

Analysis of Platelet Rich Plasma: A Comparison of Manual Method and Commercial Separation System

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Background: In Platelet Rich Plasma (PRP) the concentration of platelets is crucial for wound healing as they quickly aggregate at damaged site & release range of growth factors along with cytokines linked to healing, hastening and regeneration of soft tissues. It can be considered as magic medicine for treating acute and chronic conditions as it is autologous so it reduces the risk of cross contamination as well as immune rejecting reactions. **Objective:** To Compare the Manual method of PRP preparation with the commercially available Kits. **Methods:** After signing the informed consent from the patients, 180 ml of blood was isolated from each subject and PRP was prepared according to manufacturer's guidelines in accordance with the PRP kits- Dr PRP kit (REMI Healthcare), Smart Prep3 (Harvest Terumo), Magellan (Arteriocyte Medical Systems), ACP (Device Technologies), Angel (Arthrex Angel System) and for manual PRP preparation method. **Results:** PRP prepared using manual method and Magellan system produced highest Platelet count. The concentration of growth factors and Platelet conc. factor (PCF) and Platelet recovery percent was maximum in the PRP prepared using manual method followed by Magellan and other PRP preparation systems. **Conclusion:** Manual method provides the optimum concentration of platelets & growth factors, can be considered as more efficacious, easy and cost-effective method for preparing PRP.

Key Words: Platelet rich Plasma, Growth factors, Platelet Concentration factor, Platelet recovery factor.

Plain Language Summary

What is the context?

PRP is a generic term referring to sample of autologous plasma that contains platelet concentration above baseline blood values. Because of its growing popularity several investigations are conducted to elucidate its role in managing multiple conditions including hair loss, tissue and wound healing, chronic ulcer management & in gynecological conditions such as thin endometrium & for infertility. Currently there are multiple methods for preparing PRP including: apheresis, commercial separation systems & manual PRP preparation methods. All of these focus on fractionating the whole blood into composite parts but each method creates variability isolating the required cellular composition which creates methodological challenges.

What is new and its Impact?

- In this study, we have estimated the concentration of growth factors, platelet conc. factor, platelet dose and platelet recovery %, in PRP prepared using different commercial separation systems and manual method of PRP preparation according to their protocols.
- Based on results, our analysis suggests that there was considerable difference observed in the conc. of growth factors, platelet count, platelet conc. factor, platelet dose and platelet recovery % when PRP is prepared using manual method, though the percentage was nearly similar in a few commercial separation systems but considering the kit cost, quantity of blood required, PRP preparation time etc. these factors must be considered to find an effective and efficient approach.

Introduction

PRP preparation has become more and more popular due to its extensive use in a variable medical forte. Preparation of PRP have been used since 1970s, but gained sudden popularity since 1990s. Since then, other methods for making PRP have surfaced, ranging from commercial equipment to traditional blood centrifugation[1].PRP is frequently utilized as fillers or in rejuvenating operations in the fields of cosmetic medicine and cosmetology. Treatment with PRP involves inserting concentrated platelets into wound sites to start tissue healing process by releasing adhesion proteins and biological factors that start the haemostatic cascade, new connective tissue production, & revascularization [2].PRP's capacity to release several growth factors, cytokines, and interferons is thought to be responsible for its therapeutic impact. These factors are released when platelets come into contact with exposed endothelium in wounds or damaged tissues. They cooperate with tissue-healing mechanisms like angiogenesis, Chemotaxis, Cell Proliferation, Extracellular Matrix (EM) deposition, and remodelling to support proper wound healing. Therefore, it was hypothesized that raising the number of platelets in damaged tissue would raise

levels of several bioactive substances and enhance the body's natural healing process [3]. When applied as a supplement to routine wound care procedures, they encourage angiogenesis, fibroblast proliferation, epithelial cell proliferation [4]. The basis of PRP treatment was centered on maximizing the quantity of growth factors present in α granules of platelets for regulating wound healing processes and to enhance tissue healing and regeneration.

