Characterization and Comparison of 5 Platelet-Rich Plasma Preparations in a Single-Donor Model

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Purpose: The purpose of this study was to compare the biological characteristics of platelet-rich plasma (PRP) obtained from 4 medical devices and a preparation developed in our laboratory using a single-donor model.

Methods: Ten healthy persons donated blood that was processed to produce PRP by use of 4 commercial preparation systems and a protocol developed in our laboratory. Volumes and platelet, white blood cell (WBC), and red blood cell concentrations were recorded. The platelet activation status was assessed by flow cytometry. Enzyme-linked immunosorbent assay was used to determine the concentrations of vascular endothelial growth factor, platelet-derived growth factor AB, epidermal growth factor, and transforming growth factor β1. We calculated platelet capture efficiency, relative composition, and increase factors from whole blood in platelets and WBC, as well as platelet and growth factor (GF) doses, provided from each preparation.

Results: Leukocyte-rich PRP was obtained with RegenPRP (RegenLab, Le Mont-sur-Lausanne, Switzerland) and the Mini GPS III System (Biomet Biology, Warsaw, IN) and provides PRP with higher proportions of red blood cells, WBCs, and neutrophils than leukocyte-poor PRP obtained with the Selphyl System (Selphyl, Bethlehem, PA), Arthrex ACP (Arthrex, Naples, FL), and the preparation developed in our laboratory. The highest platelet and GF concentrations and doses were obtained with the Mini GPS III System and the preparation developed in our laboratory. Different centrifugation protocols did not show differences in the percentages of activated platelets. Finally, a positive correlation between platelet doses and all the GFs studied was found, whereas a positive correlation between WBC doses and GFs was found only for vascular endothelial growth factor and epidermal growth factor.

Conclusions: In a single-donor model, significant biological variations in PRP obtained from different preparation systems were highlighted. The observed differences suggest different results for treated tissue and could explain the large variability in the clinical benefit of PRP reported in the literature.

Clinical Relevance: Our findings will help clinicians to choose a system that meets their specific needs for a given indication.

The phases of healing are similar in most tissues and involve platelets as well as the release of growth factors (GFs). Platelet-rich plasma (PRP) is defined as a suspension of platelets in plasma characterized by a platelet concentration that is higher than the concentration of the original blood collected. In brief, platelets—once activated—release GFs that improve reparative and regenerative processes. In particular, concentrated levels of platelet-derived growth factors (PDGFs), transforming growth factor β1 (TGF-β1), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor 1, or fibroblast growth factor found in PRP are known to play a critical role in cell proliferation, chemotaxis, cell differentiation, and angiogenesis. PRP is harvested from a patient’s peripheral blood, centrifuged to obtain a concentrated amount of platelets, placed in a small volume of plasma, and administered at the site of injury. Both liquid and semisolid forms of PRP are available; PRP gel is simply obtained by mixing PRP with autologous thrombin and calcium chloride. Described as an easy, fast, effective, cheap, and safe (because of its autologous origin) product, PRP has been the subject of increased clinical interest in many diverse medical fields, and the market for these preparations, estimated at $45 million in 2009, is expected to be worth $126 million by 2016. In orthopaedic injuries several uncontrolled studies have shown benefit for a variety of indications, and recently, controlled studies have...
shown less favorable results.\textsuperscript{15,16} The common point among these studies is the lack of characterization of the content of the PRP product used as therapy. Nevertheless, at least 16 commercial platelet separation systems are available today,\textsuperscript{17} using variable separation methods. Authors have shown substantial differences in the content of platelet concentrates produced by the various automated and manual protocols described in the literature.\textsuperscript{18-20} Several critical points are described as hypothetically influencing the clinical efficiency of PRP. The use of an appropriate anticoagulant that does not affect platelet functions is recommended.\textsuperscript{21} The presence (or not) of white blood cells (WBCs), in particular neutrophils, in the PRP and whether the PRP should be activated and, if so, how are issues hotly debated,\textsuperscript{22} but there is still no consensus regarding these factors. A parameter thought to influence the efficacy of PRP is the platelet increase factor corresponding to the platelet concentration increase in PRP compared with whole blood and is frequently described in scientific publications and in manufacturers’ promotional literature. Platelet concentrations in PRP below whole blood baseline values may not provide sufficient cellular response,\textsuperscript{23} and platelet concentrations higher than 6-fold compared with platelet whole blood baseline values have had an inhibitory effect on healing.\textsuperscript{24} Good clinical results were obtained with platelet concentrations from 1- to 6-fold higher compared with platelet whole blood concentrations in Achilles tendinopathy,\textsuperscript{25} anterior cruciate ligament surgery,\textsuperscript{26} or bone healing in transformational lumbar interbody fusions.\textsuperscript{13} However, the PRP increase factor in platelets from whole blood is directly linked to the volume of PRP obtained,\textsuperscript{7} and these 2 factors should not be interpreted alone. We are therefore introducing the notion of platelet and GF doses that correspond to the quantity of platelets and GF hypothetically delivered at the injection site. On the basis of the field of hematology, which first used cells as therapy, cell doses are the most relevant parameter to assess clinical efficacy and cell-dose effects are now clearly established.\textsuperscript{27}

