Quantification of Growth Factor Levels Using a Simplified Method of Platelet-Rich Plasma Gel Preparation

Regina Landesberg, DMD, PhD,* Martin Roy, PhD,† and Robert S. Glickman, DMD‡

Purpose: This study compared two methods of preparing platelet-rich plasma (PRP) gel and the levels of PDGF and TGFβ in each preparation.

Materials and Methods: Platelet-rich plasma gel was prepared by centrifugation and clotted using the ITA gelling agent (Natrex Technologies Inc, Greenville, NC) or by the addition of thrombin and calcium chloride. The levels of platelet-derived growth factor (PDGF) and transforming growth factor beta (TGFβ) generated by clot formation were assayed by enzyme-linked immunoassay (ELISA).

Results: Both methods of preparation yielded PRP gel in less than 30 minutes. However, the ITA preparation did not require thrombin to achieve adequate gel formation. The levels of PDGF and TGFβ were similar regardless of which method was used for initiation of clot formation.

Conclusion: Use of ITA for gel preparation is equivalent to using calcium chloride and thrombin, without the need for special equipment and the risk of coagulopathy.

Platelet-rich plasma (PRP) gel is derived from an autogenous preparation of concentrated platelets. PRP gel has numerous applications, particularly in the cardiac and neurosurgical areas. Recently it has undergone a significant increase in use as an adhesive with cancellous bone particles in oral and maxillofacial surgery bone grafting procedures.

The traditional method of PRP preparation involves isolating platelets with a cell separator (Medtronic, Parker, CO), followed by gel formation using calcium chloride and bovine thrombin. This procedure has several disadvantages. The equipment necessary is expensive and is generally available only in an operating room or blood bank facility, making the use of PRP in a private office extremely difficult. Furthermore, the use of bovine thrombin has been associated with the development of antibodies to clotting factors V, XI, and thrombin, resulting in the risk of life-threatening coagulopathy. We describe a new method to prepare PRP gel using a simplified armamentarium of equipment and supplies. This procedure can be used in an office setting and will yield an adequate amount of PRP gel for most minor bone grafting procedures. Additionally, this method uses an alternative to thrombin for gelling of the PRP, making it a safer preparation than that currently available.

PRP is known to contain a number of growth factors/cytokines that may aid in the accelerated maturation of a bone graft. Although platelet-derived growth factor (PDGF) and transforming growth factor-β (TGFβ) have been identified in PRP gel, the amounts have not been quantified. It is also unclear as to whether the method used to gel the PRP affects the amount of growth factors/cytokines released from the platelets. We therefore compared the levels of PDGF and TGFβ generated from PRP prepared with the ITA gelling agent (Natrex Technologies Inc, Greenville, NC) and the thrombin/calcium chloride method.

Materials and Methods

PREPARATION OF PRP

Venous blood was obtained from three healthy volunteers (2 males, 1 female). The blood was drawn into 5-mL vacutainer tubes containing either 0.5 mL 0.129 mol/L sodium citrate or 0.048 mL of 15% ethylenediaminetetra-acetic acid (EDTA) (K3) solution (Becton Dickinson, Franklin Lakes, NJ). An aliquot was removed to determine the platelet count. The tubes were then spun at either 100g or 200g for 2 to 20 minutes in a Mistral 3000i centrifuge (Sanyo Gallenkamp, Bensenville, IL). All of the plasma was
transferred to a 15-mL conical polypropylene tube with printed graduations (Corning, Corning, NY) and centrifuged at 100g, 200g, 250g, or 400g for 2 to 10 minutes. The upper half of the preparation was designated platelet-poor plasma and the lower half platelet-rich plasma (PRP). Platelet counts were performed on both fractions.

**GENERATION OF PRP GEL**

The PRP obtained from each donor was aliquoted into two 50-mL glass beakers, and PRP gel was generated using the ITA gelling agent or by addition of thrombin/calcium chloride. For the thrombin/calcium chloride gel, 5 mL of 10% calcium chloride was added to 5,000 units of bovine thrombin (GenTrac, Middleton, WI); 0.167 mL of this mixture was added to each milliliter of PRP and the mixture was allowed to solidify in the beaker at 37°C in a water bath. For the ITA method, 0.06 mL of 10% calcium chloride and 0.5 mL of ITA gelling agent were added to each ml of PRP. The mixture was allowed to gel in a glass beaker at 37°C in a water bath.

**QUANTIFICATION OF GROWTH FACTORS IN PRP GEL**

The PRP gel preparations prepared above were covered with parafilm and allowed to undergo maximal clot retraction at 4°C overnight. The contents were then transferred to 50 mL polypropylene centrifuge tubes (Corning) and centrifuged at 1,000 rpm, 20 min. The supernatant amounts were measured and stored at −70°C.

**TGFβ and PDGF IN PRP PREPARATIONS**

The PRP gel preparations prepared above were covered with parafilm and allowed to undergo maximal clot retraction at 4°C overnight. The contents were then transferred to 50 mL polypropylene centrifuge tubes (Corning) and centrifuged at 1,000 rpm, 20 min. The supernatant amounts were measured and stored at −70°C.