Platelets mainly contain three types of granules - α granules, dense granules and lysosomes. The α granules majorly contains the growth factors which includes VEGF, Insulin like growth factor-2, epidermal growth factor, transforming growth factor- β , epithelial -cell growth factor & platelet derived growth and hepatocyte GF where as dense granules contain limited amount of small molecules- serotonin, adenosine diphosphate, adenosine triphosphate, calcium ions, magnesium ions, potassium, pyrophosphate and polyphosphates as shown in the table below [5]:

Table 1. Growth factors in PRP with their functions	
Factors	
PDGF	• Powerful chemoattractant & stimulator. • Promotes proliferation & migration of inflammatory cells - neutrophils, fibroblasts, macrophages & smooth muscle cells. • Promotes DNA synthesis & chemotaxis
VEGF	• Potent stimulator of endothelial cell proliferation, sprouting, migration & tube formation. • Participate in chemotaxis of endothelial cells by promoting their proliferation, differentiation & regulation of vascular permeability.
TGF- β	• Multifactorial regulator of cellular growth in developing systems. • Prominent component of Extra cellular matrix.
IGF	• Majorly of two types- IGF-1 & IGF-2. • IGF-1 is always present in life, but decreases with age whereas IGF-2 present at fetal age only. • Stimulates protein synthesis.
EGF	• It has high affinity for epidermal GF receptor-triggers and increase in expression of certain genes that lead to DNA synthesis & cell proliferation. • Stimulates epidermal regeneration by revivifying the proliferation keratocytes & dermal fibroblasts.

These GFs released from activated PRP are believed to stimulate the transition from Telogen to Anagen phases of hair growth cycle and might extend the length of anagen phase through prevention of apoptosis as well as increase vascularisation & angiogenesis to promote delivery of nutrients to hair follicle. VEGF and PDGF is usually secreted by keratinocytes and DP cells. They are believed to promote proliferation of DP cells in bulge areas of hair follicle through increased accumulation of fibroblast growth factor-7 & β -catenin which are required for growth. Angiogenesis and vascularisation are considered crucial in launching anagen phase by growing the early follicle and through an increase in circulation. The GFs in PRP provide appropriate nutrition & oxygen to support folliculoneogenesis [6]

The Open Method and the Closed Method are the two approaches currently available for preparing PRP. Blood is exposed to the surroundings in the work area when using the open approach. On the other hand, when using commercial devices or kits in a closed system, the blood or PRP is not exposed to the environment during the PRP preparation process. In Open method- the double spin method is usually the recommended method for dermatological preparations as it is low cost, yields good amount of platelets and great volume of PRP is extracted. Three fundamental techniques are employed in the most popular commercial kits for closed preparation systems: automated cell separators, gel separators, and narrow neck tubes. Due to their ease of use and preparation, the majority of commercial kits for PRP preparation use the gel separation technique [7].

PRP is typically regarded as enriched if the concentration of platelets is at least five times that of the peripheral blood baseline. Because sample variability presents certain methodological challenges for researchers, many in vitro and in vivo studies have yielded results that are challenging to interpret in terms of the dose-dependent relationship between platelet load, growth factors (GFs) delivered to the injury site, and actual healing [8]. To facilitate preparation and aid in content standardization, a number of businesses, including REMI Healthcare, Device Technologies, Arthrex Angel systems etc, have marketed PRP preparation kits and/or methods that have been approved for clinical usage. These kits give medical professionals a proven way to regulate the injectate's composition, including the number of neutrophils or other white blood cell populations [9]. PRP can be prepared using a number of commercially available kits that separate blood into its component parts and isolate plasma with elevated platelets. The protocols of these kits vary in their method of seclusion type of collecting tubes, speed of centrifugation & other processes of production which leads to production of varying volumes of plasma along with the concentration of growth factors which might create methodological challenges for investigators [10]. There may be variations in the quantity of whole blood needed depending on the purification technique used to create PRP. Understanding the variations in ultimate product, liquid components, and GF concentrations may help PRP kits be utilized in clinically appropriate ways.

Our study's goal is to compare the platelet concentration, white blood cell, growth factors, platelet concentration factor, platelet dose & platelet recovery percentage in PRP made using five commercially available PRP kits to a novel PRP preparation method.

Material and Methods

- Commercial PRP Kit preparation

Blood samples were collected from 10 healthy subject saged between 25-40 years, after taking the informed consent. The samples were taken in accordance with the manufacturer's instructions, using the proper anticoagulant ratio as mentioned in the protocols of the PRP kits- Dr PRP kit (REMI Healthcare), Smart Prep3 (Harvest Terumo), Magellan (Arteriocyte Medical Systems), ACP (Device Technologies), Angel (Arthrex Angel System) and for manual PRP preparation method. In order to create the finished product, the samples were prepared in accordance with the manufacturer's instructions.