The purpose of this study was to compare the biological characteristics of PRP obtained from 4 medical devices and a preparation developed in our laboratory using a single-donor model. We hypothesized that significant differences should be highlighted among the different PRP preparations tested in this study. To reach this objective, we recorded the volumes; platelet activation status; platelet concentration; WBC concentration; red blood cell (RBC) concentration; and VEGF, PDGF-AB, EGF, and TGF-\(\beta1\) concentrations of each PRP obtained. From these findings, we calculated and compared platelet capture efficiency, relative composition, and increase factor in platelets and WBCs from whole blood, as well as platelet and GF dose, provided from each PRP preparation. The formulas used for our calculations are provided in Fig 1.

**Methods**

**Different PRP Preparation Systems**

Four commercial PRP separation systems and 1 preparation developed in our laboratory were selected for the study. The commercial preparations were the Selphyl System (Selphyl, Bethlehem, PA) and RegenPRP (RegenLab, Le Mont-sur-Lausanne, Switzerland), both using a gel separator system; the Mini GPS III System (Biomet Biology, Warsaw, IN), using a floating buoy separator system; and Arthrex ACP (Arthrex, Naples, FL), using a double-syringe system. The preparation developed in our laboratory was described by Bausset et al.\textsuperscript{8} Customized centrifuges were necessary for the Mini GPS III System and Arthrex ACP and were provided by the manufacturers.

**Participant Recruitment**

Ten healthy volunteer donors (7 men and 3 women; age range, 23 to 52 years; mean age [± SD], 29.4 ± 8.28 years) were included in the study from September to December 2012. They had no relevant diseases and were free of any medication known to affect platelet functions for 7 days before the study. All donors included in this study had platelet numbers over 150 \(\times 10^9\)/L and signed informed consent documents.

**Sample Collection**

A single technician collected 86.5 mL of blood by venipuncture using an 18-gauge arteriovenous fistula needle (Greiner Bio-one, Monroe, NC) connected with a 3-way stopcock (reference RO301M; Cair LGL, Civi-rieux-d’Azeruges, France), filling both anticoagulated tubes (8 mL for the Selphyl System, 8 mL for RegenPRP, and 30 mL for the preparation developed in our laboratory) and syringes (27 mL for the Mini GPS III System and 11 mL for Arthrex ACP) with the appropriate ratio of anticoagulant as recommended by the manufacturers. Approximately 2.5 mL of whole blood was collected in an EDTA-coated tube for whole blood analysis.

**PRP Preparation**

Whole blood collected with tubes was centrifuged in a Multifuge Heraus 3 S-R centrifuge (Thermo Scientific, Indianapolis, IN), whereas whole blood collected with Arthrex ACP by syringe was centrifuged in a Hettich Rotofix 32A centrifuge (Hettichlab Technology, Tutlingen, Germany) and whole blood collected with the Mini GPS III System by syringe was first transferred to a disposable separation tube and then centrifuged in a Drucker 755VES centrifuge (Drucker, Port Matilda, PA).

The instructions provided with the commercial system or from the literature were applied, and platelet-poor plasma (PPP) was removed each time it was suggested (RegenPRP, Mini GPS III System, and preparation developed in our laboratory). The protocol developed in
our laboratory was specifically associated with low centrifugation speeds to preserve platelet functionality and GF release capacity. Table 1 and Fig 2 summarize the protocol for each PRP preparation system with the macroscopic aspect of the final PRP obtained.

