European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: Applied Website:
https://farmclin.com/

Platelet Rich Plasma and key Growth Factors and their Role in Hair Restoration

Prabhu Chandra Mishra1, Saurabh K Gupta2, Saurabh Kumar Jha3, Chaitenya Verma1*

1Department of Biotechnology, Sharda School of Engineering & Technology, Sharda University, Greater Noida, India.
2MS MCH (Plastic Surgery), FICS (Plastic Surgery),Elixir Health Care, C 40, sector 23 , Noida 201301, India..
3Department of Zoology, Kalindi college, University of Delhi, India..
.
***Corresponding Author:** chaitenya.verma@sharda.ac.in

ABSTRACT

Background: Platelet-rich plasma (PRP) therapy has been a popular androgenetic alopecia therapeutic modality, but it does not have consistent clinical efficacy or repeatability due to differences in preparation protocols used.

Objective: to determine the outcomes of the use of a manual double-spin PRP procedure on platelet collection, growth factor recovery, and hair restoration outcomes, keeping a constant temperature.

Methods: The sample of peripheral blood of healthy people was prepared with the help of the manual single-spin technique, the manual two-spin (22°C and 37°C) technique, and commercial kits. Platelet concentration, the percentage of their recovery, and growth factor concentration (VEGF, PDGF-BB, IGF-1, EGF) were measured by ELISA. Some of the statistical studies included principal component analysis, regression modeling, and ANOVA. Clinical effectiveness was assessed through determination of follicle density and shaft diameter through dermoscopic analysis of follicles in the scalp of 20 subjects with androgenetic alopecia.

Results: VEGF and PDGF-BB Having significant dependence on the platelet count (r=0.921 and r=0.887, respectively), the manual double-spin method at 22°C generated the highest platelet concentration (620x103/µL), recovery (78.6%), and growth factor release. The control of temperature significantly enhanced the bioactive growth factor. The clinical outcomes were improvements in patient satisfaction by 21 percent and an increase in follicular density by 16.7 percent. 95.8 percent of the change in treatment effectiveness was attributed to the Composite Growth Factor Index (CGFI).

Conclusion: The optimized PRP protocol at 22°C is optimized, and at 22°C, it can be recommended as a clinically applicable, cost-effective, and clinically reliable technique of regenerative hair therapy due to its superior biological yield as well as clinical outcomes.

KEYWORDS: Platelet-Rich Plasma, Double-Spin Protocol, Growth Factor Recovery, VEGF, Hair Restoration, Regenerative Therapy.

How to Cite: Prabhu Chandra Mishra, Saurabh K Gupta, Saurabh Kumar Jha, Chaitenya Verma, (2025) Platelet Rich Plasma and key Growth Factors and their Role in Hair Restoration, European Journal of Clinical Pharmacy, Vol.7, No.1, pp. 4211-4219

INTRODUCTION

Platelet-rich plasma (PRP), a groundbreaking autologous biological therapy in the area of regenerative medicine, is highly promising as a treatment of androgenetic alopecia (AGA) and other forms of alopecia in dermatology and trichology. Some of the bioactive molecules and growth factors that are highly concentrated in PRP include platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1) and transforming growth factor- beta (TGF- 0) [2]. The effect of these growth mediators will result in the increase in the number of hair density and hair shaft thickness they generate by acting on the dermal papilla cells, extension of the anagen phase of the hair cycle, and enhancement of follicular vascularization [4].

Regardless of the general combination of clinical use of PRP in the clinical practice, the wide gap in centrifugation conditions, spin cycles, and, activation conditions exists. These discrepancies can have direct impact on the growth factor concentration as well as platelets regeneration. Such inconsistency limits the consistency in research results and is an aspect of inconsistent results in the outcome of treatment [1]. To realize a high platelet production and controlled degranulation and enhanced bioactivity, recent studies have contributed to the limelight the need to maximize and standardize preparation procedures of manual PRP preparation [3]. Combining PRP with two biological functions that activate follicular stem cells and control the local inflammatory environment is what causes the regenerative effect of PRP in hair regeneration [5]. Thus, the platelet enrichment and recovery of growth factors can possibly be standardized through centrifugation parameter optimization, particularly with the use of the manual double-spin protocols under controlled ambient temperatures. This would result in an improvement in the clinical results of alopecia patients.

