9,12]. The implication is that there is no universally optimal preparation. A protocol optimised for osteogenic and fibroblastic signalling is not the protocol that maximises angiogenic and epithelialising signalling, and the two cannot be obtained simultaneously from a single spin.

That dissociation reframes the long-running debate between leukocyte-rich and leukocyte-poor PRP. The question is not which is better in the abstract, but which cellular payload the target tissue needs. For the synovial space, where leukocytes are associated with catabolic cytokine release and post-injection flare, a leukocyte-poor product obtained at 500 ×g is defensible even at a small cost in platelet yield. For a chronic wound, where angiogenesis, epithelialisation and antimicrobial activity are the goals, the leukocyte-rich product obtained at 200–300 ×g is the rational choice despite its lower platelet concentration. Our recommendation of 400 ×g / 9 min as a default reflects the fact that most musculoskeletal indications are dominated by PDGF- and TGF-β-driven repair, not that it is superior for every purpose.

The four-to-five-fold concentration achieved at the optimum also speaks to the dose question. The frequently cited target of a five-fold increase originates in early maxillofacial work [6] and has never been validated as a universal therapeutic threshold; there is evidence that excessive platelet concentration can be inhibitory rather than additive. What our data establish is that a single spin can reach the conventional target without resorting to a second processing step, and that pushing beyond it by increasing force is counterproductive.

Limitations

Several limitations qualify these findings. The study was performed in healthy volunteers aged 25–45 with normal blood counts; patients receiving PRP are frequently older, and platelet reactivity and growth factor content vary with age and sex [12]. Aliquots of 1.5 mL were processed in a fixed-angle rotor, and sedimentation geometry differs in the larger tubes and swing-out rotors used in some clinical systems, so absolute concentration factors may not transfer exactly even at identical relative centrifugal force. Growth factors were measured in releasate after exogenous activation, which reports total available payload rather than the kinetics of release in tissue [15]. Only single-spin protocols were tested, so no direct comparison with double-spin preparation is possible from these data. Finally, and

most importantly, composition is a surrogate: this study does not demonstrate that the protocol with the best in vitro profile produces the best clinical outcome.

Future directions

The natural extension is a randomised clinical comparison of the recommended protocol against a commonly used higher-force protocol, with a clinical rather than compositional endpoint. Parallel work should test whether the leukocyte-derived VEGF and EGF signal identified here translates into measurably better angiogenesis in wound models, and should repeat the factorial mapping in older and comorbid populations in whom baseline platelet function differs. Standardised reporting of relative centrifugal force, rotor geometry, aspiration depth and final leukocyte content — as required by the DEPA and PAW frameworks [5,14] — would allow future trials to be pooled meaningfully, which is currently impossible.

Conclusion

Centrifugation parameters determine the composition of single-spin PRP more than any other controllable variable, and relative centrifugal force dominates spin time by roughly thirty to one in its effect on platelet yield. A single spin at 400 ×g for 9 minutes concentrated platelets 4.85-fold while keeping CD62P expression at 10.7% and preserving discoid morphology in 89% of platelets, and it simultaneously maximised PDGF-BB and TGF-β1. It ranked first on a composite desirability index and is recommended as the default single-spin protocol for musculoskeletal and dental applications.

The corollary is equally important. Because VEGF and EGF derive predominantly from leukocytes rather than platelets, no single protocol optimises every growth factor. Preparation should be selected against the biological requirement of the target tissue: 400 ×g / 9 min when platelet-derived signalling is the objective, 500 ×g / 9 min when a leukocyte-poor intra-articular product is required, and 200–300 ×g for 5–7 minutes when leukocyte-associated angiogenic and antimicrobial activity is wanted. Adopting relative centrifugal force as the reported unit, and stating leukocyte content alongside platelet concentration, would remove much of the heterogeneity that currently prevents PRP trials from being compared.

Declarations

The study was approved by the institutional research ethics committee (protocol number [insert]) and conducted in accordance with the Declaration of Helsinki. All donors provided written informed consent.

Competing interests

The authors declare no competing interests. Any commercial relationship with manufacturers of the haematology analyser, multiplex immunoassay platform or centrifugation equipment used in this study should be disclosed here.

Funding

No funding.

Availability of data

The complete 36-protocol dataset is available from the corresponding author on reasonable request.

Authors' contributions

Conceptualisation and study design: [MHK, CAPMR]. Donor recruitment and blood collection: [MHK, BF, CGB]. Centrifugation and sample processing: [MHK, BF, CGB]. Flow cytometry and electron microscopy: [MHK, BF, CGB]. Immunoassay: [MHK, BF, CGB]. Statistical analysis: [MHK, BF, CGB]. Manuscript drafting: [MHK, CAPMR]. Critical revision and final approval: all authors.

Acknowledgements

The authors thank the phlebotomy and laboratory staff who supported donor recruitment and sample processing.

References

1. Perez AGM, Lana JFSD, Rodrigues AA, Luzo ACM, Belangero WD, et al. (2014) Relevant aspects of centrifugation step in the preparation of platelet-rich plasma. ISRN Hematol. 2014:176060.
2. Araki J, Jona M, Eto H. (2012) Optimized preparation method of platelet-concentrated plasma and noncoagulating platelet-derived factor concentrates: maximization of platelet concentration and removal of fibrinogen. Tissue Eng Part C Methods. 18(3):176-85.
3. Amable PR, Carias RBV, Teixeira MVT. (2013) Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. Stem Cell Res Ther. 4(3):67.
4. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. (2009) Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol. 27(3):158-167.
5. DeLong JM, Russell RP, Mazzocca AD. (2012) Platelet-rich plasma: the PAW classification system. Arthroscopy. 28(7):998-1009.
6. Marx RE. (2001) Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent. 10(4):225-228.
7. Anitua E, Sánchez M, Orive G, Andía I. (2007) The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials. 28(31):4551-60.
8. Sundman EA, Cole BJ, Fortier LA. (2011) Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am J Sports Med. 39(10):2135-40.
9. Boswell SG, Cole BJ, Sundman EA, Karas V, Fortier LA. (2012) Platelet-rich plasma: a milieu of bioactive factors. Arthroscopy. 28(3):429-39.
10. Mazzocca AD, McCarthy MBR, Chowaniec DM. (2012) Platelet-rich plasma differs according to preparation method and human variability. J Bone Joint Surg Am. 94(4):308-16.
11. Everts PA, Knape JTA, Weibrich G. (2006) Platelet-rich plasma and platelet gel: a review. J Extra Corpor Technol. 38(2):174-87.
12. Weibrich G, Kleis WKG, Hafner G, Hitzler WE. (2002) Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. J Craniomaxillofac Surg. 30(2):97-102.
13. Fitzpatrick J, Bulsara MK, McCrory PR, Richardson MD, Zheng MH. (2017) Analysis of platelet-rich plasma extraction: variations in platelet and blood components between 4 common commercial kits. Orthop J Sports Med. 5(1):2325967116675272.
14. Magalon J, Chateau AL, Bertrand B. (2016) DEPA classification: a proposal for standardising PRP use and a retrospective application of available devices. BMJ Open Sport Exerc Med. 2(1):e000060.
15. Kobayashi E, Flückiger L, Fujioka-Kobayashi M. (2016) Comparative release of growth factors from PRP, PRF, and advanced PRF. Clin Oral Investig. 20(9):2353-60.