<table>
<thead>
<tr>
<th>PRP Preparation System</th>
<th>Selphyl System</th>
<th>RegenPRP</th>
<th>Mini GPS III System</th>
<th>Arthrex ACP</th>
<th>Laboratory Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling needle (gauge)</td>
<td>21</td>
<td>21</td>
<td>18</td>
<td>18-20</td>
<td>21</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>Sodium citrate, 1 mL</td>
<td>Sodium citrate, 1 mL</td>
<td>ACD-A, 3 mL</td>
<td>ACD-A, 1 mL</td>
<td>ACD-A, 6 mL</td>
</tr>
<tr>
<td>Net volume of blood collected (mL)</td>
<td>8</td>
<td>8</td>
<td>27</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td>No. of centrifugation steps</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Common or specific centrifuge</td>
<td>Common</td>
<td>Common</td>
<td>Specific</td>
<td>Specific</td>
<td>Common</td>
</tr>
<tr>
<td>Speed and time</td>
<td>1,100g and 6 min</td>
<td>1,500g and 9 min</td>
<td>3,200 rpm and 15 min</td>
<td>1,500 rpm and 5 min</td>
<td>130g and 15 min, 250g and 15 min</td>
</tr>
<tr>
<td>Removal of PPP</td>
<td>No</td>
<td>Yes, 2 mL</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Platelet resuspension</td>
<td>Return 7-fold</td>
<td>Gently return</td>
<td>Gently return during 30 s</td>
<td>No</td>
<td>Gently resuspend</td>
</tr>
</tbody>
</table>

ACD-A, anticoagulant citrate dextrose solution A.

Volume Measurements

The PRP volumes produced from each protocol were recorded after PPP was removed when necessary by use of a 10-mL graduated pipette (Sarstedt, Nümbrecht, Germany).

**Table 1. Protocol for Each Preparation System Provided by Either Commercial System Instructions (Selphyl System, RegenPRP, Mini GPS III System, and Arthrex ACP) or Publications (Laboratory Preparation)**

**Fig 1.** Formulas used for calculations of PRP characteristics. G/L, giga/L ($\times 10^9$/L).

**Platelet Capture Efficiency (\%)**

\[
\text{Volume of PRP obtained (ml) x Platelets concentration in PRP (G/L)} \\
\text{Net volume of whole blood collected (ml) x Platelets concentration in whole blood (G/L)}
\]

**Relative composition in platelets (\%)**

\[
\text{Platelets concentration in PRP (G/L)} \\
\text{Platelets concentration in PRP (G/L) + WBC concentration in PRP (G/L) + RBC concentration in PRP (G/L)}
\]

**Relative composition in WBC (\%)**

\[
\text{WBC concentration in PRP (G/L)} \\
\text{Platelets concentration in PRP (G/L) + WBC concentration in PRP (G/L) + RBC concentration in PRP (G/L)}
\]

**Relative composition in RBC (\%)**

\[
\text{RBC concentration in PRP (G/L)} \\
\text{Platelets concentration in PRP (G/L) + WBC concentration in PRP (G/L) + RBC concentration in PRP (G/L)}
\]

**Factor increase in platelets or WBC concentration**

\[
\frac{\text{Platelets or WBC concentration in PRP (G/L)}}{\text{Platelets or WBC concentration in whole blood (G/L)}}
\]

**Platelets dose ($\times 10^9$) in PRP**

\[
\frac{\text{Volume of PRP obtained (ml) x Platelets concentration in PRP (G/L)}}{10^9}
\]

**Growth factors dose (pg)**

\[
\frac{\text{Volume of PRP obtained (ml) x Growth factors concentration in PRP (pg/ml)}}{}
\]
PRP Sampling

The final PRP products obtained were divided into 2 samples: The first sample, which did not undergo the exogenous activation step, enabled us to immediately assess platelet, WBC, and RBC concentrations and the percentage of activated platelets. The second sample, exogenously activated with calcium chloride, following the method described by Anitua et al., was stored at $-80^\circ C$ for future assays of VEGF, PDGF-AB, TGF-$\beta_1$, and EGF.

Quantification of Platelet, WBC, and RBC Concentrations

Platelet, WBC, and RBC concentrations from whole blood and from each PRP preparation were determined with a hematology analyzer (Advia 2120; Siemens Diagnostic Solutions, Tarrytown, NY).

Platelet Activation Status

The percentage of activated platelets before exogenous activation was assessed as previously described. In brief, the percentage of platelets expressing P-selectin corresponded to the percentage of activated platelets and was recorded by flow cytometry with Cytomics FC500 and StemCXP automatic analysis software (Beckman Coulter, Fullerton, CA).

Quantification of GF Concentration

VEGF, PDGF-AB, EGF, and TGF-$\beta_1$ quantification assays were performed in duplicate for the 50 PRP
preparations using enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) following the manufacturer’s instructions.

Statistical Analysis
Statistical analyses were performed with SPSS statistical software, version 16.0 (SPSS, Chicago, IL). The 5% level of significance was used for all statistical tests. Data are presented as mean ± standard deviation.