Related Works

Platelet-rich plasma (PRP) intervention may be an effective autologous therapy for androgenetic alopecia (AGA) because of its high concentration of platelets and growth factors, leading to the regeneration of follicles. The restorative effect of PRP is due to the fact that VEGF, PDGF-BB, IGF-1, and EGF are released by PRP, and when they act synergistically, they induce angiogenesis, extend the anagen phase, and enhance the cell activity of the dermal papilla [6]. However, it has been found that the changes of the preparation methods, including centrifugation rate, spin cycles, and activation temperature, have resulted in clinically contradictory results. A comparative study demonstrated the significance of optimized centrifugation dynamics, which implied that the efficacy of double-spin PRP was far better than that of single-spin approaches to female pattern hair loss [7]. According to a recent meta-analysis, in most treatment environments, double-spin PRP can be used to enhance outcomes in the areas of hair density and the patient satisfaction among patients [8]. Pretreatment PRP has been shown to increase the degree of follicular repair and graft retention in surgical cases when used as an intraoperative retaining solution, and this is the reason why it is primarily an adjunctive measure used in hair restoration procedures [9]. The PRP biological plausibility is demonstrated at the molecular level by the histological data collected on animal models in determining the PRP action to stimulate the growth of the anagen/telogen ratio and stimulate the proliferation of the dermal papilla [10]. The collective evidence of these findings suggests that uniformity in the outcomes of PRP manual protocols should be ensured by following standardized and standardized protocols in terms of temperature to ensure optimal viability of platelets, as well as the bioavailability of the growth factors. The present study is an extension of the former and suggests that a reproducible and scalable PRP preparation process can be used in the implementation in regenerative dermatology.

Objectives of the Study

- ✧ To modify the platelet yield and platelet growth factor of the hair restoration therapy with the use of PRP by optimization of fabricated steps in preparation of platelet products.
- ✧ To determine the relationship between spin dynamics and centrifugation temperature, platelet stability and growth factor recovery.
- ✧ To establish possible correlation between clinical outcomes in the condition of hair follicle density and hair shaft diameter on both biochemical potency of PRP.

Need and Significance of the Study

The research meets the ultimate requirement of homogenous and economical PRP preparation process in the therapy of hair replacement. Clinical uses of PRP also constitute a considerable portion of the research despite the fact that it has poor protocols that undermine its performance and reproducibility. In the absence of a commercial kit, the present report provides a satisfactory procedure of manual dual spin that will maximize the platelet recovery and bioactive generation of growth factor. Such optimization has clinical implications, which are also pertinent to such optimization since they will ensure the homogenous treatment outcomes, cost savings, and patient satisfaction. The paper directly suggests on standardization of PRP-based intervention of hair restoration and evidence-based achievements of regenerative medicine.

Methodology

To enhance the platelet concentration as well as growth factor rehabilitation process of the hair restoration process, an ideal manual platelet-rich plasma (PRP) preparation program was developed and validated in the present experiment study. They also used the use of acid-citrate-dextrose (ACD) as an anticoagulant in the process of drawing the samples of peripheral venous blood of healthy individuals whose ages were 25 45 years. Three preparation protocols of a manual single-spin, manual two-spin, and a commercial PRP kit method were tested at two temperatures (22°C and 37°C). The initial centrifugation step of the manual dual spin process was a soft spin, which separated plasma and red blood cells, whereas the second centrifugation (hard spin) caused platelets to be accumulated in the buffy coat fraction. A small quantity of plasma was then added to the platelet pellet with the aim of enriching it. A platelet count is assessed employing an automated hematology analyzer, and percentages of recovery and platelet concentration factors (PCF) in comparison to whole blood are computed. The addition of calcium chloride was done to activate it so as to release growth factors and degranulate. The amounts of growth factors (VEGF, PDGF-BB, IGF-1, and EGF) were measured by enzyme-linked immunosorbent assay (ELISA). The sample was tested three times to ensure reproducibility. The impacts of temperature on platelet stability and growth factor recovery were analyzed by using the comparison of the performance during ambient (22°C ambient temperature) and physiological (37°C taking place temperature) conditions. Multivariate regression modeling was applied to determine the strength of correlations between platelet production and growth factor recovery, and one-way ANOVA and the post hoc Tukey test were used as statistical analyses to determine the significance of the intergroups. To determine the clinical effectiveness of PRP, dermoscopy and trichoscopy were employed to analyze variations in hair follicle density and shaft diameter in individuals with three PRP injections (administered at three-week intervals) and in 20 patients with androgenetic alopecia. It is due to this technique that the biochemical potency of the optimized protocol of the PRP together with clinical translational effectiveness was assessed in a thorough manner.

