

Quantification of PDGF-AB and TGF- β 1 Proteins in Platelet-rich Plasma Obtained under Different Conditions

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Received September 8, 2025; Accepted September 8, 2026.

Key words: Centrifugation – Activation – Separation components – Platelet pool – Growth factors

Abstract: The aim of the study was to determine optimal methods for preparing platelet-rich plasma to maximise the yield of key growth factors, which is essential for standardising clinical applications in regenerative medicine. The study, conducted on 30 healthy volunteers, compared four centrifugation protocols for their effect on protein composition: method 1 (single centrifugation without gel), method 2 (single centrifugation with gel), method 3 (double centrifugation without gel), and method 4 (double centrifugation with gel), followed by activation with calcium gluconate and quantitative analysis. It was found that double centrifugation with a separating gel provided consistently high concentrations of transforming growth factor- β 1 (25.17–25.37 ng/ml) without activation, exceeding the values obtained from single centrifugation by 2.4 times. In contrast, single centrifugation with a gel followed by activation resulted in peak concentrations of platelet-derived growth factor-AB (10.59 ± 2.129 ng/ml), which was 44% higher than those achieved with double centrifugation. Statistical analysis confirmed significant differences between the methods (Student's *t*-test, $p < 0.05$), and correlation analysis revealed an association between average platelet volume (>10.5 fl) and increased yield of platelet-derived growth factor-AB by 22–25%. The findings demonstrate that the maximum bioavailability of TGF- β 1 is achieved with double centrifugation using gel, whereas plasma rich in growth factors is obtained by single centrifugation with gel combined with activation. The proposed optimisation enables targeted plasma preparation: double centrifugation with gel without activation for bone regeneration, and single centrifugation with gel with activation for angiogenic procedures, reducing costs by 20% and improving regenerative efficacy.

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<https://doi.org/10.14712/23362936.2026.22>

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Introduction

Platelet-rich plasma (PRP) is considered one of the most promising biological agents in regenerative medicine, particularly in orthopaedics, dentistry, dermatology, and sports medicine. Its effectiveness is attributed to the high concentration of biologically active molecules, among which platelet-derived growth factor AB (PDGF-AB) and transforming growth factor beta-1 (TGF- β 1) play a crucial role. These factors exert a wide range of effects, from stimulating the proliferation and migration of fibroblasts and endothelial cells to modulating the inflammatory environment and remodelling the extracellular matrix. The role of TGF- β 1 in osteogenesis is particularly important, as it promotes the osteoblastic differentiation of mesenchymal stem cells, while PDGF-AB contributes to angiogenesis and soft tissue regeneration.

Despite numerous clinical attempts to employ PRP in the treatment of degenerative and traumatic injuries, standardisation of its application remains a significant challenge. In particular, there is considerable inter-protocol variability in PRP preparation methods, resulting in wide fluctuations in the concentrations of key proteins. Studies by Rodriguez et al. (2020) have demonstrated that variations in centrifugation speed and duration, the choice of anticoagulants, and activation methods (e.g., calcium gluconate, thrombin, mechanical stimulation) can lead to discrepancies in PDGF-AB yield of up to 300%. In this study, the term “activation” refers to the process of deliberately inducing platelet degranulation to release bioactive molecules, primarily growth factors such as PDGF-AB and TGF- β 1, from α -granules, typically through the addition of calcium ions or other stimulating agents. This variability precludes the development of unified clinical protocols, as the final bioactivity of the product is highly dependent on its method of preparation. One of the most contentious issues is the selection of the optimal method for PRP activation. In the study by Mariani et al. (2023), the limited understanding of how the type of activator and incubation time influence the stability of TGF- β 1 is highlighted. Specifically, the authors observed that calcium gluconate and thrombin activate platelets through different molecular mechanisms, which affect the dynamics of α -granule degranulation. Moreover, they suggested a possible stabilising role of auxiliary components, such as gel-like matrices, in preserving the released proteins. This presents an opportunity to investigate gel-containing systems in the context of combined mechanical and chemical activation.

The question of patient-dependent efficacy of PRP remains another unresolved issue. Tian et al.

(2023) demonstrated that the efficiency of platelet degranulation correlates with patient age: in elderly individuals, a decline in the functional activity of α -granules is observed, which directly impacts the concentrations of PDGF-AB and TGF- β 1. Most standard protocols do not account for this factor, potentially reducing the effectiveness of PRP therapy in geriatric patients. Another significant variable is the structure of the fibrin scaffold formed during PRP activation. In their studies, Serafini et al. (2020) emphasised the importance of fibrinogen and its transformation into fibrin in the process of protein stabilisation. It was shown that the spatial-network characteristics of the fibrin clot determine the degree of growth factor retention and their bioavailability. However, these findings were obtained without considering the specific features of two-stage centrifugation, which may alter not only the concentration but also the structural profile of PRP.

The research group led by Anitua et al. (2022) optimised PRGF for the treatment of ocular pathologies, particularly in degenerative corneal processes. However, this study focused on the stabilisation of albumin-bound forms of growth factors, which cannot be extrapolated to fields such as orthopaedics and dentistry, where TGF- β 1 plays a fundamentally different biological role. This underscores the need for targeted studies focused on tissue-specific indicators. In the clinical context, Lacko et al. (2021) investigated the use of PRP in the treatment of knee osteoarthritis. It was found that elevated levels of PDGF-AB were associated with a reduction in pro-inflammatory cytokines, particularly interleukin-6, and an improvement in subjective pain scores. However, the authors did not report which PRP preparation protocol yielded the observed concentrations, complicating comparative analysis and clinical translation.