The differences in mean volumes, platelet and WBC concentrations, relative composition, GF concentrations, platelet doses, and GF doses in the different PRP preparations were analyzed by 1-way analysis of variance (ANOVA), and Bonferroni post hoc testing enabled us to make pairwise comparisons of the preparations. Linear correlations between platelet and GF concentrations, relative composition, GF concentrations, platelet doses, and GF doses were analyzed by Pearson correlation.

Results

Whole Blood Characteristics
Table 2 summarizes mean values of platelet and WBC concentrations and the relative composition of whole blood from the 10 healthy donors, which were all within the ranges of normal biological values.

Volume of PRP After Processing
The greatest volumes of PRP were obtained from the Selphyl System and Arthrex ACP, with 4.1 ± 0.43 mL and 4.03 ± 0.35 mL, respectively. ANOVA showed significant differences among the different systems (P = .0002) (Table 3), and pairwise analysis confirmed that the Selphyl System and Arthrex ACP provided significantly higher volumes of PRP than the Mini GPS III System and RegenPRP.

Platelet Capture Efficiency
ANOVA showed a significant difference among the different systems concerning platelet capture efficiency (P < .0001) (Table 3). Pairwise analysis showed that the protocol developed in our laboratory provided significantly lower platelet capture efficiency (29.63% ± 9.10%) than Arthrex ACP (48.23% ± 7.41%), RegenPRP (55.28% ± 18.43%), and the Selphyl System (59.89% ± 15.73%).

Relative Composition of PRP: Tool to Assess PRP Purity
Relative Composition in Platelets. ANOVA showed a significant difference among the different systems concerning the relative composition in platelets (P < .0001) (Table 3). Pairwise analysis showed that Arthrex ACP (80.96% ± 3.10%), the preparation developed in our laboratory (80.72% ± 3.79%), and the Selphyl System (73.86% ± 19.72%) delivered PRP that was significantly purer than the PRP delivered by the Mini GPS III System (51.84% ± 18.48%) and RegenPRP (45.97% ± 24.70%).

Table 2. Mean Whole Blood Characteristics From 10 Healthy Donors

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet concentration (× 10^{9}/L)</td>
<td>284.80 ± 54.72</td>
</tr>
<tr>
<td>WBC concentration (× 10^{9}/L)</td>
<td>7.24 ± 1.46</td>
</tr>
<tr>
<td>Relative composition in platelets (%)</td>
<td>5.41 ± 0.90</td>
</tr>
<tr>
<td>Relative composition in WBCs (%)</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>Relative composition in RBCs (%)</td>
<td>94.45 ± 0.91</td>
</tr>
</tbody>
</table>

Table 3. Mean PRP Characteristics Obtained From Different Preparations and ANOVA Comparison Among Different Systems

