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Standardized Double-Spin Preparation of Autologous Platelet-Rich Plasma in Rabbits: Optimization and Hematological Characterization from cartilage regeneration study

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ABSTRACT: Platelet-rich plasma (PRP) is an emerging autologous biological therapy widely used in regenerative medicine due to its high concentration of growth factors that support tissue repair. The present study aimed to standardize a double-spin centrifugation protocol for preparing autologous PRP in rabbits and to evaluate its hematological characteristics. Blood samples collected from ten healthy rabbits were processed using sequential centrifugation without commercial kits. The protocol produced a substantial increase in platelet concentration compared with baseline whole blood values. A significant reduction in red blood cell contamination and stable leukocyte levels indicated effective cellular separation and controlled composition of the final product. Overall, the standardized technique was simple, reproducible, and suitable for preparing PRP for experimental intra-articular regenerative applications in rabbits.

KEYWORDS: Platelet-rich plasma, Double-spin centrifugation, Autologous PRP, Rabbits, Platelet enrichment, Regenerative medicine.

I.INTRODUCTION

Platelet-rich plasma (PRP) has emerged as a promising autologous biological therapy in regenerative and orthopedic medicine. PRP is defined as plasma containing a platelet concentration higher than baseline whole blood levels (1,2). Initially developed in hematology for the management of thrombocytopenia, PRP has since expanded into multiple medical disciplines, including orthopedics, sports medicine, maxillofacial surgery, and dermatology (3).

The therapeutic potential of PRP is attributed to the high concentration of bioactive growth factors stored within platelet α-granules. These include platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), and fibroblast growth factor (FGF) (3, 4). Upon activation, platelets release these mediators, which play crucial roles in angiogenesis, cellular proliferation, extracellular matrix synthesis, and modulation of inflammatory responses (5, 6). Through these mechanisms, PRP supports tissue repair and regeneration.

In the context of musculoskeletal disorders, PRP has gained particular attention as a potential disease-modifying therapy due to its regenerative and anti-inflammatory properties.

Despite its expanding clinical and experimental applications, considerable heterogeneity exists in PRP preparation protocols. Variations in centrifugation speed, duration, number of centrifugation cycles, and plasma handling techniques directly influence platelet concentration, leukocyte content, and overall biological activity, thereby affecting therapeutic consistency (1, 2). Among the available preparation techniques, the double-centrifugation

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(double-spin) method is frequently employed, as it enables higher platelet enrichment while remaining relatively simple and cost-effective (2).

Given that the biological efficacy of PRP is closely associated with its preparation methodology, standardization of protocols is essential to ensure reproducibility and reliability in experimental research. Therefore, the present study describes a structured double-spin protocol for the preparation of autologous PRP designed to achieve substantial platelet concentration suitable for intra-articular therapeutic application.

II. MATERIALS AND METHODS

Approximately 8.5 mL of venous blood was obtained aseptically from the jugular vein of 10 rabbits and collected into sterile 10 mL vacuum tubes (Mediblioo^{R}) containing 1.5 mL of acid citrate dextrose (ACD) as an anticoagulant. An aliquot of 0.5 mL was separated prior to processing for baseline platelet quantification.