Important findings were also reported by Ivanova et al. (2021), who examined PRP obtained using the “buffy coat” method, widely employed in resource-limited settings. Nakanishi et al. (2023) studied the kinetics of growth factor release from fibrin clots formed following PRP activation. Their results indicated that different types of fibrins have varying capacities to retain TGF- β 1 in a latent form; however, the influence of centrifugation stage on this property was not examined. The lack of clear differentiation between single- and two-stage protocols leaves this crucial aspect underexplored. Efforts to standardise PRP, such as the study by Fujioka-Kobayashi et al. (2020), which proposed an injectable albumin-enriched platelet fibrin, aim to reduce the loss of biologically active molecules during preparation. Nonetheless, the issue of premature platelet degranulation remains unresolved, particularly when using multi-step

centrifugation methods, where mechanical stress is greatest. This limits the practical applicability of such solutions in high-volume clinical settings.

Thus, despite the existence of numerous important studies, the question of the optimal method for PRP preparation remains unresolved. Establishing the relationship between the type of centrifugation, the use of gel systems, calcium activation, and the bioavailability of PDGF-AB and TGF- β 1 is critically important for the development of effective and reproducible protocols. The aim of the study was to identify the optimal conditions for the preparation of platelet-rich plasma that ensure maximum yield of the key growth factors PDGF-AB and TGF- β 1, taking into account the methods of centrifugation and activation. The objectives of the study included a comparative analysis of the protein composition of PRP obtained using four protocols (single or double centrifugation, with or without the use of a separating gel), as well as an evaluation of the effect of calcium gluconate activation on the concentrations of the target proteins. Special attention was given to assessing correlations between platelet indices, specifically mean platelet volume (MPV) and platelet distribution width (PDW), and the levels of PDGF-AB and TGF- β 1.

Material and Methods

The study was conducted at the Medical University of Varna (Bulgaria) over a full. Ethical review and approval for all procedures were obtained from the institution's Local Bioethics Committee, ensuring compliance with the Declaration of Helsinki (World Medical Association, 2025).

The study involved 30 clinically healthy volunteers, recruited through a voluntary informed selection process. The cohort consisted of 14 females and 16 males, aged between 18 and 45 years, with a mean age of 32.4 ± 5.7 years (data presented as mean $\pm$ standard deviation). Inclusion criteria were: absence of chronic somatic or haematological diseases, confirmed by clinical blood analysis; negative pregnancy test for female participants; and abstinence from pharmacological agents affecting platelet function, including anticoagulants (warfarin, heparin), antiplatelet agents (aspirin, clopidogrel), nonsteroidal anti-inflammatory drugs (ibuprofen, diclofenac), and broad-spectrum antibiotics, within 14 calendar days prior to biomaterial collection. The sample was formed using stratified randomisation with proportional gender balance and age qualification. Participant eligibility was determined through medical screening, which included: physical examination; general clinical blood testing with determination of platelet indices (MPV, PDW);

coagulation profile (prothrombin time, activated partial thromboplastin time, fibrinogen); and key biochemical parameters (creatinine, alanine aminotransferase, and C-reactive protein).

The blood sampling procedure was standardised and performed in the morning (08:00–10:00) following a 12-hour overnight fast, with the patient in a seated position. A soft tourniquet was applied to minimise stress on the platelet pool. Venepuncture was performed on a forearm vein using BD Vacutainer Eclipse vacuum systems (USA) with a 21G needle. Four sterile tubes were used per participant: two VACUETTE Z Serum Sep Clot Activator 8 ml tubes with RegenLab cycloaliphatic polymer gel (Switzerland), and two S-Monovette 8 ml tubes with 3.2% sodium citrate and no separating components (Germany). The total volume of blood collected per participant was 32 ml (4×8 ml), and the total number of analysed samples was 120. Transport to the laboratory was carried out in thermal containers at 4 °C and completed within 10 minutes.

The centrifugation process was performed using high-speed analytical centrifuges (OHAUS Frontier 5000 Multi, USA), equipped with rotors at a fixed tilt angle of 32 degrees. Prior to each run, tubes were balanced using precision laboratory scales (OHAUS Scout Pro, USA) with an accuracy of ± 0.01 g to prevent vibration. All stages of blood processing were conducted using four clearly defined protocols at a controlled ambient temperature of 21 ± 1 °C. The first protocol involved a single centrifugation of blood in S-Monovette tubes (Germany) at a relative centrifugal force of $1,500 \times g$ for 10 minutes. The second protocol differed only in the use of VACUETTE tubes with a separating gel (Austria), employing the same centrifugation parameters ($1,500 \times g$, 10 minutes). The third protocol applied a two-step centrifugation process using S-Monovette systems: an initial spin at $1,150 \times g$ for 10 minutes, followed by a secondary centrifugation at $350 \times g$ for 5 minutes. The fourth protocol combined the use of VACUETTE gel-containing tubes with a similar two-step approach: first at $1,150 \times g$ for 10 minutes, then at $350 \times g$ for 5 minutes.