Table 2. Commercial PRP Preparation Systems

Device	Manufacturer	Whole Blood volume (ml)	Anticoagulant: Type & Quantity(ml)	Procedure	1 st Centrifuge: RPM & Time	2 nd Centrifuge: RPM & Time	PRP Volume (ml)
Angel (2%)	Arthrex Angel System	52	ACD-A; 8	Double Spin	3500; 3 min.	3000 ; 9 mins	3.00±0.50
ACP	Device Technologies	16	ACD-A; 1.5	Single Spin	1500 ;15 mins.		4-7
Dr. PRP	Remi Healthcare	18	2	Double Spin	3000 ; 3-4 mins	3200 ; 6 mins	4.00 ± 0.50
Magellan	Arteriocyte Medical Systems	52	ACD-A; 8	Double Spin	2800	3800	5.3±1.6
Smart PreP	Harvest Terumo	54	ACD-A; 6	Double Spin	2500 ;1-3 mins	2300-2400 ; 6-9 mins	7
Manual Method		18	Heparin (5000 IU); (1:10)	Double Spin	1800 ; 12 mins	3200 ; 6 min	7±0.50

For preparing PRP manually following method was followed:

- Manual Preparation of PRP from whole blood.

Eighteen ml of whole blood was collected under sterilised conditions, samples were transferred to the falcon tubes containing 1ml heparin (5000 IU) as anticoagulant. The samples were mixed gently with the anticoagulant, following to that first spin was performed at 1800rotations per minute (RPM) for 12 mins. The

sample got separated into 3 layers- upper-most layer (platelet poor plasma), Centre layer (buffy coat), bottom-most layer (red blood cells). In a separate falcon tube upper and middle layers are collected and second spin is performed at 3200 rpm for 6 mins, platelets form a soft pellet at the bottom of the tube, upper plasma is discarded and the remaining plasma is rich with platelets.

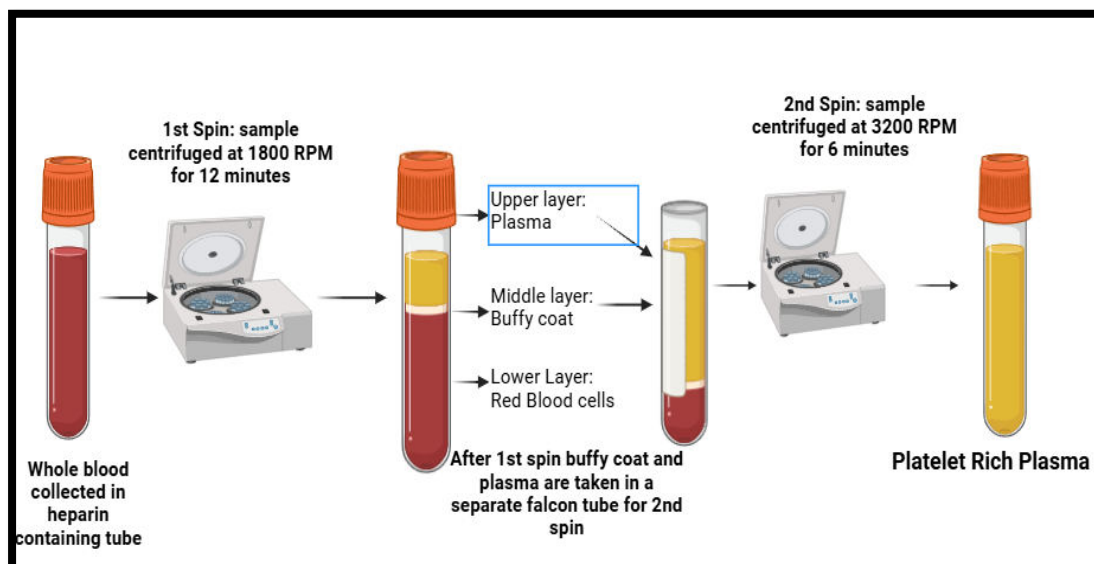


Fig. 1 :Steps for Manual Method For PRP Preparation

- **Quantification**

Platelet rich plasma collected from all the commercially available kits and manual preparation method was assessed for the platelet counts, WBC count, WBC recovery, platelet yield, PRP volume, Platelet concentration factor, Platelet Recovery Percentage, PRP volume consistency (ie. The proportion of times a treatment uses the same whole blood input to produce the same PRP volume), PRP protocol time (ie. Centrifuge duration for PRP production), cost of PRP kit and PRP kit cost per billion platelets. Following points were considered for comparing the PRP from commercial kits from manual methods as these points can be used as measures of reproducibility, efficacy & true cost of PRP kit that must be considered wisely. The above-mentioned parameters can be calculated using following formula's: [11]