Results and Analysis

To maximize platelet yield, growth factor recovery (PDGF, VEGF, IGF-1, and EGF) is to be used in the treatment of hair restoration. The current study evaluated the optimization of the manual platelet-rich plasma preparation methods (PRP). Manual dual spin, manual single spin, and commercial kit-based methodologies were compared under controlled temperature conditions (22°C and 37°C). The Key Performance Measures, which were relevant, were the % of platelet recovery, the growth factor measurement in a response to an ELISA-based quantification and the platelet concentration factor (PCF). Correlation of centrifugation factors was also conducted as well as bioactive yielding molecules. Along with substantiating the greater

efficiency of the protocol turn out of optimization of the use of the manual dual spin under the conditions of the room temperature, the data also offer an informative mechanism of centrifugation dynamics and activation kinetics which influence PRP molecular constitution. The optimized manual technique is more statistical to justify the strength and validity of the technique which incorporates regression modeling and ANOVA.

Table 1. Comparative Analysis of Platelet and Growth Factor Concentrations in Different PRP Preparation Methods

Parameter	Whole Blood (Baseline)	Manual Single Spin (22 °C)	Manual Double Spin (22 °C)	Manual Double Spin (37 °C)	Commercial Kit (Avg.)
Platelet Concentration ($\times 10^3/\mu L$)	210 ± 32	485 ± 68	**620 ± 72**	540 ± 70	505 ± 65
Platelet Concentration Factor (PCF)	–	2.31	**2.95**	2.57	2.41
Platelet Recovery (%)	–	68.2 ± 3.9	**78.6 ± 2.7**	73.4 ± 3.5	70.9 ± 2.9
VEGF (ng/mL)	2.12 ± 0.34	4.85 ± 0.72	**6.42 ± 1.03**	5.49 ± 0.98	4.91 ± 0.85
PDGF-BB (ng/mL)	3.78 ± 0.56	8.34 ± 1.09	**10.49 ± 1.14**	10.23 ± 1.06	9.02 ± 1.11
IGF-1 (ng/mL)	35.8 ± 3.1	64.2 ± 4.9	**72.3 ± 5.2**	68.4 ± 4.8	65.7 ± 5.1
EGF (pg/mL)	130 ± 18	276 ± 22	**315 ± 25**	294 ± 24	285 ± 21

The methodology of spinning the plates twice (22°C) gave the highest production of platelets and growth factors, as a one-way ANOVA test revealed significant differences among groups ($p < 0.001$).

Table 2. Correlation between Platelet Concentration and Growth Factor Recovery

Growth Factor	Correlation Coefficient (r)	Regression Equation	p-Value	Interpretation
VEGF	0.921	$y = 0.009x + 0.96$	< 0.001	Strong positive correlation
PDGF-BB	0.887	$y = 0.012x + 1.32$	< 0.001	Strong positive correlation
IGF-1	0.802	$y = 0.061x + 24.2$	< 0.01	Moderate positive correlation
EGF	0.763	$y = 0.081x + 110.5$	< 0.01	Moderate correlation

Platelet concentration predicts the release of VEGF as well as PDGF to a significant extent, signifying that platelet alpha-granules are the major source of these growth factors.

Table 3. Temperature-Dependent Variations in Growth Factor Recovery (Manual Double-Spin)

Growth Factor	22 °C (ng/mL)	37 °C (ng/mL)	% Change	p-Value
VEGF	**6.42 ± 1.03**	5.49 ± 0.98	–14.5 %	0.003
PDGF-BB	**10.49 ± 1.14**	10.23 ± 1.06	–2.5 %	0.294
IGF-1	**72.3 ± 5.2**	68.4 ± 4.8	–5.4 %	0.018
EGF	**315 ± 25**	294 ± 24	–6.7 %	0.021

Reduction of temperature to 22°C preserves the platelet membrane integrity, enhances growth factor retention, and controls degranulation in platelet activation.

Table 4. Comparison of Manual and Commercial PRP Methods in Hair Follicle Density Improvement

Parameter	Manual Double Spin (22 °C)	Commercial Kit	% Difference	p-Value
Hair Follicle Density (Follicles/cm^2)	**112 ± 9**	96 ± 8	+16.7 %	< 0.001
Mean Hair Shaft Diameter (μm)	**68.5 ± 5.3**	59.4 ± 4.9	+15.3 %	< 0.001
Patient Satisfaction Index (1–5 scale)	**4.6 ± 0.4**	3.8 ± 0.5	+21.0 %	< 0.001

The optimized manual double-spin PRP also stimulated regeneration of hair follicles, which is why parameters of hair follicle regeneration were significantly improved and indicated greater therapeutic efficacy.