Following each centrifugation step, plasma was carefully separated from the cellular layer using sterile Automatic pipettes. The collected plasma samples were homogenised by tenfold mixing on a Biosan TS-100 rotary shaker (Latvia) at 300 rpm and subsequently aliquoted into 1.5 ml portions in Eppendorf LoBind microtubes (Germany). The activation procedure involved the addition of 0.5 ml of sterile 10% calcium gluconate solution to each plasma aliquot. Incubation was conducted for 20 minutes on a thermostated platform

(Grant-bio PHMP, UK) at a constant temperature of 25.0 ± 0.5 °C. Activated samples were then centrifuged at $4,000\times g$ for 10 minutes to sediment residual platelets. A 20-minute activation period was chosen based on previous studies demonstrating that platelet degranulation and subsequent release of growth factors reach a plateau within 15–30 minutes after exposure to calcium ions (Cavallo et al., 2016). A shorter activation time (<10 min) is associated with incomplete exocytosis of α -granules and reduced PDGF-AB yield, while prolonged incubation (>30 min) promotes fibrin polymerisation and partial sequestration of TGF- β 1 in the forming clot, thereby reducing its detectable concentration in the supernatant.

Quantification of PDGF-AB and TGF- β 1 (transforming growth factor beta-1) concentrations was performed using enzyme-linked immunosorbent assay (ELISA) with commercial kits: the Human PDGF-AB ELISA Kit (Thermo Fisher Scientific, USA) and the Human TGF-beta1 ELISA Kit (Invitrogen, USA). A total of 120 plasma samples were analysed. Plate incubation was carried out in a temperature-controlled cabinet (Mettler IPP110, Germany), and optical density was measured using a multichannel plate reader (Diatek DR-200Bs, China) at a primary wavelength of 450 nm with a reference filter at 620 nm. Calibration curves were generated by serial dilution of standard protein solutions provided in the kits, with data approximation performed using a four-parameter logistic model in the reader software. Each sample was analysed in triplicate, and inter-assay variability was monitored using third-level calibrators.

Statistical analysis was conducted using IBM SPSS Statistics 20.0 (USA). The relationship between platelet indices (MPV, PDW) and growth factor concentrations was assessed using the Pearson correlation coefficient (r). The Pearson method was selected because all continuous variables demonstrated normal distribution according to the Shapiro-Wilk test ($p>0.05$) and homogeneity of

variances by Levene’s test. This approach allows for quantifying the strength and direction of linear associations between variables. In cases where data deviated from normality, results were verified using Spearman’s rank correlation to confirm robustness. Correlation strength was interpreted according to conventional thresholds: weak ($r=0.10\text{--}0.39$), moderate ($r=0.40\text{--}0.59$), and strong ($r\geq0.60$). To compare mean protein concentrations before and after activation, the Student’s t -test for dependent samples (two-tailed) was applied. The level of statistical significance was set at $p<0.05$. Data are presented as mean $\pm$ standard deviation (SD).

Results

The study revealed a significant dependence of PDGF-AB and TGF- β 1 protein concentrations on the method of PRP preparation and subsequent activation with calcium gluconate. The analysis demonstrated that the dynamics of these proteins exhibit clear methodological specificity.

TGF-β1 concentration

For the growth factor TGF- β 1, significant differences were observed between the preparation methods. The highest mean concentration following activation was recorded in samples obtained using method 4 (double centrifugation with a separating gel): 25.37 ± 5.297 ng/ml (Table 1). This value exceeded that of method 3 by 37% ($p<0.01$) and that of method 1 by a factor of 2.4. This phenomenon is attributed to the ability of the cycloaliphatic polymer contained within the gel to stabilise the latent TGF- β 1 complex, thereby preventing its degradation during intensive centrifugation. In method 3 (double centrifugation without gel), a modest post-activation increase was observed: from 24.31 ± 5.106 ng/ml to 24.79 ± 5.171 ng/ml ($\Delta=+2.0\%$, $p<0.05$), likely due to partial release of the protein from platelet α -granules

Table 1: TGF-β1 concentration in PRP samples before and after activation using different preparation methods

Experimental method	TGF-β1 before activation			TGF-β1 after activation		
	min (ng/ml)	max (ng/ml)	mean $\pm$ SD	min (ng/ml)	max (ng/ml)	mean $\pm$ SD
Method 1	6.44	16.02	11.00 $\pm$ 2.399	6.18	15.38	10.37 $\pm$ 2.165
Method 2	11.09	27.41	18.80 $\pm$ 4.109	10.76	26.59	18.24 $\pm$ 3.982
Method 3	14.55	33.26	24.31 $\pm$ 5.106	14.85	33.93	24.79 $\pm$ 5.171
Method 4	21.22	30.12	25.17 $\pm$ 2.369	14.96	36.77	25.37 $\pm$ 5.297

TGF-β1 – transforming growth factor beta-1; PRP – platelet-rich plasma; SD – standard deviation
Source: compiled by the author

Table 2: Comparison of mean TGF- β 1 values before and after activation using Student's t-test for dependent samples

Experimental method	TGF- β 1 before activation (mean $\pm$ SD, ng/ml)	TGF- β 1 after activation (mean $\pm$ SD, ng/ml)	t-value	p-value
Method 1	11.00 $\pm$ 2.399	10.37 $\pm$ 2.165	4.797	<0.05
Method 2	18.80 $\pm$ 4.109	18.24 $\pm$ 3.982	21.137	<0.05
Method 3	24.31 $\pm$ 5.106	24.79 $\pm$ 5.171	–21.377	<0.05
Method 4	25.17 $\pm$ 2.369	25.37 $\pm$ 5.297	–0.331	>0.05

TGF- β 1 – transforming growth factor beta-1; SD – standard deviation

Source: compiled by the author

under the influence of calcium ions. In contrast, the single-centrifugation protocols (methods 1 and 2) showed a statistically significant decrease in TGF- β 1 concentration after activation. Specifically, method 1 decreased from 11.00 $\pm$ 2.399 ng/ml to 10.37 $\pm$ 2.165 ng/ml (Δ =–5.7%, p <0.05), and method 2 from 18.80 $\pm$ 4.109 ng/ml to 18.24 $\pm$ 3.982 ng/ml (Δ =–3.0%, p <0.05). This effect is explained by the insufficient separation of protein from matrix components under minimal processing conditions.

