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Original Article

Autologous Platelet Rich Plasma Preparation for Regenerative and Intra-Articular Applications

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Abstract

Background: Platelet-rich plasma (PRP) has been used in many regenerative and intra-articular therapies due to its regenerative probabilities with high growth factor content. However, lack of standardized protocols for preparation limits reproducibility and comparison across studies.

Methods: A reproducible protocol for autologous PRP preparation with usage of a manual double-spin method was applied in ten patients. 25 ml of peripheral venous blood were drawn under aseptic condition into 5 ml of 3.8% sodium citrate as anticoagulant. The first centrifugation performed at 2300×g (gravitational acceleration) for 3 minutes to separate red blood cells. The upper plasma portion was transferred and centrifuged again at 2300×g for 8 minutes. Platelet-poor plasma was discarded, and the platelet pellet was resuspended in 3 mL of plasma.

Results: The protocol consistently produced a homogeneous PRP with a platelet concentration of at least four times baseline were achieved in all preparations. Samples were successfully aspirated into sterile syringes without technical difficulty.

Conclusion: The described technique provides a simple, cost-effective, and reproducible method for PRP preparation that can be performed in operating room with usage of a centrifuge and sterile empty tubes. This protocol supports consistent platelet concentration and may facilitate clinical use and intraoperative applications.

Keywords: Platelet Rich Plasma, PRP, Regeneration, Intra-articular, and Cost-effective.

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Introduction

Platelet-rich plasma (PRP) is an autologous blood derivative that has gained significant interest for its regenerative potential. Platelets release a wide array of growth factors and cytokines that modulate inflammation, stimulate regeneration, and enhance tissue repair. Consequently, PRP has been employed across several medical disciplines, including orthopedics, dermatology, and plastic and reconstructive surgery.(1)

Despite its popularity, PRP is not a standardized product. Preparation methods differ substantially in terms of centrifugation force, spin duration, anticoagulant used, and final plasma volume. This variability directly influences platelet concentration, thereby affecting biological activity and clinical efficacy.(2) Furthermore, many studies report centrifugation in revolutions per minute (RPM) without reference to rotor radius, making reproducibility across different laboratories difficult. Because relative centrifugal force (RCF) reflects the true physical parameter.(3)

In addition to inconsistencies in preparation, routine quality-control measures such as platelet counting and growth factor profiling are often omitted. This omission limits the ability to validate protocols and hinders the establishment of evidence-based guidelines. The development of simple, reliable, and cost-effective techniques remains essential, particularly in resource-limited settings where commercial PRP kits are not feasible.(4)

The present study describes a reproducible manual double-spin protocol that requires only centrifuge and basic laboratory equipment such as sterile tubes and syringes in room temperature and can be implemented in the operating room immediately before or even during surgical procedure. Emphasis is placed on detailed reporting of centrifugation parameters, anticoagulant ratios, and final PRP volume, thereby optimizing reproducibility and facilitating comparison across future studies.(5)

Materials and Methods

The study protocol was conducted in adherence to the Declaration of Helsinki and was reviewed and approved by the Faculty of Medicine, Sohag University Ethics Committee and represents a secondary analysis of a thesis under approval number (21-06-15) Informed consent was obtained from all participants before blood collection.

Blood Collection Procedure

Peripheral venous blood was collected immediately before the planned intervention under aseptic conditions. The venipuncture site was prepared using an alcohol swab for at least 30 seconds, covering approximately 2 cm around the puncture site. A sterile 50 mL syringe prefilled with 5 mL of 3.8% sodium citrate was used to collect 25 mL of blood, yielding a total of 30 mL. Care was taken to avoid excessive aspiration force to minimize platelet activation and hemolysis. The syringe was gently inverted several times to ensure thorough mixing.

PRP Preparation Protocol

Blood samples were divided equally into two sterile tubes. The first centrifugation “soft spin” was performed at 2300×g for 3 minutes, producing an upper plasma fraction containing platelets and a lower fraction of red blood cells. The plasma layer was carefully aspirated into two fresh tubes, avoiding contamination from the buffy coat or red cells.

The second centrifugation “hard spin” was then performed at 2300×g for 8 minutes. This step resulted in a platelet pellet at the bottom of each tube. The upper platelet-poor plasma (PPP) was discarded, leaving approximately 1.5 mL of plasma above each pellet. The pellets were gently resuspended, and the two fractions were combined to produce approximately 3 mL of PRP. The final PRP was aspirated into sterile syringes for application (Figure 1)