**Table 1. First Spin Platelet Yields from a Representative Sample**

<table>
<thead>
<tr>
<th>Force/Time</th>
<th>Platelets/mL</th>
<th>% Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>2.68 × 10^8</td>
<td>—</td>
</tr>
<tr>
<td>100g 10 min</td>
<td>5.12 × 10^8</td>
<td>191</td>
</tr>
<tr>
<td>200g 10 min</td>
<td>6.15 × 10^8</td>
<td>229</td>
</tr>
<tr>
<td>200g 20 min</td>
<td>4.66 × 10^8</td>
<td>174</td>
</tr>
</tbody>
</table>
The ITA gelling agent was developed to be used without bovine thrombin. The current armamentarium prevents this from being performed in an office setting. Additionally, the use of bovine thrombin is contraindicated because it may cause life-threatening coagulopathy. Moreover, the use of bovine thrombin is contraindicated because it may cause life-threatening coagulopathy. The current armamentarium prevents this from being performed in an office setting. Additionally, the use of bovine thrombin is contraindicated because it may cause life-threatening coagulopathy.

The use of PRP gel in oral and maxillofacial surgical procedures has technical benefits and may enhance bone regrowth when used in conjunction with autologous bone grafts. Production of PRP gel involves the use of an expensive autotransfusion system and solidification of the gel with bovine thrombin. The current armamentarium prevents this from being performed in an office setting. Additionally, the use of bovine thrombin is contraindicated because it may cause life-threatening coagulopathy.

In 3 separate preparations, 40 mL of donor blood yielded approximately 8 mL of PRP gel. This amount of gel is adequate for most minor surgical procedures, including bilateral sinus grafts, onlay grafts, ridge preservation, and repair of 1- to 2-cm bone defects. Although a laboratory centrifuge was used for preparing the PRP, the low speeds and short times needed can be achieved with the inexpensive clinical centrifuge used in most hematology laboratories.

Our results showed that significant and approximately equal amounts of TGFβ1 and PDGF were present in the PRP gels, regardless of the method of preparation. The platelet-free plasma was also assayed after removal of all residual platelets. The amounts of TGFβ1 and PDGF present in the platelet-free plasma were minimal (Tables 3 and 4), indicating that almost all of the growth factor/cytokine present in the PRP gel is derived from the platelets.

In these experiments, the quantification of TGFβ1 and PDGF in PRP gel was performed on the serum derived 24 hours after clot formation. It was collected after a hard centrifuge spin to recover all the available fluid from the compacted clot. Although a significant

### Table 2. SECOND SPIN PLATELET YIELDS

<table>
<thead>
<tr>
<th>g Force/Time</th>
<th>Platelets/mL (% Enrichment)</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>2.06 × 10⁸</td>
<td>2.94 × 10⁸</td>
<td>1.73 × 10⁸</td>
<td></td>
</tr>
<tr>
<td>100g, 10 min</td>
<td>3.13 × 10⁸</td>
<td>6.85 × 10⁸</td>
<td>3.80 × 10⁹</td>
<td></td>
</tr>
<tr>
<td>200g, 10 min</td>
<td>5.57 × 10⁸</td>
<td>9.35 × 10⁸</td>
<td>5.70 × 10⁹</td>
<td></td>
</tr>
<tr>
<td>250g, 10 min</td>
<td>5.23 × 10⁸</td>
<td>8.00 × 10⁸</td>
<td>5.70 × 10⁹</td>
<td></td>
</tr>
</tbody>
</table>

Mean % increase ± SE

1. 100/10     101.7 ± 30.7
2. 200/10     205.7 ± 22.2*
3. 250/10     185.0 ± 27.7*

NOTE. Plasma from 1st spin of 200g, 10 minutes.
*Significantly different from 100g, 10 minutes (P < .05).

### Table 3. TGF-β1 PRODUCTION (TOTAL ng/3 mL PRP GEL)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>3.89</td>
<td>4.97</td>
<td>7.66</td>
<td>5.51 ± 1.37</td>
</tr>
<tr>
<td>Thrombin/Ca²⁺</td>
<td>157.35</td>
<td>113.70</td>
<td>107.36</td>
<td>126.14 ± 19.25</td>
</tr>
<tr>
<td>ITA</td>
<td>155.36</td>
<td>127.71</td>
<td>130.30</td>
<td>137.79 ± 10.80</td>
</tr>
</tbody>
</table>

### Table 4. PDGF (AB) PRODUCTION (TOTAL ng/3 mL PRP GEL)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>3.11</td>
<td>2.00</td>
<td>1.88</td>
<td>2.33 ± 0.48</td>
</tr>
<tr>
<td>Thrombin/Ca²⁺</td>
<td>118.02</td>
<td>123.88</td>
<td>115.99</td>
<td>119.30 ± 2.90</td>
</tr>
<tr>
<td>ITA</td>
<td>119.93</td>
<td>130.94</td>
<td>126.91</td>
<td>123.26 ± 7.08</td>
</tr>
</tbody>
</table>
amount of clot retraction is achieved by 2 hours, maximal retraction takes place by 24 hours.\textsuperscript{18} Whereas there may be growth factors/cytokines still sequestered within the remaining clot, enzymatic breakdown by fibrinolysis would be necessary for their release. Our method of assaying the TGF\textsubscript{β1} and PDGF levels allowed the amounts of growth factor that are immediately available to the surrounding bone and tissue surfaces to be quantitated. However, it does not permit a determination of the biologic activity, which can be affected by the method of preparation.

It should be noted that, because some clot retraction takes place immediately, a small amount of liquid appears to remain unincorporated into the gel. We are aware that some practitioners blot the PRP gel before placement in the graft site. Because this fluid has significant amounts of growth factors, we recommend adding it to graft rather than discarding it.

Acknowledgment

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References


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