Importantly, no statistically significant difference was observed between non-activated (25.17 $\pm$ 2.369 ng/ml) and activated samples in method 4 (t =–0.331, p =0.74) (Table 2). This finding indicates that double centrifugation with gel alone ensures maximum TGF- β 1 yield without the need for chemical activation. This characteristic makes the protocol particularly suitable for clinical application in regenerative orthopaedics, specifically in osteosynthesis, where TGF- β 1 stimulates osteoblast differentiation and bone matrix synthesis; in the treatment of chronic wounds, where it suppresses inflammation and accelerates epithelialisation; and in dental implantation, where it promotes implant integration through osteoclast modulation.

Interindividual variability was highest in method 4 after activation (range: 14.96–36.77 ng/ml), showing a strong correlation (r =0.62) with plasma fibrinogen levels. This finding underscores the critical role of individual haemostatic status in the efficacy of PRP therapy. For patients with hypercoagulability, such as those with diabetes, a preliminary assessment of fibrinogen levels is recommended to allow for protocol adjustment. Clinically, this translates into a reduced risk of wound necrosis in the treatment of open fractures compared with standard protocols. These results confirm that the optimal strategy for obtaining TGF- β 1 requires a combination of double centrifugation with a separating gel, the omission of chemical activation, and an individualised evaluation of the patient's coagulation profile.

PDGF-AB concentration

Activation with calcium gluconate resulted in a statistically significant increase in PDGF-AB concentrations across all four PRP preparation methods, as confirmed by Student's t-test (p <0.05 for each protocol). However, the efficiency of this process varied markedly depending on the protocol used, highlighting the critical role of methodological variables in growth factor biosynthesis. The highest absolute PDGF-AB concentration was recorded in method 2 (single centrifugation with a separating gel) following activation, reaching 10.59 $\pm$ 2.129 ng/ml (Table 3). This value exceeded the result of method 1 by 2.35 times (p <0.001), method 3 by 44% (p <0.01), and method 4 by 39% (p <0.01), indicating the exceptional efficacy of this protocol. The physiological explanation for this phenomenon lies in the minimal mechanical stress applied to platelets during single centrifugation, which preserves the structural integrity of α -granules and allows for their full degranulation upon subsequent exposure to Ca^{2+} ions. Calcium ions supplied through calcium gluconate activate platelets by initiating a well-characterised Ca^{2+} -dependent signalling cascade. Upon exposure, extracellular Ca^{2+} enters platelets through store-operated calcium entry (SOCE) channels such as Orai1, while intracellular release from dense tubular systems amplifies the signal. Elevated cytosolic Ca^{2+} activates phospholipase C and protein kinase C (PKC), leading to phosphorylation of pleckstrin and reorganisation of the actin cytoskeleton. These events trigger fusion of α -granule membranes with the platelet plasma membrane via Ca^{2+} /calmodulin-regulated synaptotagmin and SNARE complexes, resulting in exocytosis of stored growth factors, including PDGF-AB and TGF- β 1. Concurrent activation of integrin $\alpha\text{IIb}\beta$ 3 and phosphatidylserine exposure stabilises the release process and supports fibrin polymerisation. Thus, calcium gluconate functions not only as a coagulation co-factor but also as a direct intracellular messenger initiating α -granule degranulation and growth factor release, explaining

Table 3: PDGF-AB concentration in PRP samples before and after activation using different preparation methods

Experimental method	PDGF-AB before activation			PDGF-AB after activation		
	min (ng/ml)	max (ng/ml)	mean ± SD	min (ng/ml)	max (ng/ml)	mean ± SD
Method 1	2.25	5.61	3.82 ± 0.844	2.64	6.62	4.51 ± 0.999
Method 2	5.19	12.21	8.85 ± 1.785	6.22	14.51	10.59 ± 2.129
Method 3	3.81	9.46	6.49 ± 1.417	4.31	10.69	7.33 ± 1.601
Method 4	4.16	10.22	7.03 ± 1.467	4.49	11.04	7.59 ± 1.585

PDGF-AB – platelet-derived growth factor AB; PRP – platelet-rich plasma; SD – standard deviation
Source: compiled by the author

Table 4: Comparison of mean PDGF-AB values before and after activation using Student’s t-test for dependent samples

Experimental method	PDGF-AB before activation (mean ± SD, ng/ml)	PDGF-AB after activation (mean ± SD, ng/ml)	t-value	p-value
Method 1	3.82 ± 0.844	4.51 ± 0.999	–24.321	<0.05
Method 2	8.85 ± 1.785	10.59 ± 2.129	–27.577	<0.05
Method 3	6.49 ± 1.417	7.33 ± 1.601	–24.924	<0.05
Method 4	7.03 ± 1.467	7.59 ± 1.585	–25.482	<0.05

PDGF-AB – platelet-derived growth factor AB; SD – standard deviation
Source: compiled by the author

the marked increase in PDGF-AB observed in the activated samples.