- Platelet Conc. factor = PRP Platelet count/ Blood platelet count
- WBC recovery= WBC in PRP/ WBC in whole blood
- Platelet Dose = PRP platelet count * PRP Volume
- Platelet recovery percentage = Platelet dose * total platelets
- Platelet yield= (PRP platelet count/ PRP volume)/ (platelets in whole blood/ volume of whole blood)

Results

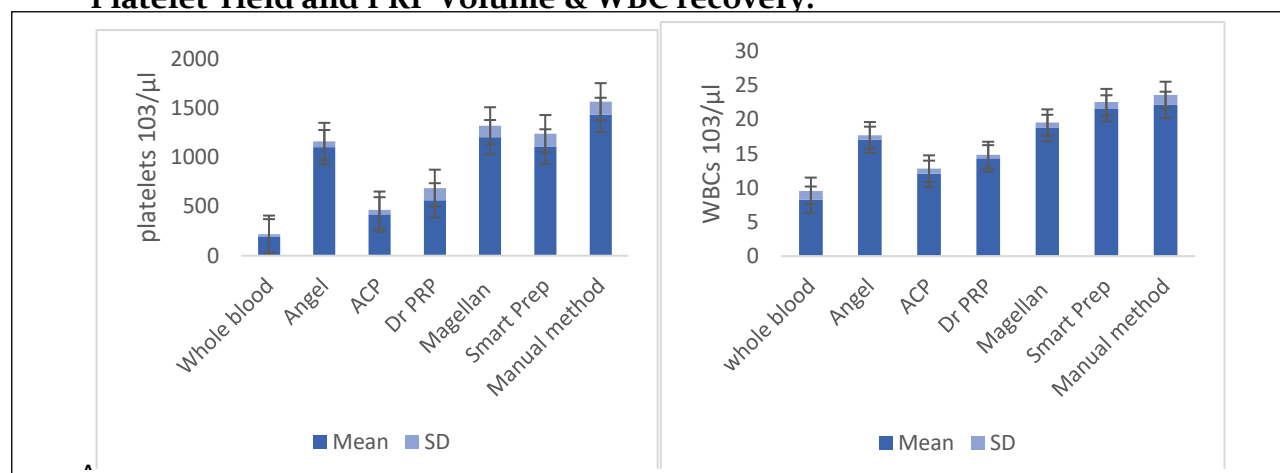
After preparing the PRP from both the Commercial preparation systems and manual method we further analyzed the cellular composition (Platelets and white blood cells) and quality of PRP prepared. For analyzing the quality, we preferred calculating the

WBC recovery, platelet yield, platelet dose, platelet recovery %, platelet concentration factor and PRP volume produced in each method.

Platelet and White Blood Cells Concentration

The total blood platelets were comparatively higher in the manual method of PRP preparation (1433.68 ± 135.53) from the baseline followed by Magellan kit preparatory method, similar concentration of platelets was isolated in the Angel and Smart Prep kit preparations i.e. 1104.76 ± 60.57 & 1112.56 ± 131.88 . Similarly, the concentration of WBCs was higher in manual method of PRP preparation (22.17 ± 1.44) from the baseline quantity, followed by the Smart Prep commercial kits rather than Magellan system, nearly equal number of WBCs were isolated in Magellan and Angel PRP kit preparations as seen below in figure 1

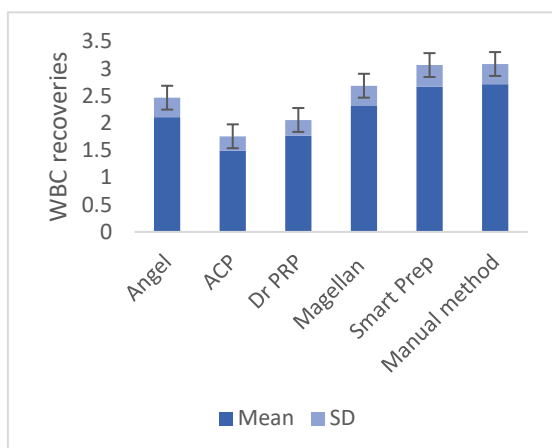
Platelet Yield and PRP Volume & WBC recovery.