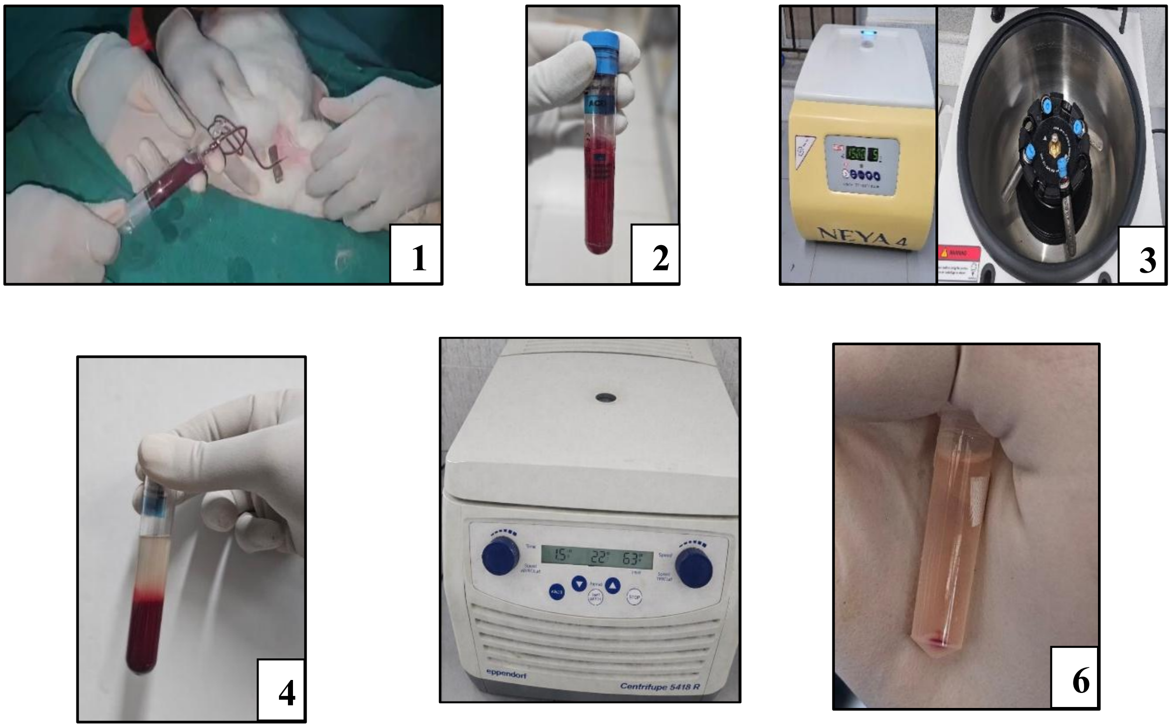
The remaining blood sample was subjected to a primary centrifugation at 1500 rpm for 5 minutes to facilitate separation of plasma from erythrocytes. Following this step, the plasma layer was carefully transferred to a sterile tube while avoiding disturbance of the buffy coat interface.

Subsequently, a second centrifugation was performed at 6300 rpm for 20 minutes to concentrate the platelets. After centrifugation, a portion of the platelet-poor plasma was removed, and the platelet pellet was gently resuspended in the remaining plasma to produce the final PRP preparation.

This procedure yielded a platelet concentration approximately 10.25-fold higher than that of baseline whole blood.

To develop a protocol suitable for routine clinical application, all procedures were performed at ambient room temperature and centrifugation was carried out without refrigeration.

The protocol is as follows (Figure 1)



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1. Collection of 9ml blood from Jugular vein under aseptic conditions using a sterile syringe 2. Whole blood sample collected in an ACD anticoagulant-containing tube prior to centrifugation 3. First centrifugation; 1500 rpm for 5 min 4. Distinct separation of blood components following centrifugation, showing plasma and cellular fractions 5. Second centrifugation of extracted plasma; 6300 rpm for 20 min 7. Following centrifugation, the upper layer consisted of platelet-poor plasma (PPP), while the lower fraction contained platelet-rich plasma (PRP) with a visible platelet button (pellet) at the bottom of the tube.

To simulate routine clinical conditions, blood component analysis was carried out immediately after PRP preparation. Both whole blood and PRP samples were evaluated using an automated hematology analyzer (Mindray bc 30 Vet). The platelet concentration factor was determined by calculating the ratio of platelet count in PRP to that in baseline whole blood.

III. RESULTS

Blood collection was successfully performed in all ten clinically healthy rabbits, and baseline hematological values were within normal reference ranges. A comparison of cellular components between whole blood and platelet-rich plasma (PRP) is summarized in Table 1.

Parameter	Baseline Values		Platelet-Rich Plasma		P value**
	Mean ± SE	Range	Mean ± SE	Range	
Platelets	237.30 ± 9.70	208–318	4726.00 ± 671.87	1553–9315	< 0.01
RBC (×10⁶/µL)	5.03 ± 0.15	4.28–5.85	0.56 ± 0.25	0.01–2.26	< 0.01
WBC	7.93 ± 0.30	6.35–9.74	8.68 ± 3.35	0.52–28.81	0.769
Lymphocytes	2.60 ± 0.20	1.67–4.56	8.46 ± 2.45	0.38–23.74	0.234

Table 1: Comparison of hematological parameters at baseline and following PRP preparation. Values are expressed as $\times 10^3/\mu L$ unless otherwise specified *Wilcoxon signed-rank test.*

Preparation of PRP using the standardized double-centrifugation technique resulted in a substantial elevation in platelet concentration relative to baseline whole blood. The mean platelet count increased from 237.30 ± 9.70 $\times 10^3/\mu L$ to 4726.00 ± 671.87 $\times 10^3/\mu L$, corresponding to an average 19.91-fold enrichment ($p < 0.01$).

