Component of Manual Preparation of Platelet-Rich Plasma

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Background: *Popularity of using Platelets Rich Plasma (PRP) in the orthopedics practice is increasing, however, commercial kits for preparation are typically expensive.*
Objective: *To clarify the best protocols of manual PRP preparation that can obtain the highest platelet concentration with low amount of white blood cells and red blood cell.*
Material and Method: *A 22 ml blood samples was taken from 10 healthy volunteers. Each specimen was equally separated into 4 syringes for 4 different protocols of centrifugation duration; 1,000 rpm/2 mins, 1,000 rpm/3 mins, 1,000 rpm/5 mins and 1,000 rpm/7 mins. Platelet counts from each protocol was analyzed.*
Results: *The protocol of 2 and 3 minutes spinning duration have shown higher platelets concentration compared to other protocols. The highest amount of platelet concentration was obtained from the 3-minute duration protocol at a mean of $438.1 \pm 124.733 \times 10^3/mm^3$ (1.7 fold than baseline) and also lower WBC contamination when compared to 2-minute duration protocol.*
Conclusion: *The spinning of blood at 1,000 rpm for 3 minutes is the best protocol to gain plasma that has a high platelet concentration and low WBC contamination.*

Keywords: *Platelet-rich plasma, Manual PRP preparation, Platelet concentration*

Platelets rich plasma (PRP) is known as an autologous preparation concentrates platelets in small volume of plasma[1]. The therapeutic potential of PRP is based on the cytokine released from the alpha granules of platelets to enhance the body's natural healing response[2]. Moreover, specific growth factors such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and transforming growth factor beta-1 (TGF-B1), fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1) play an important role in the therapy. In addition to growth factors, platelets also release a multitude of bioactive proteins that not only promote removal of degenerated and necrotic tissue but also enhance tissue regeneration and healing process[3]. In clinical studies data of PRP, showed the benefit in facilitating tissue repair, modulating inflammation tendon, ligament, and joint[4-11].

In Thailand, PRP has gained attention as the treatment of orthopedic patients. Due to the fact that the equipments involved in general PRP preparation provided by the commercial markets are expensive. The authors have initiated the manual technique which is simple, sterile and inexpensive. This technique involved centrifugation blood samples by centrifuge machines, which are available in every hospital in Thailand. However, the basic data of blood components of this preparation technique is important, especially this preparation use different of spinning time that has not been studied.

The purpose of this study is to elucidate the protocols of platelet preparation which can obtain the highest platelet concentration with low amount of white blood cells and red blood cell.

Material and Method

The study was approved by review board and ethics committee of HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University (SWUEC/E-393/2559). Ten healthy volunteers (8 males, 2 females) were recruited in the study. All of the subjects

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were over 20 years old. None of them had an underlying of hematological diseases and informed consents were obtained before taking blood samples.

At the orthopedics outpatient department, the manual PRP preparation was done under sterile technique. Firstly, a 22 ml of blood was obtained from each volunteer subject for CBC (2 ml) baseline and PRP preparation (20 ml). Then, the blood sample was divided into 5 ml in four syringes. The syringes inside has a thin coat of heparin. Each plastic syringe was prepared for four different blood spinning protocols as follow; 1,000 rpm for 2 mins, 1,000 rpm for 3 mins, 1,000 rpm for 5 min and 1,000 rpm for 7 mins. The tip of syringes was closed with a lock tip cap and the plunger was cut in the level of finger grip and wrapped with an adhesive tape before being taken to the centrifuge machine (Digisystem Model DSC-200T- voltage 220V) (Fig. 1A, B). After the spinning, the blood in the syringes was separated into two layers: a plasma layer and a red cells pack layer (Fig. 1C). The tip cap was opened, then the plasma layer was pipetted with a new syringe and analyzed the platelets concentration in each protocol (Fig. 1D). The data obtained from different protocols
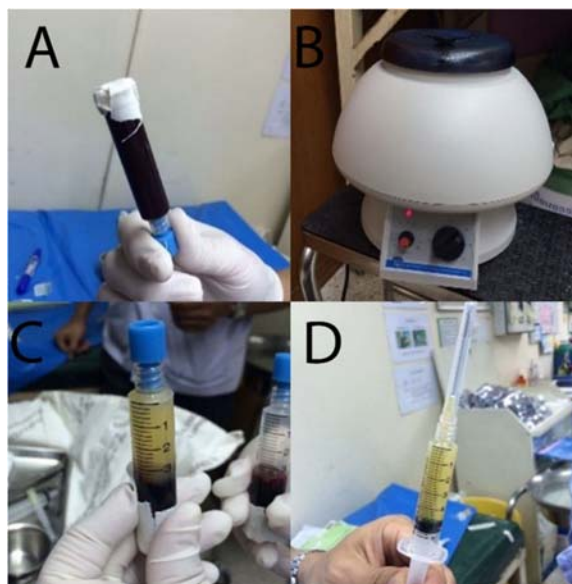