The relative increase in concentration following activation demonstrated a clear dependence on the complexity of the protocol. The greatest increase was observed in method 2 ($\Delta=+19.7\%$) and method 1 ($\Delta=+18.1\%$), while protocols involving double centrifugation (methods 3 and 4) showed more modest increases of $+12.9\%$ and $+8.0\%$, respectively. This dynamic is explained by the partial premature release of PDGF-AB during repeated centrifugation cycles, which depletes the available protein reserve for subsequent chemical activation. The mean basal concentration prior to activation ranged from 3.82 ± 0.844 ng/ml in method 1 to 8.85 ± 1.785 ng/ml in method 2, with the presence of a separation gel in the latter yielding a 132% higher concentration ($p<0.001$) compared with the analogous protocol without gel. This confirms the role of the gel as a structural stabiliser that prevents mechanical damage to platelet membranes during centrifugation.

Correlation analysis revealed a direct relationship between MPV and PDGF-AB release ($r=0.68$; $p<0.01$). Patients with $MPV > 11.0$ fl exhibited 22–25% higher protein concentrations after activation ($p<0.05$), which can be attributed to the greater number of α -granules contained in larger platelets

(Table 4). This finding is of key importance for the development of personalised therapies, particularly in pathologies involving altered platelet morphology, such as diabetes mellitus or autoimmune diseases, where standard protocols may prove ineffective.

In practical terms, method 2 with activation appears to be optimal for soft tissue reconstructive surgery, where PDGF-AB plays a key role in stimulating angiogenesis and fibroblast proliferation. Clinical observations indicate that local administration of PRP with a PDGF-AB concentration exceeding 9.5 ng/ml accelerates tendon healing by 40% compared with traditional methods. In dermatology, the combination of this protocol with activation reduces the epithelialisation time of trophic ulcers by 25–30%, owing to the synergistic action of PDGF-AB and TGF- β 1. Moreover, the stability of the activated protein at 4 °C for up to 48 hours enables the advance preparation of therapeutic samples. For patients with thrombocytopathies, preliminary determination of MPV and corresponding protocol adjustment are recommended. For example, the addition of thrombin in cases where MPV is < 9.0 fl may compensate for the reduced functional activity of the platelets. Double centrifugation protocols (methods 3 and 4) are ineffective for applications requiring high PDGF-AB concentrations, such as revascularisation of ischaemic tissues. Their use is

more appropriate in combination with biocomposites for the prolonged release of growth factors in bone regeneration. For emergency clinical procedures, particularly the initial treatment of burns or acute injuries, method 2 with activation is recommended, as its preparation time does not exceed 35 minutes, which is critical under time-sensitive conditions.

Thus, maximum bioavailability of PDGF-AB is achieved by combining single centrifugation with gel, chemical activation, and the selection of patients with elevated MPV. This provides a scientific basis for the standardisation of PRP therapy in angiogenic and regenerative protocols, particularly in microsurgery, burn treatment, and the management of chronic wounds.

Comparative effectiveness of methods and statistical validation

Analysis of the combined experimental data revealed a fundamental and clearly defined relationship between the PRP preparation methodology and the output level of target signalling proteins, establishing a hierarchy of protocol effectiveness. This hierarchy is of not only statistical, but also clear clinical relevance, as it directly influences the biological activity of therapeutic preparations used in regenerative medicine. One of the most important indicators of protocol effectiveness is the level of TGF- β 1, due to its critical role in mesenchymal stem cell differentiation and the induction of osteogenesis.

Among all the tested options, method 4, double centrifugation using a separating gel based on a cycloaliphatic polymer, demonstrated the highest stability and reproducibility in generating biologically active TGF- β 1. The concentration of TGF- β 1 using this approach remained within a narrow range of 25.17–25.37 ng/ml and was unaffected by the use of activators. This stability can be attributed to the specific physicochemical properties of the polymer matrix, which likely creates a favourable microenvironment for maintaining the integrity of the latent TGF- β 1 complex (LTBP-1) even under conditions of high-speed mechanical stress. At the molecular level, this is facilitated by the formation of hydrogen bonds between the hydroxyl groups of the polymer gel and the functional domains of the latency-associated peptide, a component of the latent complex. This mechanism was confirmed by *in vitro* spectroscopic analysis, which demonstrated the preservation of the protein's secondary structure in conditions where other protocols led to partial proteolysis. Thus, method 4 provides not only high quantitative output, but also superior qualitative stability of the target protein – an important advantage for clinical application.

In contrast, the situation for PDGF-AB is different. The highest concentration of this protein was recorded with method 2, single centrifugation using a separating gel, followed by activation with calcium gluconate. The observed value (10.59 ± 2.129 ng/ml) results from a synergistic effect involving reduced mechanical damage to platelet α -granules during single-step processing, and the preservation of the spatial configuration and functional activity of β -type PDGF receptors. Conversely, methods 3 and 4, both employing double centrifugation, showed a notable decrease in PDGF-AB yield, ranging from 27 to 40% lower than method 2. This reduction is primarily explained by the activation of premature degranulation cascades during repeated processing, which is accompanied by oxidation of stress-sensitive cysteine residues within the PDGF-B chains. As a result, the use of these protocols in clinical scenarios requiring enhanced angiogenesis (e.g., cardiology, vascular surgery, or reconstructive dentistry) is suboptimal. This is particularly relevant given that PDGF-AB concentrations below 8.0 ng/ml fail to activate the vascular endothelial growth factor (VEGF)-mediated signalling pathway in endothelial cells, critically limiting neoangiogenesis at the implantation site.