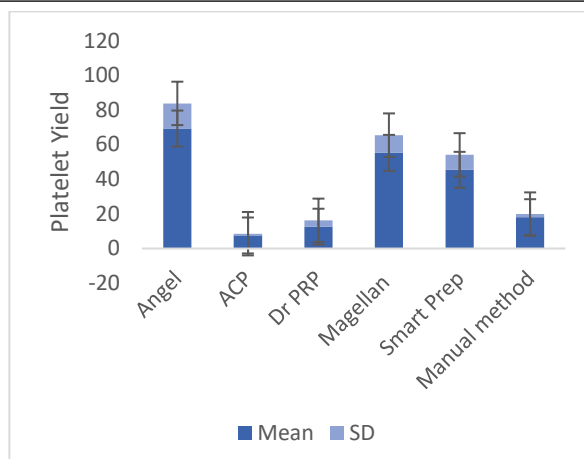
The volume of PRP prepared was maximum in Manual method of PRP preparation i.e. 7.32 ± 0.52 , followed by Magellan System and Smart Prep PRP kit preparation, but the platelet yield was maximum in Angel system of PRP, further it was observed that though the number of platelets isolated and volume of PRP prepared was maximum in the manual method the yield was comparatively lower in the manual method i.e. 18.1 ± 1.78 .