Fig. 1 Manual PRP preparation (A) 5 ml of blood in syringe was locked with cap at the tip and wrapped with adhesive tape at the plunger to prevent move (B) a centrifuge machine, Digisystem Model DSC-200T, was used in this preparation (C) plasma was separated from RBC after centrifugation (D) plasma was pipetted from the syringe to a new tube for further analysis.

were compared by using SPSS software.

Results
Platelets concentration

The analysis of each protocols in each subject is shown in Fig. 1. The centrifugation of blood sample at 1,000 rpm for 3 minutes showed the highest platelets concentration of $438.1 \pm 124.733 \times 10^3/mm^3$, which was 1.66-fold higher than baseline platelet count, followed by centrifugation at 1,000 rpm for 2 minutes which yield $401.9 \times 10^3/mm^3$ of platelets concentration (Table 1). Both protocols had a significantly higher level of platelets concentration compared to other protocols and CBC baseline. The lowest platelet concentration was from the spinning at 1,000 rpm for 7 minutes. The spinning at 1,000 rpm for 2 minutes was more contaminated with WBC than the other protocols. But, it was not a statistically significant different. The lowest contamination of white blood cell was the spinning of blood sample at 1,000 rpm for 5 minutes. For RBC count, all protocols showed low contamination of RBC. When compared among all protocols, the amount of RBC from protocol 1,000 rpm/3 mins, 1,000 rpm/5 mins and 1,000 rpm/7 mins, were of statistically significant differences (Table 1).

Pairwise comparison of platelets concentration of PRP by manual PRP preparation at different spinning time is shown in Table 2. All protocol showed statistically significant difference except the comparison between the spinning at 1,000 rpm for 2 and 3 minutes.

Matching comparison of the amount of WBC from the manual preparation indicated a significant difference between the 1,000 rpm/3 mins protocol and the 1,000 rpm/5 mins protocol (p-value = 0.029) (Table 3). Similarly, pairwise comparison of RBC revealed that the amount of RBC from protocol 1,000 rpm/3 mins,
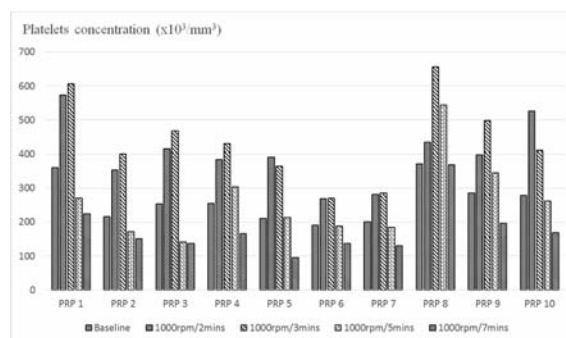

Fig. 2 The comparison of platelets concentration from 4 different preparations of 10 volunteers.