Red blood cell (RBC) levels were significantly decreased in PRP samples compared to baseline measurements ($p < 0.01$), indicating efficient erythrocyte separation during processing. In contrast, total white blood cell (WBC) and lymphocyte counts demonstrated variability between baseline and PRP samples; however, these differences were not statistically significant ($p > 0.05$).

IV. DISCUSSION

Platelet-rich plasma is commonly prepared using a double-centrifugation technique consisting of an initial low-speed ("soft") spin followed by a higher-speed ("hard") spin. The first centrifugation separates whole blood into three principal fractions: erythrocytes at the bottom, a buffy coat layer containing platelets and leukocytes and plasma in the upper layer. The second centrifugation concentrates platelets by sedimenting them within the plasma phase, thereby allowing separation of platelet-rich plasma (PRP) from platelet-poor plasma (PPP) (2, 7). The present study adopted a standardized double-spin protocol in rabbits, performed under controlled laboratory conditions without the use of a commercial kit, to ensure reproducibility and biological safety.

Using this protocol, a marked increase in platelet concentration was achieved. The mean platelet count increased nearly twenty-fold compared to baseline whole blood values. This degree of enrichment exceeds the minimum therapeutic threshold of 3–5-fold platelet concentration commonly suggested in the literature for regenerative applications (8,9). The substantial platelet enrichment observed in the present study may be attributed to the defined centrifugation parameters and careful plasma handling during the second spin, which facilitated effective platelet recovery.

A significant reduction in red blood cell (RBC) concentration was observed in PRP samples, indicating efficient erythrocyte separation during processing. Minimizing RBC contamination is considered desirable, as excessive erythrocytes may contribute to oxidative stress and inflammatory responses within the joint environment (10). Therefore, the marked decrease in RBC count in the current study supports the purity of the prepared PRP.

In contrast, total white blood cell (WBC) and lymphocyte counts did not differ significantly between baseline and PRP samples. Variability in leukocyte concentration is frequently reported across different preparation techniques, largely due to differences in centrifugation force, duration, and plasma aspiration methods (7). Some protocols yield leukocyte-rich PRP, whereas others aim to minimize leukocyte inclusion depending on the intended therapeutic indication (9). In the present study, the absence of a significant increase in leukocytes suggests that the protocol produced a preparation with controlled leukocyte content, which may be advantageous for intra-articular applications where excessive inflammation is undesirable.

The variability observed in platelet enrichment among individual rabbits is consistent with previous reports demonstrating that baseline hematological characteristics influence final PRP composition (11). Such inter-individual variability underscores the importance of reporting absolute platelet counts and concentration ratios to improve comparability between studies. Several classification systems—including PAW, PLRA, and DEPA—have emphasized the need to describe platelet concentration, leukocyte content, RBC presence, and activation methods when characterizing PRP preparations (9,10,11).

Standardization of PRP preparation protocols remains a critical challenge in both experimental and clinical research. Differences in centrifugation speed, spin duration, and handling techniques significantly influence the biological profile of the final product, thereby affecting reproducibility (12). By clearly defining centrifugation parameters and quantifying platelet enrichment, the present study contributes to improved methodological transparency in preclinical PRP research.

Nevertheless, certain limitations must be acknowledged. Growth factor concentrations were not quantified, and thus the biological activity of the prepared PRP was inferred from platelet enrichment rather than direct molecular assessment. Additionally, although the protocol demonstrated high platelet concentration in healthy rabbits, further validation in clinical or pathological models is required to confirm therapeutic efficacy.

Overall, the standardized double-spin method described herein achieved substantial platelet enrichment with effective reduction of erythrocyte contamination, producing a PRP preparation suitable for intra-articular regenerative applications.

V. CONCLUSION

A standardized double-spin technique was successfully employed for the preparation of autologous platelet-rich plasma (PRP) in rabbits using routine laboratory equipment without commercial kits. The method achieved a 19.91-fold increase in platelet concentration with significant reduction in erythrocyte contamination, while being performed at room temperature without refrigeration.

The protocol was simple, cost-effective, and reproducible, indicating its suitability for experimental and intra-articular regenerative applications in rabbit models.

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