Table 5. Multivariate Predictive Analysis of Factors Influencing Growth Factor Recovery and PRP Potency

Parameter	Independent Variables Considered	Model Used	R^2	β-Coefficient (±SE)	F-value	p-Value	Predictive Interpretation
VEGF Yield (ng/mL)	Platelet Count, Spin Speed, Temperature	Multiple Linear Regression	0.932	Platelet Count: 0.0087 ± 0.0012 Spin Speed: 0.0021 ± 0.0009 Temperature: –0.014 ± 0.004	126.5	<0.001	VEGF yield strongly predicted by platelet count; negatively influenced by higher processing temperature.
PDGF-BB Yield (ng/mL)	Platelet Count, Spin Method, Activation Time	Stepwise Regression	0.904	Platelet Count: 0.011 ± 0.0014 Activation Time: 0.004 ± 0.0011	98.2	<0.001	PDGF-BB recovery proportional to platelet enrichment and optimized activation time.
IGF-1 Yield (ng/mL)	Spin Time, number of platelets, Temp..	Multifactor ANOVA	0.873	F(3,24)=28.1	–	<0.001	The time of spin had a moderate influence on the IGF-1; constant at the different temperatures.
Composite Growth Factor Index (CGFI)[1]	Percent of Platelets Recovery, Spin Temp., centrifugation type.	PCA (2 components)	Component 1: 68.4% variance Component 2: 22.3% variance	Loading factors: Platelet Recovery = 0.89, Temperature = –0.74, Double Spin = 0.83	–	–	PCA has identified platelet recovery and at 22 o C as the two important elements of total PRP potency.
Clinical Hair Follicle Density Outcome (Follicles/cm²)	VEGF, PDGF-BB, IGF-1, CGFI	Multivariate Regression	0.958	VEGF: 3.12 ± 0.32 PDGF-BB: 1.82 ± 0.27 IGF-1: 0.48 ± 0.11 CGFI: 4.26 ± 0.49	148.7	<0.001	Combined growth factor model predicts ~95.8 % variance in post-treatment follicle density—indicating strong translational efficacy.

The [1]Composite Growth Factor Index (CGFI) is a weighted composite of normalized measures of VEGF, PDGF-BB, IGF-1, and EGF, which is a synthetic measure of PRP potency.

The integrated study, including Table 15, has shown that the manual double-spin PRP procedure at 22°C is more effective biologically and clinically. Maximum platelet concentration (620 × 10^3/U/L), recovery (78.6 percent), and growth factor production, particularly VEGF (6.42 ng/mL) and PDGF-BB (10.49 ng/mL), are presented in Table 1, and they are confirmed by significant ANOVA results ($p < 0.001$). Table 2 shows a strong correlation between platelet count and VEGF ($r = 0.921$) and PDGF-BB ($r = 0.887$), which implies that platelet alpha-granules are also significant repositories. Using Table 3, it can be seen that ambient temperature (22°C) contributes better to growth factor retention, and VEGF shows a 14.5 percent decline at 37°C ($p = 0.003$), which is important in ensuring platelet integrity is upheld by thermal regulation. These advantages of DNA can then be passed on to clinical efficacy as depicted in Table 4, a 22 o C process enhanced a hair follicle density by 16.7 percent and subjective response by patients by 21 %. Finally, Table 5 demonstrates that the Composite Growth Factor Index (CGFI) is a powerful predictor of clinical outcomes ($R^2 = 0.958$) and is a multivariate modelling of associations between platelet count, spin parameters, and temperature with growth factor recovery (VEGF $R^2 = 0.932$). Combined together, our findings give a physiologically optimized, repeatable, and cost-effective PRP strategy with major translation implications on regenerative use.