Table 1. Mean and SD of platelets concentration and white blood cells count from 4 different PRP preparation in 10 volunteers

Time used in spinning at 1,000 rpm (min)	Mean±SD of platelets concentration (x 10^3/mm³)	Fold compared to baseline	Mean of RBC count (x10^6/mm³)	Mean of WBC count (mm³)
2	401.9±95.124	1.550	3,344±5475.09	0.183±0.50
3	438.1±124.733	1.660	138±155.05	0.190±0.01
5	261.6±117.97	0.990	13±31.29	0.012±0.01
7	176.6±75.893	0.650	92±151.13	0.003±0.01

Table 2. Pairwise comparison of platelets concentration of PRP by manual PRP preparation at different spinning time

Time used for centrifugation at 1,000 rpm (min)		Mean difference ±SD (x 10^3/mm³)	p-value	95% confidence interval	
				Lower bound	Upper bound
2	3	-36.20±27.56	0.221	-98.538	26.138
2	5	140.30±39.83	0.006	50.193	230.407
2	7	225.30±30.08	<0.001	157.263	293.337
3	5	176.50±28.78	<0.001	111.393	241.607
3	7	261.50±23.72	<0.001	207.841	315.159
5	7	85.00±18.47	0.001	-126.779	-43.221

Table 3. Pairwise comparison of WBC of PRP from the manual preparation

Time used for centrifugation at 1,000 rpm (min)		Mean difference ±SD (mm³)	p-value	95% confidence interval	
				Lower bound	Upper bound
2	3	3,206±1,742.50	0.099	-735.817	7147.817
2	5	3,331±1,731.65	0.087	-586.268	7248.268
2	7	3,252±1,749.25	0.096	-705.082	7209.082
3	5	125±48.17	0.029	16.027	233.973
3	7	46±61.36	0.473	-92.803	184.803
5	7	-79±51.24	0.158	-194.911	36.911

1,000 rpm/5 mins and 1,000 rpm/7 mins were statistically significant differences ($p<0.05$) (Table 4).

Discussion

It has been known worldwide and proved by many clinical studies that the PRP has a benefit in clinical practices[8-11]. In Thailand, the physicians are currently interested in using the PRP for the treatment of orthopedic patients. However, the technique for preparation of PRP is costly. Consequenrly, the authors initiated a technique for PRP preparation which is simple and low-cost compared to those using commercial kit.

In the previous study, it is still not clear regarding the proper concentration of the platelets. According to one *in vitro* study, the concentration of platelets above 2.5 folds of baseline has inhibitory effect on osteoblast and fibroblast proliferation[12]. Moreover, the centrifugation rate and time appear to be important parameters which influence the platelet concentration.

Table 4. Pairwise comparison of RBC of PRP from the manual preparation

Time used for centrifugation at 1,000 rpm (min)		Mean difference ±SD (mm³)	p-value	95% confidence interval	
				Lower bound	Upper bound
2	3	0.164±0.158	0.328	-0.194	0.522
2	5	0.171±0.160	0.313	-0.191	0.533
2	7	0.180±0.160	0.290	-0.182	0.542
3	5	0.007±0.003	0.025	0.001	0.013
3	7	0.016±0.003	<0.001	0.010	0.022
5	7	0.009±0.002	0.001	0.005	0.013

This study used the centrifuge machine brand Digisystem Model DSC-200T- voltage 220V Rotor AR 1,506 type 45°, speed 300 to 3,000 rpm and g-force of 1,066 g which is different from the commercial kits which use Hettich Rotofix 32 centrifuge with 90° Swing Out Rotor, 220 V, RPM 4,000, and g-force of 2,558 g. The different types of centrifuge machines with different g-force, velocity and duration of blood spinning may provide different results of platelets concentration[13]. The results of our study suggest that the manual PRP preparation by spinning blood at 1,000 rpm for 3 minutes using Digisystem centrifuge was considered the best protocol to prepare platelets rich plasma as it provided higher platelets concentration and lesser WBC and RBC contamination than the other protocols.

