Optimizing Platelet-Rich Plasma Gel Formation by Varying Time and Gravitational Forces During Centrifugation

Chris H. Jo, PhD
Young Hak Roh, MD
Ji Eun Kim, MS
Sue Shin, PhD
Kang Sup Yoon, PhD

Despite the increasing clinical use of topical platelet-rich plasma (PRP) to enhance tissue healing and regeneration, there is no properly standardized method of autologous PRP gel preparation. This study examined the effect of the centrifugation time and gravitational force (g) on the platelet recovery ratio of PRP and determined the most effective centrifugation conditions for preparing PRP. Two-step centrifugations for preparing PRP were used in 39 subjects who had consented prior to the study’s start. The separating centrifugation (Step 1, used to separate whole blood into its two main components: red blood cells and plasma) was tested from 500g to 1900g at 200g increments for 5 minutes (min), and from 100g to 1300g at 200g increments for 10 minutes. After separating centrifugation, upper plasma layer was transferred to another plain tube for the condensation centrifugation and remaining lower cell layer was discarded. The condensation centrifugation (Step 2, used to condense the platelets in the separated plasma) was tested at 1000g for 15 min, 1500g for 15 min, 2000g for 5 min and 3000g for 5 min, additionally at 1000g for 10 min and 1500g for 10 min. Platelet gelation was induced by adding 10% calcium gluconate to final PRP with volume ratio of 1:10. The optimal separating centrifugation conditions were followed by 900g for 5 minutes and the condensation conditions were followed by 1500g for 15 minutes, of which recovery ratios were 92.0 ± 3.1% and 84.3 ± 10.0%, respectively.

Key Words: platelet-rich plasma, platelet gel, preparation, centrifugation, recovery ratio

INTRODUCTION

Topically applied platelet-rich plasma (PRP) derived from autologous blood was recently introduced to facilitate tissue healing and regeneration and widely used in almost all fields of surgery for the acceleration of bone and soft tissue formation and for the management of chronic nonhealing wounds. The use of PRP combines the advantage of an autologous fibrin clot that will aid in hemostasis as well as the provision of growth factors in high concentration to the site of a tissue defect or a region requiring augmentation after platelet release.

Despite the increasing application of PRP for local tissue healing and regeneration, there still remains some controversy about clinical benefits of PRP, and the heterogeneous PRP from different preparation protocols in each study made it difficult to compare the results. The actual quantity of platelets is a prior requirement for the effectiveness of PRP and there has, nevertheless, been no properly standardized preparation method systematically verified in terms of platelet recovery ratio. For the evaluation of the effectiveness of preparation method within this study, both the platelet...
concentration and the volume of the final PRP are important. Therefore, the evaluation of preparation protocol with recovery ratio, the total platelet counts after centrifugation divided by those before centrifugation, is more reasonable than that with platelet concentration of final PRP alone.

This study examined the most effective centrifugation condition, centrifugation speeds measured in gravitational forces (g) and times, in terms of the recovery ratio using routine laboratory equipment and reagents. In addition, the gelation of PRP was attempted without thrombin, which may cause unnecessary early platelet activation.

**Materials and Methods**

After obtaining signed informed consent, 39 blood samples from 41 volunteers were used. Two samples with serologic or hematologic abnormalities were excluded (one has hepatitis B antigen and the other has thrombocyte counts $<150,000/\mu$L). Finally, there were 16 males and 23 females with an average age of 53 ± 17 years. The average platelet concentration of 39 blood samples measured by cell count machine (XE-2100, Sysmex, Ill., USA) was $247.3 \times 10^3 \pm 64.0/\mu$L.

A total of 40 mL of whole blood was collected from each of 39 volunteers. These samples were further divided into 9 mL sub-samples, which resulted in a total of 156 samples, to which 1.25 mL citrate phosphate dextrose adenine-1 (CPDA-1) was mixed and agitated gently in a plain tube (BD, Franklin Lakes, NJ). Before and after centrifugation, the platelet concentration was checked using an automated cell counter machine (XE-2100, Sysmex).