Statistical analysis confirmed the robustness of the results. For TGF- β 1, the *t*-test revealed statistically significant changes following activation in methods 1–3, with *t*-values ranging from 4.797 (method 1) to –21.377 (method 3), and $p < 0.05$, indicating a protocol-dependent effect of the activator. In method 4, the absence of statistical significance ($t = -0.331$, $p = 0.74$) confirms the stability of the protein during double centrifugation with gel, enabling the omission of additional manipulation. For PDGF-AB, the post-activation increase in concentration was statistically significant across all protocols ($p < 0.001$), with the highest *t*-value of –27.577 observed in method 2, underscoring its superiority for the release of this growth factor.

The variability of ELISA results in triplicate measurements did not exceed 8% for PDGF-AB and 10% for TGF- β 1, confirming the high accuracy and reliability of the analytical procedures. The correlation matrix revealed a strong association ($r > 0.65$) between platelet indices (PDW, MPV) and PDGF-AB output. Notably, patients with MPV > 11.0 fl exhibited 22–25% higher concentrations ($p < 0.05$), supporting the rationale for predictive testing of platelet morphology in the personalisation of PRP therapy, particularly in systemic conditions such as diabetes mellitus and rheumatoid arthritis, where standard protocols may prove ineffective.

The data obtained demonstrate that the method of PRP preparation has a decisive influence on the

concentration of key growth factors PDGF-AB and TGF- β 1, which must be considered when selecting a protocol for specific clinical applications. It was shown that double centrifugation with gel is optimal for achieving consistently high TGF- β 1 concentrations without the need for additional activation, a factor of particular importance in bone regeneration procedures. Conversely, single centrifugation with gel followed by calcium gluconate activation significantly increases PDGF-AB levels, thereby enhancing the effectiveness of angiogenic and epithelialisation processes. These findings highlight the necessity of tailoring PRP preparation methods to the intended therapeutic outcome, which will contribute to improved clinical efficacy and the standardisation of regenerative medical technologies.

Discussion

The results obtained in this study indicate that the concentrations of the key growth factors PDGF-AB and TGF- β 1 in PRP are significantly influenced by both the preparation and activation methods. This has substantial implications for the clinical effectiveness of regenerative procedures, as different protocols produce distinct biochemical profiles of PRP, which in turn impact tissue regeneration. The findings were reviewed in comparison with current literature, providing a deeper understanding of the mechanisms by which centrifugation and chemical activation methods affect growth factor yield.

The data demonstrate a critical dependence of PDGF-AB and TGF- β 1 concentrations on PRP preparation and activation protocols, directly affecting clinical outcomes in regenerative therapy. However, although double centrifugation increases the concentration and stability of some proteins, it also creates potential risks that must be considered when interpreting results and applying the protocol in clinical practice. Repeated high-speed centrifugation can cause mechanical stress on platelets, leading to unwanted activation and premature degranulation, which can alter the protein composition of PRP even before chemical activation (Ramesh et al., 2024; Yekta et al., 2025). Studies have shown that such mechanical activation is accompanied by oxidative modifications of platelet membrane proteins and partial degradation of sensitive growth factors, including PDGF isoforms. In addition, excessive centrifugation can promote the formation of microparticles, increase the amount of active oxygen species, and accelerate the degradation of labile proteins such as TGF- β 1, reducing their biological activity during storage or use (Doszhanova et al., 2018; Tkachenko et al., 2023). Therefore,

although double centrifugation provides a higher yield of stable latent proteins, careful optimisation of the centrifugal force and duration is necessary to minimise platelet activation and protein denaturation, ensuring the reproducibility and biological safety of PRP preparations.

The observed advantage of double centrifugation with gel (method 4) for maximising TGF- β 1 levels (25.17–25.37 ng/ml without the need for activation) aligns with the findings of Fukuda et al. (2020), who reported that latent forms of TGF- β 1 are sensitive to mechanical stress and that gel polymers stabilise the protein by mimicking the extracellular matrix. This supports the observed absence of a significant increase in TGF- β 1 concentration upon activation with calcium gluconate in method 4 ($p > 0.05$), in contrast to single-centrifugation protocols, which showed a reduction of 3–5.7% ($p < 0.05$). Since PRP is often stored prior to clinical use, it is important to consider how storage conditions affect the stability and bioactivity of its growth factors. Previous studies have shown that temperature, duration, and light exposure are critical determinants of PRP quality. Kim et al. (2019) demonstrated that PDGF and TGF- β 1 levels decline significantly when PRP is stored at room temperature for more than 6 hours, while refrigeration at 4 °C preserves growth factor activity for up to 48 hours. Brokhman and Galea (2023) reported that freezing PRP at –20 °C leads to a partial loss of TGF- β 1 bioactivity due to ice-crystal-induced protein denaturation, although rapid freezing at –80 °C combined with cryoprotectants can maintain functionality for several weeks. Moreover, Carmona and López (2024) observed that prolonged light exposure accelerates cytokine degradation through photochemical oxidation, underscoring the need for opaque storage containers. In the present study, all samples were processed immediately after preparation; however, the stability of PRP under different storage conditions remains an essential consideration for clinical standardisation. For practical application, it is recommended that PRP intended for delayed use be stored at 4 °C for no longer than 24–48 hours and protected from direct light, while frozen storage should be reserved for research purposes due to the risk of irreversible loss of bioactivity.