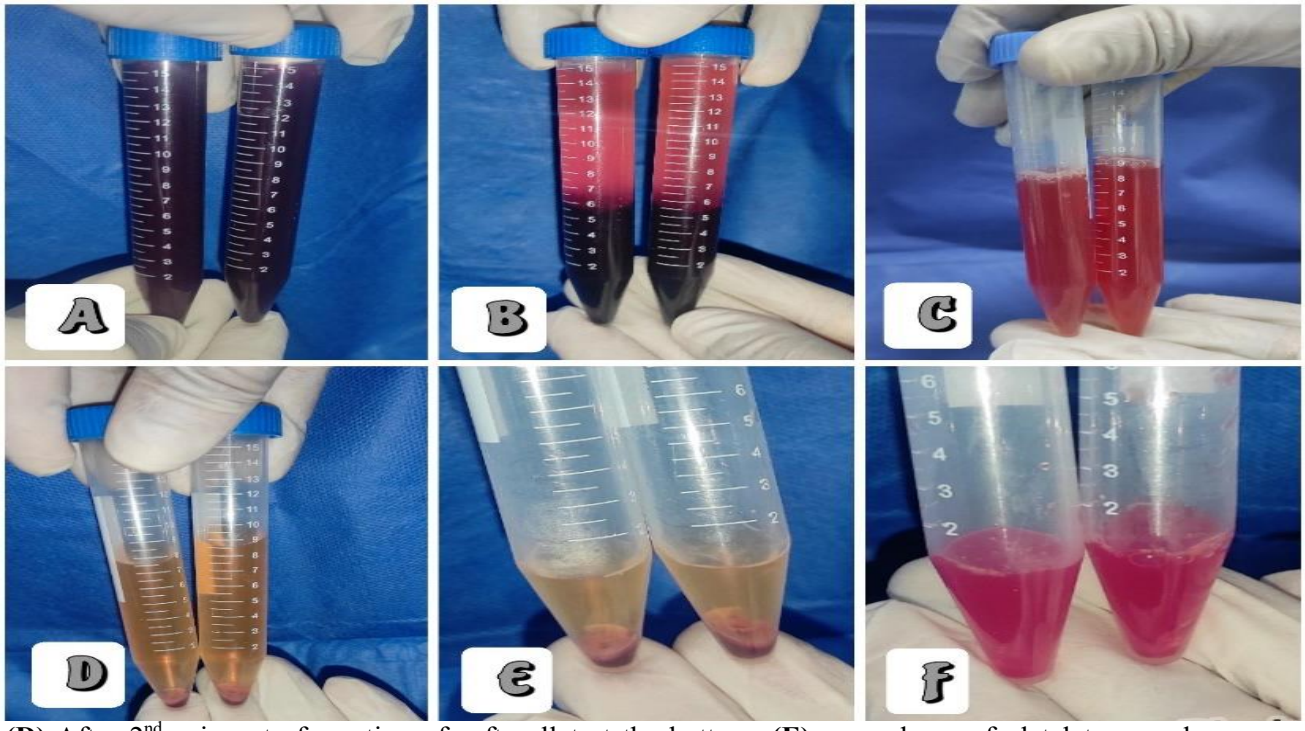
Where feasible, platelet counts were measured in baseline whole blood and final PRP samples. Platelet concentration factors were calculated by dividing the PRP count by baseline count. Samples were visually inspected for clarity, absence of hemolysis, and absence of clot formation.

Results

The described protocol was successfully performed in all ten patients. A final volume of approximately 3 mL PRP was consistently obtained from 30 mL of anticoagulated whole blood. Visual inspection revealed clear phase separation after each centrifugation step, and the final PRP appeared homogeneous (Figure1).

In cases where platelet counts were performed, concentrations of at least four times baseline values were achieved. The PRP was easily aspirated into sterile syringes and demonstrated favorable handling properties, with no technical difficulties encountered during preparation.

Figure 1: PRP preparation steps **(A)** 30 ml of collected blood divided into 2 equal (15ml) sterile tubes. **(B)** After 1st spin note sample divided into upper plasma layer and lower RBC layer. **(C)** Plasma layer transferred into another 2 empty



tubes. **(D)** After 2nd spin note formation of soft pellet at the bottom. **(E)** upper layer of platelet poor plasma removed leaving only 1.5 cc with soft pellet. **(F)** After suspension of platelets with PRP produced.

Discussion

This study presents a reproducible double-spin protocol for autologous PRP preparation that is both cost-effective and feasible in operating room conditions. The use of defined RCF values and sodium citrate anticoagulant contributed to the preservation of platelet integrity and minimization of premature activation. The resulting PRP demonstrated consistent concentration and favorable handling properties.

One of the main strengths of this protocol is its simplicity. Unlike commercial systems, which require higher cost of consumables and equipment, this method can be applied with standard laboratory centrifuges and tubes. This is particularly important in resource-limited healthcare environments, where accessibility to commercial kits may be limited.[6] By ensuring reproducibility through standardized centrifugation parameters and anticoagulant ratios, the present protocol provides a reliable framework for clinical and research applications.

The achieved platelet concentration of four or more the baseline is comparable to values reported using commercial PRP systems. Previous studies have indicated that clinical efficacy correlates with platelet enrichment, with a target of 3–5× baseline often recommended. Therefore, the present method

provides a high platelet concentration without the additional cost of commercial kits.

The limitations of this study include that platelet counts were not routinely quantified in all cases, and levels of growth factor were not measured. However, while the reproducibility of the protocol was confirmed visually and through concentration data from selected cases, further validation using platelet and cytokine assays would strengthen its biological characterization.

Future studies should focus on the biological features of PRP prepared with this method and clinical outcomes in regenerative and intra-articular applications.

Conclusion

The manual double-spin protocol described in this study offers a simple, reproducible, and cost-effective method for production of autologous PRP. It can be used in operating room settings without the need for specialized kits or equipment. The protocol consistently yields concentrated platelets suitable for regenerative and intra-articular applications, this particularly valuable in clinical usage with limited resources.

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