According to the results, the spinning with 1,000 rpm for 3 minutes with centrifuge machine, Digisystem Model DSC-200T with 1,066 g, had the highest platelets concentration of $438.1\pm124.733\times10^3$/mm³, which is 1.7 fold higher than baseline platelet count. This platelet concentration result is near the platelet concentration level from commercial kits that are on the market in Thailand, which increased platelet concentration 2 times from the baseline[14].

The limitation of this study is the lack of control in each protocol. Further study with controling of human error factors such as skill or occupation may be needed.

Conclusion

The manual platelets-rich plasma (PRP) preparation by spinning at the speed of 1,000 rpm for 3 minutes is the best protocol when using the Digisystem centrifuge Model DSC-200T.

What is already known on this topic?

In the preparation of PRP, the centrifugation rate and time appear to be important parameters, which influence the platelets concentration.

What this study adds?

The manual PRP preparation by centrifuge machines spinning at 1,000 rpm for 3 minutes can prepare the platelets rich plasma about 1.7-fold concentration above baseline which is higher than the other experiments with different durations.

Potential conflict of interest

None.

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ส่วนประกอบของเกล็ดเลือดเข้มข้นที่ได้จากการเตรียมเกล็ดเลือดเข้มข้นด้วยมือ

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ภูมิหลัง: เกล็ดเลือดเข้มข้นได้รับความสนใจมากขึ้นในการนำมาใช้รักษาทางด้านศัลยกรรมกระดูกและข้อ อย่างไรก็ตามวิธีการเตรียมเกล็ดเลือดเข้มข้น โดยทั่วไปที่มีตามท้องตลาดนั้นมีราคาค่อนข้างสูง

วัตถุประสงค์: เพื่อทำให้ได้ทราบถึงวิธีการที่ดีที่สุดในการเตรียมเกล็ดเลือดเข้มข้นจากการเตรียมเกล็ดเลือดด้วยมือ เพื่อให้ได้ส่วนประกอบเกล็ดเลือดที่สูงที่สุด และมีเม็ดเลือดขาวและเม็ดเลือดแดงที่ต่ำ

วัสดุและวิธีการ: เก็บเลือด 22 มิลลิลิตร จากอาสาสมัครแต่ละรายจำนวน 10 ราย โดยแบ่งเลือดเพื่อทำการเตรียมเกล็ดเลือด 4 วิธี ได้แก่ การปั่นเหวี่ยง ที่ความเร่ง 1,000 รอบต่อนาทีที่เป็นเวลา 2 นาที 3 นาที 5 นาที และ 7 นาที จากนั้นนำเกล็ดเลือดเข้มข้นที่ได้จากแต่ละวิธีไปตรวจวิเคราะห์

ผลการศึกษา: การเตรียมเกล็ดเลือดเข้มข้นด้วยมือที่ปั่นด้วยช่วงเวลา 2 และ 3 นาที พบว่ามีจำนวนเกล็ดเลือดเข้มข้นที่สูงกว่าเมื่อเทียบกับช่วงเวลาอื่น ซึ่งวิธีการเตรียมด้วยช่วงเวลา 3 นาทีจะได้ค่าความเข้มข้นของเกล็ดเลือดที่สูงที่สุดโดยมีค่าเฉลี่ยเท่ากับ $438.1 \pm 124.733 \times 10^3$ ต่อลูกบาศก์มิลลิเมตร หรือประมาณ 1.7 เท่าจากค่าเกล็ดเลือดพื้นฐาน และมีการปนเปื้อนของเม็ดเลือดขาวที่น้อยกว่าเมื่อเทียบกับการเตรียมด้วยช่วงเวลา 2 นาที

สรุป: วิธีการเตรียมเกล็ดเลือดเข้มข้นด้วยเครื่องปั่นเหวี่ยงที่ความเร็ว 1,000 รอบต่อนาทีที่เป็นเวลา 3 นาที เป็นวิธีการเตรียมเตรียมเกล็ดเลือดเข้มข้น ด้วยมือที่ดีที่สุด ซึ่งได้ปริมาณเกล็ดเลือดเข้มข้นที่สูงและเม็ดเลือดขาวปนเปื้อนต่ำ