Platelet Concentration Factor, Platelet Dose and Platelet Recovery %

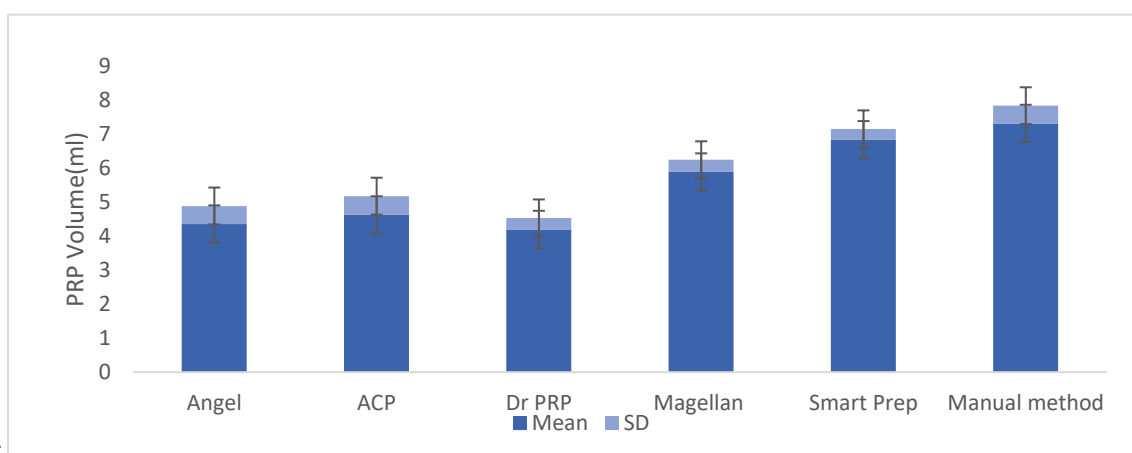
A



B



C



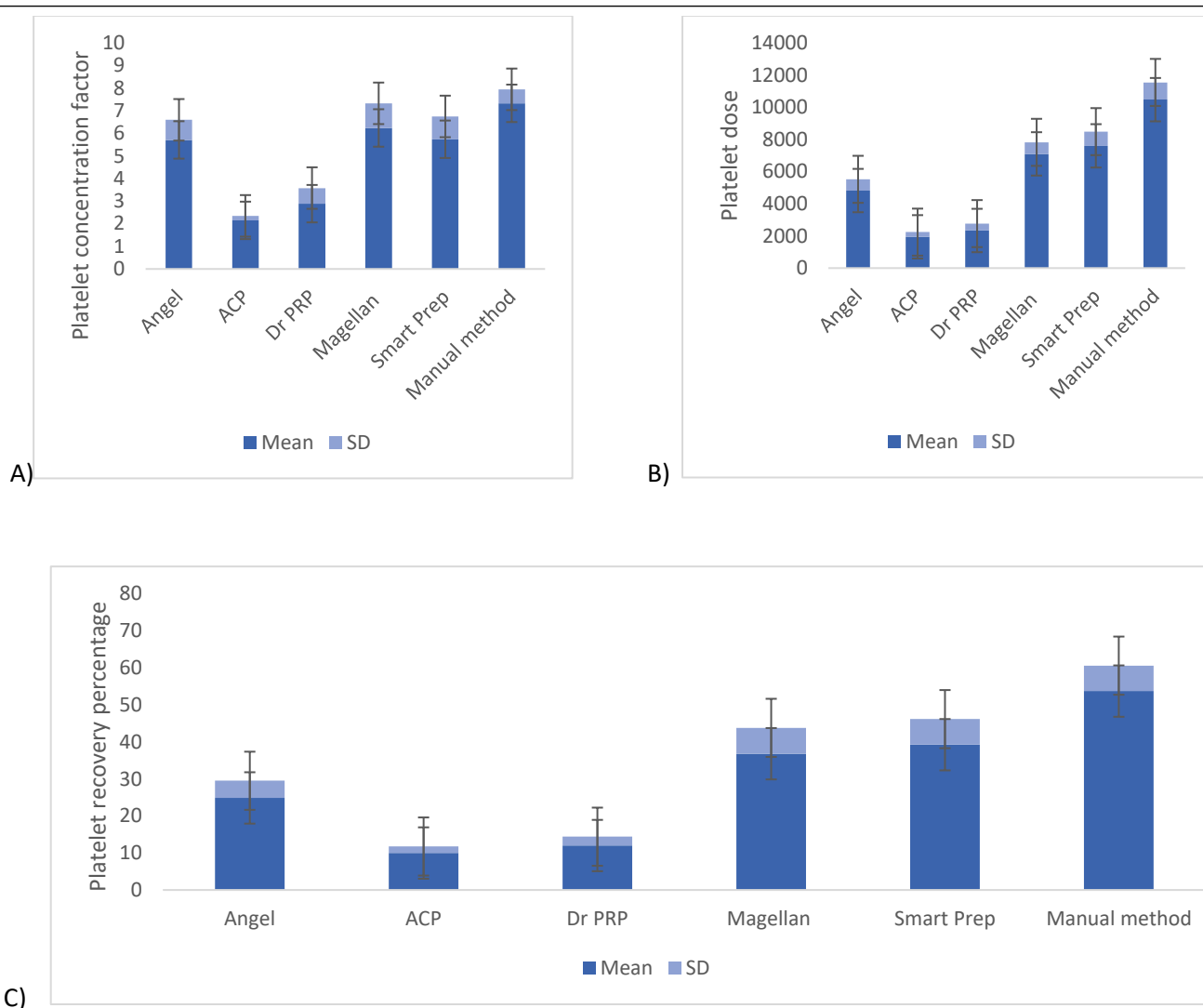


Figure 3. A B & C The platelet concentration factor, platelet dose and platelet recovery percent were maximum in manual method of PRP preparation, the platelet concentration factor was higher in Magellan PRP kit. B & C, the platelet dose and recovery was nearly similar in Magellan and Smart Prep. The platelet concentration factor, platelet dose and recovery percentage was minimum in ACP PRP kit.

The platelet concentration factor, platelet dose and platelet recovery % was maximum in manual method of PRP preparation i.e. 7.33 ± 0.62 , 10476.3 ± 1072.8 and 53.71 ± 6.87 respectively. In Magellan system and Smart Prep preparation kit nearly equal amount of platelet dose and platelet recovery was observed i.e. 7103.8 ± 719.3 & 7603.2 ± 883.6 respectively. The minimum platelet concentration factor and platelet dose and platelet recovery % was observed in Dr PRP kit & ACP kit respectively as shown in the table below.