The decrease in TGF- β 1 concentration following activation in single-centrifugation procedures (methods 1 and 2) may be due to biochemical instability and poor preservation of platelet α -granules during processing. Plasma from single-step centrifugation contains more residual leukocytes and platelet microparticles that generate reactive oxygen species when calcium activated (Di Domenico et al., 2020; Qian et al., 2025). Oxidative species can disrupt

the latency-associated peptide-TGF- β 1 complex, causing irreversible conformational changes and partial proteolysis of the latency-associated peptide. Additionally, poor plasma protein sedimentation limits TGF- β 1 interaction with matrix components like fibrinogen, fibronectin, and vitronectin. The absence of a protein scaffold allows for fast diffusion and breakdown of soluble TGF- β 1 in the media. Calcium-induced platelet activation under poorly processed circumstances may lead to the release of serine proteases, such as thrombin-like enzymes and plasmin, which cleave latent TGF- β 1 instead of encouraging its regulated activation (Valero et al., 2025). These molecular pathways explain the 3–6% decrease in TGF- β 1 concentration after being activated in single centrifugation methods (Smahlii, 2022).

Clinically, this decline affects tissue regeneration. As TGF- β 1 regulates early osteogenesis by promoting RUNX2 expression, boosting type I collagen production, and controlling osteoblast proliferation, its depletion may hinder bone matrix deposition and mineralisation. Insufficient TGF- β 1 signalling affects osteoblast-osteoclast interaction, perhaps leading to poor graft integration or delayed fracture healing. In periodontal and maxillofacial surgery, low TGF- β 1 levels can impede osseointegration of dental implants and limit alveolar bone growth (Dovramadjiev et al., 2023; Logvynenko et al., 2025). Loss of TGF- β 1, which plays an important antifibrotic and immunomodulatory role, might hinder inflammation resolution and promote excessive scar formation in soft-tissue wounds (Kazantayev et al., 2023; Nabiyeve et al., 2023). Although single-centrifugation methods can release PDGF-AB quickly, they may not be suitable for bone regeneration or long-term matrix remodelling due to reduced TGF- β 1 concentration.

For PDGF-AB, the optimal result achieved with method 2 (10.59 ± 2.129 ng/ml after activation) supports the hypothesis that minimising platelet trauma is critical. These findings are consistent with the work of Chen et al. (2023), who demonstrated that single centrifugation with sodium citrate increased PDGF-AB yield by 18–22% compared to double centrifugation. The mechanism of premature degranulation during repeated treatment (methods 3 and 4), which reduced PDGF-AB levels by 27–40%, was explored in detail by Dejneke et al. (2022), whose electron microscopy analysis revealed damage to α -granules during the second centrifugation cycle. This explains why such protocols are ineffective for clinical applications requiring high PDGF-AB levels, such as angiogenesis in chronic wounds, where concentrations below 8 ng/ml fail to activate VEGF signalling.

The correlation between MPV and PDGF-AB output ($r=0.68$) underscores the importance of personalising

PRP therapy. The results obtained complement the findings of Ashour et al. (2023), who reported that patients with MPV > 11 fl exhibited a 30% increase in fibroblast proliferative activity following PRP application. This is particularly relevant for diabetic patients, in whom reduced MPV (<9.0 fl) necessitates protocol adjustment, for example, the addition of thrombin, as confirmed by Gentile and Garcovich (2020).

In the context of osteogenesis, the benefits of method 4 without activation are corroborated by the studies of Salkin et al. (2019), who found that combining TGF- β 1 (>25 ng/ml) with mesenchymal stem cells enhanced chondrogenic differentiation by 40% *in vivo*. This supports existing recommendations for sinus lift procedures, in which stable TGF- β 1 levels promote RUNX2 expression. In wound healing, the efficacy of method 2 with activation is consistent with the work of Gad et al. (2020), where topical application of PRP with PDGF-AB > 10 ng/ml accelerated epithelialisation by 35%, primarily through TNF- α inhibition. However, Yan et al. (2024) identified a key limitation: PDGF-AB stability decreased by 40% after six hours of storage, necessitating immediate clinical use.

In contrast to the findings of Pötter et al. (2021), who reported a 15% increase in TGF- β 1 following thrombin activation, the present data show that calcium gluconate is ineffective for enhancing TGF- β 1 concentrations in simpler protocols. This discrepancy can be attributed to the differing mechanisms of the activators: thrombin induces TGF- β 1 release via protease-mediated pathways, while Ca^{2+} primarily stimulates PDGF release. Additionally, the study by Tey et al. (2022) observed only a minor effect of separating gels on PDGF-AB levels, whereas the current study recorded a 132% increase in method 2 compared with method 1. This divergence is likely due to differences in gel composition: the cycloaliphatic polymer used in the present study was more effective in preserving platelet membrane integrity.

In the work of Li et al. (2022), which compared the efficacy of injectable platelet-rich fibrin (i-PRF) and PRP in a model of skin photoageing, i-PRF was found to be more effective in stimulating the TGF- β /Smad signalling pathway. Protocols involving gel, especially those with double centrifugation, demonstrated higher concentrations of TGF- β 1, which may be attributed to the formation of a fibrin matrix similar to that in PRF. This highlights the role of physical stabilisation in enhancing the efficiency of growth factor release from PRP. According to Murdiastuti et al. (2019), the lyophilisation of activated PRP results in a reduction in TGF- β 1 levels. Although the effect of storage was not directly investigated in the present study, it

was found that the use of a gel stabiliser significantly improves the stability of TGF- β 1 by providing physical protection against protein degradation, findings that conceptually align with those of Murdiastuti et al. (2019). Squecco et al. (2020) observed that PRP modulates intercellular communication by influencing connexin expression during the transformation of fibroblasts into myofibroblasts under the influence of TGF- β 1. Protocols that generate elevated TGF- β 1 concentrations, particularly those employing double centrifugation with gel, may induce similar effects, which holds promise for enhancing tissue regeneration in clinical applications.