**Centrifugation method**

Two-step centrifugation is generally used to prepare PRP but various centrifugation techniques and the heterogeneous nature of PRP according to different preparation methods or commercial preparation devices have been reported. In the present study, PRP was prepared by using a 2-step centrifugation with a tabletop centrifuge machine (Allegra X-15R, Beckman Coulter, Brea, Calif., USA, Figure 1), in which step 1 centrifugation (separating centrifugation, Figure 2) used relative low g force for several minutes so that erythrocytes are sedimented but platelets remain in suspension, and step 2 centrifugation (condensation centrifugation, Figure 3) used relative high g force for several minutes to condense the platelets. After step 1 centrifugation, upper plasma layer was transferred to another plain tube for the condensation centrifugation and the remaining lower cell layer was discarded (Figure 2). After step 2 centrifugation, supernatant layer (platelet-poor plasma) was discarded and lower 2 mL plate-rich plasma was preserved, in which the platelet pellet was resuspended (Figure 3).

To determine the most effective separating spin, the initial anticoagulated whole blood was centrifuged from 500g to 1900g for 5 minutes and from 100g to 1300g for 10 minutes at 200g increments. The range between the two most effective centrifugation conditions was subdivided into 50g increments. For condensation spin, the samples centrifuged by the most effective first separating spin, were recentrifuged at 1000g for 15 min, 1500g for 15 min, 1000g for 10 min and 1500g for 10 min, 2000g for 5 min, and 3000g for 5 min.

**Gelling test**

Calcium gluconate (0.2 mL, 10%) was added to final 2 mL PRP samples, which were prepared by 2-step centrifugation condition having highest recovery ratio, and the volume of platelet gel was measured at 10 minutes, 20 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, and 24 hours (Figure 4). Bovine thrombin was not used in order to prevent the early loss of growth factors from thrombin-mediated PRP activation, and the platelet and fibrinogen counts in the remaining plasma after gel formation were measured to evaluate the amount of platelets remaining in the platelet gel.
FIGURES 2–4. FIGURE 2. The separating centrifugation (Step 1). The erythrocytes are sedimented but platelets remain in suspension. (a) Initial anticoagulated blood. (b) After separating centrifugation. (c) A separated red blood cell layer. (d) The upper plasma layer was transferred to another plain tube. FIGURE 3. The condensation centrifugation (Step 2). (a) Plasma layer before condensation centrifugation. (b) Plasma layer after condensation centrifugation. (c) Platelet pellet after condensation centrifugation. (d) Supernatant layer (platelet-poor plasma) was discarded and lower 2 mL platelet-rich plasma was preserved. FIGURE 4. Gelling test. (a) Final 2 mL PRP, in which the platelet pellet was resuspended. (b) Calcium gluconate (0.2 mL, 10%) was added to final 2 mL PRP sample. (c) Platelet gel at 1 hour. (d) Platelet gel at 24 hours.
The recovery ratio, which is a measurement to validate the centrifugation efficiency, was determined using the following formula:

\[
\text{Recovery ratio (\%)} = \left( \frac{\text{PLT concentration of extracted plasma after centrifuge} \times \text{Volume of extracted plasma after centrifuge}}{\text{PLT concentration of plasma (whole blood) before centrifuge} \times \text{Volume of plasma (whole blood) before centrifuge}} \right) \times 100
\]

The measured data is presented as the arithmetic mean and standard deviation (SD). The recovery ratio of each centrifugation condition were analyzed using a Kruskall-Wallis test (nonparametric 1-way analysis of variance) and Bonferroni post-tests were used to compare the multiple values from each preparation condition. A P-value < .05 was considered significant.