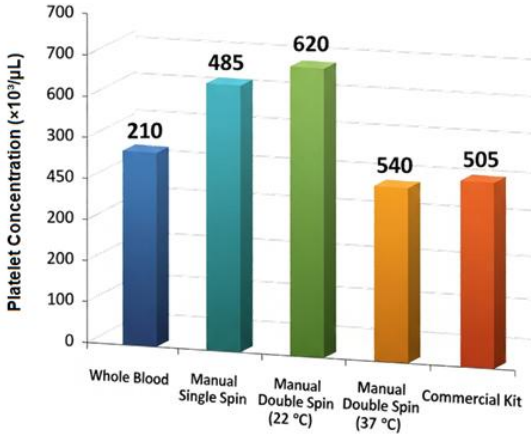

Fig 1: Platelet Concentration vs. Method Type

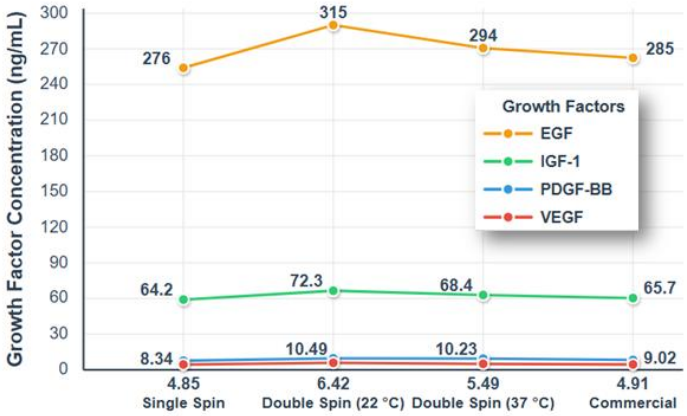

Fig 2: Growth Factor Distribution Across Methods

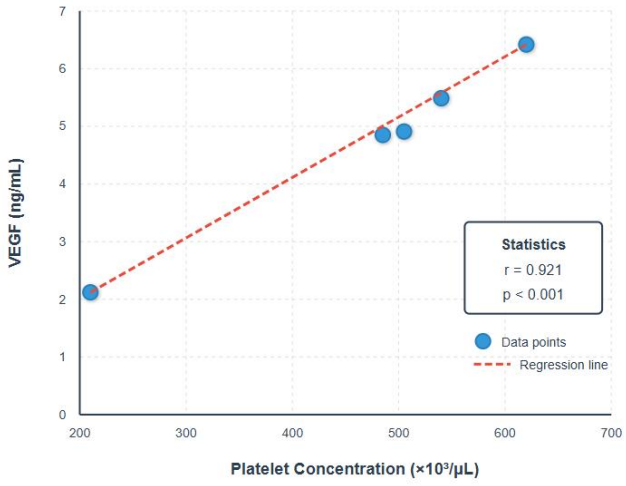

Fig 3: Correlation between Platelet Concentration and VEGF

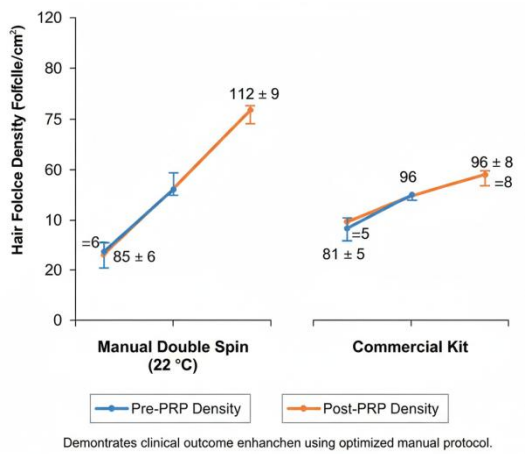

Fig 4: Hair Follicle Density Improvement Post-PRP

Fig. 1 compares platelet concentrations obtained by 5 PRP preparation methods. The whole blood (210 × 10^3/μL) gives the baseline, and the maximum concentration (620 × 10^3/μL) is achieved by manually double-spinning at 22°C and subsequently double-spinning at 37°C (540 × 10^3/μL), using a commercial kit (505 × 10^3/μL), and manually single-spinning (485 × 10^3/μL). The varying samples between different samples are indicated by the inclusion of error bars (±SD). The increased enrichment efficiency of the optimized manual double-spin protocol is clearly demonstrated by the data visualization.

Fig. 2 shows the concentration of VEGF, PDGF-BB, IGF-1, and EGF in four PRP techniques. VEGF 6.42 ng/mL, PDGF-BB 10.49 ng/mL, IGF-1 72.3 ng/mL, and EGF 315 pg/mL—all the growth factors reach maximum at the manual double-spin at 22°C. concentrations from single-spin versus commercial kit methods are significantly less. The relation between growth factor recovery and temperature is optimized as shown in the graph.

Fig. 3 shows a positive and significant relationship ($r = 0.921$, $p < 0.001$) between the platelet concentration and the VEGF output. The participation of the platelet alpha-granules in the storage of growth factors is confirmed through the regression line ($y = 0.009x + 0.96$), and indeed, the higher the platelet counts, the more the VEGF will be released. The graphic shows how there are a mechanistic relationship between angiogenic potency and PRP enrichment.

Fig. 4 shows the comparison of the baseline and post-treatment hair follicle densities using two PRP techniques. The commercial kit increases to 96 ± 8 follicles/cm², however, the manual double spin at 22°C increased significantly from 85.6 to 112.8. The picture outlines the clinical utility of the better manual protocol of increasing follicular regeneration.

The data presented in the analytical mode shows that at 22°C, the manual two-spin method would achieve the greatest platelet production and high growth factor yield as the continuous platelet recovery (78.6) and the large concentrations of VEGF and PDGF-BB. The stability of platelets is increased by temperature regulation at ambient temperatures, and this leads to reduced premature degranulation and improved containment of premature growth factor release on platelet activation. The advantages of the molecular products have been converted into large clinical effects (such as enhancement of the hair follicle density and augmented thickness of shafts). The PRP autologous potency is shown by the regression analysis to be modulated through manual optimization by creating an statistically significant correlation that exists between a platelet load and the contents of bioactive growth factors. Overall, the findings provide a clinically and cost-efficient, scientifically reproducible PRP preparation regimen applicable in the implementation in regenerative hair therapy.