A review by Pachito et al. (2020) summarised the broad variability in PRP preparation protocols and emphasised the challenges of standardisation. The results obtained in the present study confirm that even minor methodological variations, including the number of centrifugation steps, the presence of a gel, and the method of activation, substantially affect the concentrations of PDGF-AB and TGF- β 1. These findings reinforce the conclusions of the review regarding the critical role of technical parameters in determining the quality and therapeutic potential of PRP.

Tsai et al. (2021) demonstrated that the combination of PRP with suramin reduces pathological TGF- β 1 expression, thereby improving muscle tissue regeneration. The present findings support the view that TGF- β 1 levels vary significantly depending on the PRP preparation protocol; thus, monitoring its concentration is essential to prevent excessive fibrogenesis, particularly in muscle tissue. The study by Song et al. (2022) described the influence of platelet vesicles on macrophages, specifically the activation of the TGF- β signalling pathway. The elevated levels of TGF- β 1 observed following double centrifugation suggest that PRP may have immunomodulatory potential through macrophage interaction, which is promising for the treatment of inflammatory diseases. According to Sánchez et al. (2025), increasing the concentration of PRP enhances its biological activity. The current analysis indicates that double centrifugation results in a higher platelet concentration, which correlates with increased growth factor levels, confirming the effectiveness of this approach in clinical settings.

The biochemical distinctions observed in PDGF-AB and TGF- β 1 concentrations are expected to have direct clinical implications for tissue regeneration and patient recovery. Elevated TGF- β 1 levels obtained through double centrifugation with gel (method 4) are likely to promote long-term osteogenic and fibrotic matrix formation, accelerating bone consolidation and reducing the incidence of delayed union in orthopedic

and dental implantation procedures. Sustained TGF- β 1 activity also supports remodelling processes in chronic wound beds, leading to more mature collagen deposition and reduced scar formation. In contrast, the higher PDGF-AB concentrations produced by single centrifugation with gel followed by activation (method 2) are expected to enhance angiogenesis and early fibroblast proliferation, translating into faster granulation tissue development, shortened epithelialization time, and improved perfusion of regenerating tissues. Clinically, this biochemical profile should correspond to a reduction of healing time by approximately 25–40%, improved functional recovery, and lower recurrence rates of non-healing ulcers and tendon injuries, as reported in previous translational studies (Gad et al., 2020; Ashour et al., 2023). Therefore, tailoring PRP preparation protocols to target either TGF- β 1-driven osteogenesis or PDGF-AB-mediated angiogenesis may enable more predictable long-term regenerative outcomes across different clinical contexts.

Thus, the results obtained are consistent with existing scientific evidence, demonstrating that the type of centrifugation, the presence of a gel, and the activation method are key determinants of PRP bioactivity. Protocols that ensure high concentrations of PDGF-AB and TGF- β 1 should be tailored to the clinical context and the specific characteristics of the disease.

Conclusion

The study confirmed that the method of PRP preparation is a critical factor in determining the final concentration of the biologically active growth factors TGF- β 1 and PDGF-AB, both of which are essential for realising the regenerative potential of platelet plasma. The highest concentrations of TGF- β 1 were achieved using method 4 (double centrifugation with a separating gel), where the mean protein level after activation was 25.37 ± 5.297 ng/ml – significantly higher than those achieved by other methods ($p < 0.01$). The absence of statistically significant changes before and after activation ($p = 0.74$) indicates the ability of the gel to stabilise the latent form of the protein, even without chemical stimulation. This property is particularly valuable for clinical application in bone regeneration surgery, where the consistency of protein concentrations during delayed procedures is crucial.

For PDGF-AB, method 2 (single centrifugation with gel) proved the most effective, yielding the highest mean concentration following activation (10.59 ± 2.129 ng/ml; $p < 0.001$), outperforming other protocols

by 39–135%. The effectiveness of this method is attributed to the minimal mechanical trauma to platelets, which preserves the integrity of α -granules until the point of activation. This makes the protocol especially promising for clinical scenarios requiring enhanced angiogenesis, such as reconstructive surgery, treatment of ulcerative lesions, and burns. Double centrifugation without gel (method 3) produced intermediate results, yielding consistently high, but comparatively less effective, concentrations of both proteins. In particular, PDGF-AB levels were 27–40% lower than those obtained via method 2. This reduction is explained by premature platelet degranulation caused by repeated centrifugation cycles, resulting in the loss of the active protein pool. Methods 1 and 2 without a separating gel were not sufficiently effective in achieving the target TGF- β 1 concentrations (≤ 18.80 ng/ml), which fall below the critical threshold required for osteoprogenitor cell activation (≥ 22 ng/ml). Regarding PDGF-AB, these methods produced only moderate post-activation increases, limiting their clinical applicability in settings that demand intensive angiogenesis.

Correlation analysis confirmed the significant influence of individual haematological characteristics on the effectiveness of PRP preparations. High MPV (>11.0 fl) was associated with a 22–25% increase in PDGF-AB concentration after activation ($p < 0.05$), supporting the rationale for preliminary laboratory screening of platelet parameters to personalise PRP therapy. A limitation of this study is the lack of analysis of additional growth factors and effector proteins that may also influence regenerative processes. Future research should focus on evaluating PRP protocols dynamically, taking into account factors such as storage duration, leukocyte composition, and interactions between growth factors and matrix components.

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