**RESULTS**

**Separating centrifugation**

The mean platelet concentration of whole blood with CPDA-1 (9 mL whole blood with 1.25 mL CPDA-1) was 151.0 ± 49.6 x 10^3/\mu L (Figure 5). The average recovery ratio of the first step (separation) centrifugation, which was tested from 500g to 1900g at 200g increments for 5 minutes, ranged from 63.1 ± 9.8% to 92.1 ± 4.9% (n = 5, Figure 6). The first step centrifugation at 900g for 5 min and 1100g for 5 min showed the highest recovery ratio.
TABLE 1

<table>
<thead>
<tr>
<th>g force</th>
<th>900</th>
<th>950</th>
<th>1000</th>
<th>1050</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum volume (mL)</td>
<td>4.80 ± .52</td>
<td>4.79 ± .48</td>
<td>4.81 ± .48</td>
<td>4.80 ± .51</td>
</tr>
<tr>
<td>Platelet concentration (× 10^3/µL)</td>
<td>310.7 ± 78.5</td>
<td>308.9 ± 78.3</td>
<td>302.4 ± 81.5</td>
<td>301.4 ± 81.6</td>
</tr>
<tr>
<td>Recovery ratio (%)</td>
<td>92.0 ± 3.1</td>
<td>91.7 ± 3.6</td>
<td>90.1 ± 5.1</td>
<td>89.7 ± 7.7</td>
</tr>
</tbody>
</table>

*g force = gravitational force.

of 92.1 ± 4.9% and 88.5 ± 6.4% respectively. The mean platelet concentration after the first step centrifugation under these conditions ranged from 260 ± 108 × 10^3 /µL to 399 ± 151 × 10^3 /µL.

The average recovery ratios of the first separation centrifugation, which was tested from 100g to 1300g at 200g increments for 10 minutes, ranged from 38.6 ± 10.7% to 79.5 ± 10.9% (n = 4, Figure 7). Centrifugation at 500g for 10 min and 300g for 10 min showed the highest recovery ratio of 79.5 ± 10.9% and 73.6 ± 14.2%, respectively. The average platelet concentration after the first step centrifugation under these conditions ranged from 127 ± 53 × 10^3 /µL to 344 ± 77 × 10^3 /µL.

The range between the two highest centrifugation conditions (900g for 5 min and 1100g for 5 min) was subdivided at 50g increments, and 900g for 5 min showed the highest recovery ratio of 92.0 ± 3.1%, of which the platelet concentration and measured plasma volume under this condition were 310.7 ± 78.5 × 10^3 /µL and 4.80 ± 0.52 mL, respectively (n = 7, Table 1).

Condensation centrifugation

For the second step condensation centrifugation, the samples obtained by the most effective first separating spin of 900g for 5 min were centrifuged at 1000g for 15 min, 1500g for 15 min, 2000g for 5 min, and 3000g for 5 min, and the lower 2 mL volume of condensed plasma was separated. The platelet pellet was suspended into the plasma. Centrifugation at 1000g for 15 min and 1500g for 15 min showed the highest recovery ratio of 84.9 ± 8.0% and 83.3 ± 5.6%, respectively, with a mean platelet concentration ranging from 364.0 ± 185.7 × 10^3 /µL to 530.3 ± 65.5 × 10^3 /µL. Finally, the 1000g for 10 min and 1500g for 10 min conditions were compared with 1000g for 15 min and 1500g for 15 min. The 1500g for 15 min condition showed the highest recovery ratio of 84.3 ± 10% (n = 6, Table 2). The platelet concentration after 1500g for 15 min condensation centrifugation was 633.2 ± 91.6 × 10^3 /µL, which is 4.2 times higher than that of the initial anticoagulated whole blood, 152.8 ± 28.9 × 10^3 /µL.

Gelling process

Six samples of PRP were prepared by the most efficient centrifugation condition (900g for 5 min separating and 1500g for 15 min condensation centrifugations). The volumes of the platelet gel after adding 0.2 mL 10% calcium gluconate to the final 2 mL of PRP at 10 minutes, 20 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, and 24 hours were 2.08 ± 0.29 mL, 1.52 ± 0.79 mL, 0.76 ± 0.38 mL, 0.35 ± 0.15 mL, 0.21 ± 0.11 mL, 0.17 ± 0.08 mL, and 0.12 ± 0.04 mL, respectively. (n = 6, Figure 8) The platelet concentration of the remaining plasma ranged from 0.67 × 10^3 /µL to 9.67 × 10^3 /µL and no fibrinogen was detected in the remaining plasma.

TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>1000g-15 min</th>
<th>1500g-15 min</th>
<th>2000g-5 min†</th>
<th>3000g-5 min†</th>
<th>1000g-10 min</th>
<th>1500g-10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum volume (mL)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Platelet concentration (× 10^3/µL)</td>
<td>609.2 ± 121.8</td>
<td>633.2 ± 91.6</td>
<td>451.6 ± 72.6</td>
<td>352.0 ± 175.2</td>
<td>600.0 ± 107.1</td>
<td>617.0 ± 88.7</td>
</tr>
<tr>
<td>Recovery ratio (%)</td>
<td>80.4 ± 10.1</td>
<td>84.3 ± 10.0</td>
<td>69.3 ± 12.0</td>
<td>57.9 ± 25.1</td>
<td>79.4 ± 9.2</td>
<td>82.0 ± 8.5</td>
</tr>
</tbody>
</table>

*g = gravitational force; min = minute.
†Significant difference (P < .05) between centrifugation conditions. (Kruskall-Wallis test).
‡Significant different (P < .05) in post-tests for multiple comparison.
Discusssion

Despite the increasing applications of PRP for local tissue healing and regeneration in human and animal studies, there is no properly standardized preparation procedure. In this study, a 900g for 5 min separation spin and 1500g for 15 min condensation spin was determined to be the most effective centrifugation condition, which equated to 27 000g-min (900g × 5 min + 1500g × 15 min) and did not exceed the point of platelet damage. The final platelet recovery ratio of 900g for 5 min (first step) and 1500g for 15 min (second step) centrifugation was 84.9 ± 8.0%, and the concentration of 2 mL PRP from the initial 9 mL whole blood was 633.2 ± 91.6 × 10^3/μL, which is 4.2 times higher than that of the initial anticoagulated whole blood.

Zimmermann et al obtained 0.3 mL PRP with 980 ± 450 × 10^3/μL from 50 mL of whole blood and Ladesberg et al prepared 8 mL of platelet gel from 40 mL of whole blood after obtaining a PRP with an approximately 2 times higher platelet concentration. Commercial preparation devices for PRP show recovery ratios ranging from 32% to 68%. Our preparing method obtained a final 2 mL PRP from 9 mL of whole blood and showed a relative high recovery ratio of 84.9 ± 8.0%. The final platelet concentration of PRP reached 1.26 × 10^7/μL when the platelet pellet was suspended in 1 mL plasma.

The optimal platelet concentration of PRP for tissue healing and regeneration is believed to be 3 to 5 times higher than that of whole blood, and 1000 × 10^3/μL is commonly considered to be an effective platelet concentration for an efficient local cellular response. Accordingly, this study also aimed to obtain a PRP of 1000 × 10^3/μL. Supposing that the mean platelet concentration is 250 × 10^3/μL, preparation of 2 mL PRP from 10 mL whole blood with an 80% recovery ratio might result in a final platelet concentration of 1000 × 10^3/μL. However, 1.25 mL CPDA-1 was mixed with 9 mL whole blood in present study and the final platelet concentration of PRP did not reach the theoretic value. This deficiency of platelet concentration of whole blood after adding CAPD-1 might be related with the microclotting during the laboratory handling process. Platelet concentration of PRP could be adjusted by controlling the plasma volume, in which the platelet pellet was suspended. Moreover, the platelet gel after 30 minutes gelling process, of which volume was 0.76 ± 0.38 mL, was considered to exceed the target concentration.

Landesberg et al reported that PRP prepared with ethylenediaminetetraacetic acid (EDTA) showed a higher platelet count than that with citrate but showed microscopic findings of platelet damage. Accordingly, CPDA-1 for anticoagulation and the lowest volume of 1.25 mL, in which blood clotting was not activated, were used. Instead, the conditions reported in the literature were used.