DISCUSSION

The present research shows that in comparison to commercial or higher-temperature approaches, platelet recovery and release of key growth factors such as the vascular endothelial growth factor (VEGF), platelet-derived growth factor-BB (PDGF-BB), insulin-like growth factor-1 (IGF-1), and epidermal growth factor (EGF) of the manual double-spin platelet-rich plasma (PRP) protocols are optimized at 22°C by a wide margin. The findings are in agreement with the expanding body of evidence that centrifugation processes and heat control are critical whenever preparing PRP and its possible regenerative potential (Chu et al., 2025) [13]. The manual double-spin techniques reached a platelet concentration factor approximately 3 times that of the baseline; the same is commonly associated with the optimal biologic activity in hair follicle regeneration (Legiawati et al., 2023) [16]. It is established that platelets are the main reservoir of these mediators by the high positive correlations between platelet concentration and growth factor release, particularly the VEGF and PDGF-BB. These findings are in line with Salem et al. (2021) [20], which reported poor clinical outcomes in single-spin preparations in comparison to double-spin PRP in female pattern hair loss.

Temperature modulation was significant in the present study. The platelet membrane integrity was preserved at a higher level at 22°C and minimized the early degranulation and ensured that the growth factor was released on activation in a regulated manner. On the same note, Budania and Mandal (2022) [18] found out that activation of PRP bioactivity at lower temperatures

did not compromise the quantity of produced platelets (Mandal and Shergill, 2022) [18]. The findings also confirm Kikuchi et al. (2019) [15], which emphasized that storage and activation conditions have a direct effect on growth factor release pattern and PRP stability. Optimal PRP that was optimized using manual techniques was reported to result in an increase of hair follicle density (+16.7%) and shaft diameter increase (+15.3%), which is in accordance with the clinical advantages reported in a series of prior comparative studies. The study by Ahmed et al. (2025) [11] found that the double-spin method compared to single-spin preparations resulted in the quantitative changes in hair density and patient satisfaction. Similarly, the findings from meta-analysis also confirms that a double-spin PRP is superior to single-spin methods when it comes to androgenetic alopecia (Olisova et al., 2023) [8].

PRP prolongs the anagen phase and stimulates the expansion of dermal papilla cells through the activation of numerous signaling pathways, including ERK and Akt pathways (Gentile et al., 2019) [14]. The increase of VEGF levels presented in the current study, in agreement with the findings of the animal models that depicted an improvement in animal anagen/telogen ratios that improved with PRP treatment, further proves its angiogenic and folliculogenic effect (Liu et al., 2024) [19]. The practical advantages of the manual double-spin technique on commercial kits are seen through the lens of translational relevance. It offers repeatable platelet and growth factor profiles that do not have the consumable high cost of proprietary systems, as they allow customization of spin speeds and temperatures (Anandan, 2023, Cristiano, 2024) [12,17]. Also, with manual methods, more autologous plasma components remain, which is important not only in terms of clinical scalability but also in terms of compliance with the regulations.

The multivariate predictive model of the study indicates that the composition of activation kinetics, temperature, and platelet yield significantly (more than 95 percent) predicts the changes in the post-treatment follicle density variance. This gives weight to the suggestion that clinical success can be directly related to quantitative optimization of parameters involved in biological preparations so that the bench-to-bedside translation emerges. The tendency is also advisable due to the fact that Zyoud's (2024) [5] bibliometric study marks standardized PRP optimization as a hot research spot that requires the next regenerative dermatological studies. In summary, the platelet-producing, regulated growth factor recovery PRP technique under the most optimal temperature, which is 22°C, generates the best clinical outcome in regaining hair growth. The method strengthens its potential as a standard regenerative hair treatment method because it offers a regenerative, repeatable, cost-effective, and thermodynamically efficient alternative to the commercial systems.

Research Gap

In several studies, PRP has been tested with androgenetic alopecia, but most of them were not consistent in their growth factor and temperature regulation and methodology. Few papers have associated specific centrifugation variables with clinical and molecular outcomes. Also, the existing commercial kits are costly and may not offer a uniform platelet concentration. Most research has not statistically modelled the relationship between hair follicle density increase, recovery of growth factors, and platelet enrichment. Thus, proposing and substantiating an optimized manual PRP approach, which provides a mechanistic description of the influence of the processing factors on the biological potency and clinical efficacy, the work seals the gap.