Parameters	Angel	ACP	Dr PRP	Magellan	Smart Prep	Manual method
Platelet concentration factor	5.71 ±0.89	2.15 ±0.20	2.89 ±0.69	6.24 ±1.09	5.74 ±1.01	7.33 ±0.62
Platelet Dose	4823 ±698.7	1946.2 ±291.7	2339.4 ±429.2	7103.8 ±719.3	7603.2 ±883.6	10476.3 ±1072.
Platelet Recovery %	24.86 ±4.64	9.98 ±1.76	12 ±2.39	36.80 ±6.98	39.23 ±6.91	53.71 ±6.87

Discussion

The primary objective of the large field of human regenerative medicine is to replace damaged human cells, tissues & organs that have been impacted by aging, illness or birth abnormalities. The most often used platelet-based bioproduct is PRP & its therapeutic use is a comparatively new strategy in regenerative medicine that has clinical benefits across many different medical specialties [14]. By immediately administering a high concentration of platelet-derived growth factors and other cytokines to the injured area, PRP therapy—which can be identified in clinical practice using a variety of diagnostic techniques—aims to speed up the healing processes. The amount of whole blood taken by the patient, the anticoagulant type, the separator's design, the centrifugation time & speed, extraction and activation process used, and other factors all affect how these kits vary from one another. They also differ in how many WBCs are present in the finished product and what approximate concentration they allow to be reached.

Without giving comprehensive information, it is impossible to accurately evaluate the efficacy of treatments using various autologous platelet-rich plasma preparations due to the large no. of variables involved [15]. It's crucial to recognize the kits that generate various dosages of platelets since a certain "dose" may be needed to create a therapeutic effect [16]. In the current study we have focussed on analysing the cellular composition (platelets & WBCs) and quality of PRP prepared using different commercial systems and manual method of PRP preparation. It was observed that maximum number of platelets and WBCs were isolated in manual method. The quality of PRP was assessed by calculating the WBC recoveries, Platelet yield, Platelet concentration factor, platelet dose and platelet recovery % instead of assessing the quantity of growth factors, as using calculative method can be more time efficient and economically more feasible. The selection of appropriate PRP components should be taken into account the patient's indications as well as the biomolecular properties of each component. Though there are studies which have compared commercially available kits for PRP preparation, this is the first study which has compared manual method of preparation with commercial kits using more feasible parameters. Though the quantity of platelets isolated, PRP volume was maximum in manual method the

platelet yield was comparatively low which might be due to that platelet yield depends on the PRP volume and Whole blood volume used for preparing PRP, the latter one was less compared to the PRP volume. In a similar investigation, Kakudo et al. found that the average platelet count in PRP made with the Magellan method was 7.1 ± 0.79 . Fitzpatrick et al. examined four commercially available kits, including the Magellan, Smart Prep2, and ACP kits, in a different study. They observed that concentration of platelets was only 1.5 times increased in ACP kits and there was 3-6 times increase in the concentration in the other kits, the variation observed was similar in our study. Castillo et al, in their study described that platelet count of Magellan system was $780 \times 10^3 / \mu\text{l}$ and conc. of PDG and VEGF was 34.4 ± 10.7 & 1.2 ± 0.8 ng/ml whereas in our study the conc. was comparatively higher which might be due to type of Kit used, amount of blood taken and geographical variations. Because clinicians require a comprehensive explanation of PRP as a biologic drug in order to assess its efficacy, Buford et al.'s analysis took into account a number of parameters that aid in comparing PRP kits. This helps clinicians determine which parameters are clinically important when evaluating PRP kits. From that report, our study is the first one that has considered platelet concentration factor, Platelet dose and Platelet recovery % as points of comparison among commercial kits and manual method.

The cost of the disposable PRP preparation kit varies from US\$ 50 to US\$ 500, in comparison to that manual method is way cheaper and easier to use with good concentration of platelets and growth factors. PRP separation technologies come in a variety of forms, and it will be important to advance their clinical use in ways that optimize the distinct advantages of each.

Conclusion

The potential of platelet-rich plasma to promote creation of growth factors & consequently, the rise in their concentration in the secretion of proteins that can optimize the healing process at the cellular level serves as the justification for its clinical usage. Using autologous PRP reduces the possibility of cross-contamination and the spread of immunological responses or microbiological diseases. From the above analysis it is evident that manual method is more efficacious compared to other systems of PRP preparation and is inexpensive. The manual technique aids in supplying the ideal platelet concentration required to promote tissue repair or can be applied in varying dilutions to get a therapeutically desired quantity of platelets specific to a tissue or condition.

Conflict of Interest: None.

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