Most studies on PRP preparation methods used the final platelet concentration to determine the effectiveness. However, the final concentration alone could not evaluate each centrifugation step separately. For the first separation spin, the total platelet count is more important than the plasma concentration, and separating the entire plasma close to the blood cell layer is important. The platelet pellet with a sufficiently high concentration could be obtained by second condensation centrifugation and the target concentration could be achieved by controlling the plasma volume in which the pellet was suspended. Therefore, in this study, the recovery ratio instead of the platelet concentration was used to evaluate the centrifugation conditions.

The recovery ratio of PRP after centrifugation was reported to be affected by the centrifugation speed and time, respectively. Clemons et al reported that the most effective centrifugation speed and time in domestic animals differ according to the animal species. They reported that the recovery ratio was high with a fast centrifugation speed and short centrifugation time in canine, equine, and porcine, and with low centrifugation speed and long centrifugation time in bovine, caprine, and feline. They believed that these differences might be due to variations in the platelet and red blood cell volume.

While a platelet gel is formed with fibrinogen polymerization to a fibrin meshwork, the platelet begins to release growth factors in 10 minutes with thrombin activation and more than 95% of growth factors in alpha granule are exhausted within 1 hour. Because the cross-action of growth factors is not completely understood, the use of PRP for tissue healing and regeneration is more reasonable and effective than a single or combined growth factor therapy. However, there is some controversy with pre-activated PRP with thrombin...
regarding the early loss of growth factors. The half survival time of platelet-derived growth factor or transforming growth factor is less than a few minutes.\(^3,20\)

In the gelation process, only calcium without thrombin was used to make a platelet gel in this study. Gelling without thrombin can reduce the loss of growth factors by preventing early platelet activation and coagulopathy or immune reaction from bovine thrombin. Platelet gelling without thrombin in previous literature noted to take a longer time than that with thrombin,\(^6,9,12,21\) and gelation inducing enzyme, temperature or mechanical (sonication) stimuli were introduced as alternatives for thrombin to promote gelling process.\(^6,10\) In the present study, platelet gelling process (gel retraction) was induced within 30 minutes without bovine thrombin and the estimated volume of platelet gel reached \(0.76 \pm 0.38 \text{ mL}\) and \(0.35 \pm 0.15 \text{ mL}\) at 30 and 60 minutes, respectively. The platelet concentration of the remaining plasma after gel formation was \(<10 \times 10^3/\mu\text{L}\) and no fibrinogen was detected, which suggests that almost all platelets and fibrinogen were included in the platelet gel.

This study had several limitations. A certain amount of growth factor could be activated or released during platelet processing, which was not evaluated in this study. However, the total centrifugation force was limited to less than the value known to damage the platelet membrane and bovine thrombin was not used in the gelling process. Therefore, it is believed that the activation and release of growth factors in this study was minimized. Furthermore, the statistical significance of the recovery ratios are limited due to the restricted number of human samples and study design, which subdivided the centrifugation condition into 50g and 5-minute increments. To our knowledge, this is the first human study that systemically compared the centrifugation efficiency in terms of the recovery ratio; sample size estimation was infeasible from previous reference data.

This study examined the most effective centrifugation conditions for PRP using routine laboratory equipment and reagents in terms of the recovery ratio. This preparation method with table top centrifugation is simple and easy for clinical applications at out-patient clinics and operating rooms. After a 2-step centrifugation process, final 2 mL PRP with \(84.3 \pm 10\%\) recovery ratio was obtained from only 9 mL whole blood. The centrifugation speed and time were controlled to prevent cell damage and the early activation of platelets. The lack of growth factor analysis in the final PRP is the limitation of this study. Further experimental studies to determine the actual concentration of growth factors and the clinical application of PRP prepared using this centrifuge method are currently under way.

**CONCLUSION**

The actual quantity of platelets is prior requirement for the effectiveness of PRP, and our protocol optimizes the centrifugation condition in terms of recovery ratio by varying time and gravitational forces during centrifugation, of which recovery ratio resulted in 84.3%.

**ABBREVIATIONS**

CPDA-1: citrate phosphate dextrose adenine-1
PRP: platelet-rich plasma

**ACKNOWLEDGMENT**

This study was supported by grant 04-2008-0660 from the SNUH Research Fund.

**REFERENCES**