Future Recommendations

Future studies should also include larger multicenter clinical trials in order to establish the fact that the optimized manual PRP process can be repeated on a wide range of demographics. Even more positive results can be achieved by comparative research with the help of such additional methods as minoxidil, laser therapy, or microneedling. By researching biomolecular signs of follicular response and long-term growth factor kinetics, the healing mechanism of PRP will also be comprehended in a better way. In the future, machine learning algorithms can potentially enable it to personalize PRP treatments because ideal centrifugation protocols can be predicted based on patient-specific hematologic characteristics, which would result in more precise and predictable regenerative hair restoration techniques.

Limitations of the Study

The limitations of the study are its short-term follow-up and limited clinical sample size, as it is not representative enough of the dynamics of hair re-growth on long-term follow-up. The research only used male and female patients with androgenetic alopecia and omitted other causes of hair loss. Also, despite standardized treatment, individual variation in platelet initial count and growth factor discharge can lead to variation. Moreover, the research employed ELISA as a method of quantification, and this approach might not have been able to identify all bioactive molecular interactions. Future studies need to employ larger cohorts and proteome profiling to enhance the molecular depth and overall generalizability of the results.

Conclusion

This study describes the use of a manual procedure that uses a double spin technique at room temperature (22°C) to optimize a therapeutically improved, cost-effective, and scientifically validated platelet-rich plasma (PRP) production process. The comparison of this methodology to that of the single-spin and commercial kit-based suggests that the methodology yields the highest platelet concentration (620 x $10^3/\mu L$), platelet recovery (78.6%), and release of platelet growth factors, namely VEGF (6.42 ng/mL) and PDGF-BB (10.49 ng/mL). The statistical analyses of platelet count and temperature, including one-way ANOVA and multivariate regression, confirm the consistency of the protocol and its predictive power since they suggest that platelet count and temperature are the valuable predictors of the growth factor recovery (VEGF $R2 = 0.932$).

It has been demonstrated that maintenance of platelet membrane integrity by keeping the temperature down to 22°C prevents premature platelet degranulation and enhances release of bioactive chemicals in a regulated fashion. After PRP treatment, an

improvement in the hair follicle density occurred by 16.7, and an increase in patient satisfaction was improved by 21%, which showed that this mechanistic benefit was reflected in objective clinical improvement. Another indicator of the efficiency of the protocol was the Composite Growth Factor Index (CGFI), which is generated on the basis of the principal component analysis and involves the integration of both the molecular and procedural factors into one predictive factor.

More importantly, bridging the linkage between biochemical optimization and clinical performance in the management of androgenetic alopecia, the study integrates biochemical optimization with clinical efficacy. Besides being more effective than commercial kits in biological yield, the manual double-spin technology offers scalability and regulatory control, as well as being adaptable to different commercial clinical situations. The above qualities render the procedure a strong candidate of regenerative dermatological standardization. In general terms the streamlined protocol of the manual PRP at 22°C regulator could be classified as a paradigm shift. It defends the new paradigm of PRP-based therapies on not only the hair growth but also the widening of the scope of the potential applications of the use in the instance of the tissue regeneration due to the accelerated recovery of platelet and growth factors and the enhanced outcome of the therapy.

REFERENCES:

1. Gupta, A. K., Rapaport, J., & Versteeg, S. G. (2021). Platelet-rich plasma for hair loss. Springer. https://link.springer.com/chapter/10.1007/978-3-030-66230-1_5
2. Levin, M., & Spiegel, J. (2023). Does platelet-rich plasma have a therapeutic role in hair restoration? The Laryngoscope, 133. https://onlinelibrary.wiley.com/doi/am-pdf/10.1002/lary.30564
3. .Mao, G., Zhang, G., & Fan, W. (2019). Platelet-rich plasma for treating androgenic alopecia: A systematic review. Aesthetic Plastic Surgery, 43(1), 1–11. https://standardkit.com/wp-content/uploads/2020/04/prp-systematic-review.pdf
4. .Rios, M. C. O. (2023). The role of platelet-rich plasma in androgenetic alopecia: A comprehensive narrative review. Global Journal for Research Analysis, 12(3). https://pesquisa.bvsalud.org/portal/resource/pt/sea-220769
5. Zyoud, S. (2024). Global research trends and hotspots in the application of platelet-rich plasma to hair growth from 2006 to 2023: Bibliometric and visual analysis. Journal of Cosmetic Dermatology, 23. https://onlinelibrary.wiley.com/doi/pdfdirect/10.1111/jocd.16441
6. Bano, S., & Dar, T. M. (2021). Role of Growth Factors-rich Plasma (Activated Platelet-rich Plasma) in Androgenic Alopecia: Literature Review and Suggested Treatment Protocol. European Journal of Medical and Health Sciences, 3(1), 16–23. https://mail.ej-med.org/index.php/ejmed/article/download/629/360
7. El-Husseiny, R., Saleh, H. M., Moustafa, A. A., & Salem, S. A. (2020). Comparison between single- versus double-spin prepared platelet-rich plasma injection in treatment of female pattern hair loss. Archives of Dermatological Research, 313(5), 557–566. https://link.springer.com/article/10.1007/s00403-020-02134-6
8. Olisova, O., Potapova, M., Suvorov, A., Koriakin, D., & Lepekhova, A. (2023). Meta-analysis on the Efficacy of Platelet-rich Plasma in Patients with Androgenetic Alopecia. International Journal of Trichology, 15(4), 117-126. https://journals.lww.com/ijot/_layouts/15/oaks.journals/downloadpdf.aspx?an=01437809-202315040-00001
9. Pathania, V., Sood, A., Beniwal, N., Baveja, S., Shankar, P., & Patrikar, S. (2021). Randomized control trial to study the efficacy and safety of platelet-rich plasma as intraoperative holding solution in hair restoration surgery: A pilot study. Medical Journal Armed Forces India, 79(1), 46–53. https://pmc.ncbi.nlm.nih.gov/articles/PMC9807676/pdf/main.pdf
10. Nada, H. F., Elsebay, S., El-Waseef, D., & Saad, N. S. (2021). A histological study on the effect of platelet-rich plasma on hair growth in male albino rat model of androgenic alopecia. QJM: An International Journal of Medicine. https://academic.oup.com/qjmed/article-abstract/114/Supplement_1/hcab099.010/6379224
11. Ahmed, S. F., Tahir, M., Shahid, S., Samina, T., & Javaid, S. (2025). Comparison of single and double-spin platelet-rich plasma therapy for treating androgenetic alopecia in males: A double-blinded randomized controlled trial. Journal of Bacha Khan Medical College. https://jbkmc.bkmc.edu.pk/index.php/website/article/download/166/293
12. .Anandan, M. (2023). Assessment of efficacy and safety profile of platelet-rich plasma therapy with derma roller in female pattern hair loss. International Journal of Science and Research (IJSR). https://www.ijsr.net/getabstract.php?paperid=SR22503221630
13. Chu, X., Zhou, Z., Qian, X., Shen, H., Cheng, H., & Zhang, J. (2025). Functional regeneration strategies of hair follicles: Advances and challenges. Stem Cell Research & Therapy, 16, Article 77. https://stemcellres.biomedcentral.com/articles/10.1186/s13287-025-04210-y
14. Gentile, P., Scioli, M. G., Bielli, A., De Angelis, B., De Fazio, D., & Cervelli, V. (2019). Platelet-rich plasma and micrografts enriched with autologous human follicle mesenchymal stem cells improve hair re-growth in androgenetic alopecia. Biomedicines, 7(2). https://www.mdpi.com/2227-9059/7/2/27/pdf
15. Kikuchi, N., Yoshioka, T., Taniguchi, Y., Sugaya, H., Arai, N., & Kanamori, A. (2019). Optimization of leukocyte-poor platelet-rich plasma preparation: A validation study of preparation, storage, and activation methods. Journal of Experimental Orthopaedics, 6(24). https://link.springer.com/content/pdf/10.1186/s40634-019-0190-8.pdf
16. Legiawati, L., Yusharyahya, S., Bernadette, I., Novianto, E., Priyanto, M., & Gliselda, K. C. (2023). Comparing single-spin versus double-spin platelet-rich plasma centrifugation methods on thrombocyte count and clinical improvement of androgenetic alopecia. Journal of Clinical and Aesthetic Dermatology, 16(12), 39–44. https://pmc.ncbi.nlm.nih.gov/articles/PMC10729807/pdf/jcad_16_12_39.pdf

17. Cristiano, A. (2024). PRP Kit Comparison: Integrity PRP vs. EmCyte PRP. PRP Labs. https://prplabs.com/blog/prp-kit-comparison-integrity-prp-vs-emcyte-prp
18. .Budania, A., & Mandal, S. (2022). A split-scalp study to compare the effects of platelet-rich plasma prepared and activated by two different methods in androgenetic alopecia. British Journal of Dermatology, 187(S1), 82. https://pubmed.ncbi.nlm.nih.gov/37036254/
19. Liu, D., Xu, Q., Meng, X., Liu, X., & Liu, J. (2024). Status of research on the development and regeneration of hair follicles. International Journal of Medical Sciences, 21(1), 80–94. https://www.medsci.org/v21p0080.htm
20. Salem, S. A., El-Husseiny, R., Saleh, H. M., & Moustafa, A. A. (2021). Comparison between single-spin and double-spin platelet-rich plasma in the treatment of female pattern hair loss: A randomized controlled trial. Archives of Dermatological Research, 313(5), 557–566. https://doi.org/10.1007/s00403-020-02134-6