<table>
<thead>
<tr>
<th>PRP Preparation System</th>
<th>Selphyl System</th>
<th>RegenPRP</th>
<th>Mini GPS III System</th>
<th>Arthrex ACP</th>
<th>Laboratory Preparation</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of PRP obtained (mL)</td>
<td>4.10 ± 0.43</td>
<td>3.10 ± 0.61</td>
<td>3.21 ± 0.15</td>
<td>4.03 ± 0.35</td>
<td>3.41 ± 0.92</td>
<td>.0002</td>
</tr>
<tr>
<td>Platelet capture efficiency (%)</td>
<td>59.89 ± 15.73</td>
<td>55.28 ± 18.43</td>
<td>46.45 ± 12.66</td>
<td>48.23 ± 7.41</td>
<td>29.63 ± 9.10</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Relative composition in platelets (%)</td>
<td>73.86 ± 19.72</td>
<td>45.97 ± 24.70</td>
<td>51.84 ± 18.48</td>
<td>80.96 ± 3.10</td>
<td>80.72 ± 3.79</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Relative composition in WBC (%)</td>
<td>0.18 ± 0.11</td>
<td>1.04 ± 0.47</td>
<td>1.37 ± 0.36</td>
<td>0.08 ± 0.06</td>
<td>0.27 ± 0.24</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Relative composition in RBC (%)</td>
<td>25.97 ± 19.65</td>
<td>52.99 ± 24.93</td>
<td>46.79 ± 18.51</td>
<td>18.96 ± 3.05</td>
<td>19.01 ± 3.72</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Relative composition in neutrophils (%)</td>
<td>13.99 ± 11.56</td>
<td>38.40 ± 22.57</td>
<td>33.60 ± 18.88</td>
<td>25.26 ± 17.70</td>
<td>9.63 ± 16.66</td>
<td>.0031</td>
</tr>
<tr>
<td>Platelet concentration (× 10^{9}/L)</td>
<td>330.60 ± 95.64</td>
<td>453.67 ± 262.37</td>
<td>1,135.20 ± 422.15</td>
<td>372.90 ± 77.67</td>
<td>756.20 ± 195.00</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Factor increase in platelet concentration</td>
<td>1.16 ± 0.27</td>
<td>1.59 ± 0.84</td>
<td>3.93 ± 1.09</td>
<td>1.31 ± 0.15</td>
<td>2.64 ± 0.33</td>
<td>—</td>
</tr>
<tr>
<td>WBC concentration (× 10^{9}/L)</td>
<td>1.29 ± 2.02</td>
<td>10.61 ± 3.64</td>
<td>30.36 ± 9.81</td>
<td>0.39 ± 0.33</td>
<td>2.27 ± 2.01</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Factor increase in WBC concentration</td>
<td>0.18 ± 0.27</td>
<td>1.52 ± 0.46</td>
<td>4.13 ± 0.80</td>
<td>0.06 ± 0.04</td>
<td>0.35 ± 0.31</td>
<td>—</td>
</tr>
<tr>
<td>% of activated platelets</td>
<td>6.46 ± 2.45</td>
<td>6.10 ± 4.07</td>
<td>5.03 ± 2.95</td>
<td>4.27 ± 3.06</td>
<td>11.08 ± 9.85</td>
<td>.0801</td>
</tr>
<tr>
<td>Platelet dose in PRP (×10^{9})</td>
<td>1.36 ± 0.42</td>
<td>1.25 ± 0.49</td>
<td>3.61 ± 0.13</td>
<td>1.49 ± 0.29</td>
<td>2.62 ± 0.12</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Relative Composition in WBCs. ANOVA showed significant differences among the various systems concerning the relative composition in WBCs \( (P < .0001) \) (Table 3). Pairwise analysis showed that the Mini GPS III System \( (1.37\% \pm 0.36\%) \) and RegenPRP \( (1.04\% \pm 0.47\%) \) delivered PRP with a significantly higher WBC percentage than the preparation developed in our laboratory \( (0.27\% \pm 0.24\%) \), the Selphyl System \( (0.18\% \pm 0.11\%) \), and Arthrex ACP \( (0.08\% \pm 0.06\%) \).

Relative Composition in Neutrophils. ANOVA showed a significant difference among the different systems concerning the relative composition in neutrophils \( (P = .0031) \) (Table 3). Pairwise analysis showed that RegenPRP \( (38.40\% \pm 22.57\%) \) and the Mini GPS III System \( (33.67\% \pm 18.88\%) \) delivered PRP with a significantly higher neutrophil percentage than the preparation developed in our laboratory \( (9.63\% \pm 16.66\%) \). RegenPRP also delivered PRP with a significantly higher neutrophil percentage than the Selphyl System \( (13.99\% \pm 11.56\%) \).

Relative Composition in RBCs. ANOVA showed a significant difference among the different systems concerning the relative composition in RBCs \( (P < .0001) \) (Table 3). Pairwise analysis showed that RegenPRP \( (52.99\% \pm 24.93\%) \) and the Mini GPS III System \( (46.79\% \pm 18.51\%) \) delivered PRP with a significantly higher RBC percentage than the preparation developed in our laboratory \( (19.01\% \pm 3.72\%) \) and Arthrex ACP \( (18.96\% \pm 3.05\%) \). RegenPRP also delivered PRP with a significantly higher RBC percentage than the Selphyl System \( (25.97\% \pm 19.65\%) \).

Platelet Concentration
All PRP systems delivered PRP with platelet concentrations that were higher than whole blood baseline values. ANOVA showed a significant difference among the different systems \( (P < .0001) \) (Table 3), and pairwise analysis showed that the Mini GPS III System provided a significantly higher platelet concentration \( (1,135.20 \pm 262.37 \times 10^{9}/L) \) compared with the preparation developed in our laboratory \( (756.20 \pm 195 \times 10^{9}/L) \), RegenPRP \( (453.67 \pm 262.37 \times 10^{9}/L) \), Arthrex ACP \( (372.90 \pm 77.67 \times 10^{9}/L) \), and Selphyl System \( (330.60 \pm 95.64 \times 10^{9}/L) \). The preparation developed in our laboratory also had a higher platelet concentration than Arthrex ACP and the Selphyl System.

WBC Concentration
The Mini GPS III System and RegenPRP delivered PRP with a WBC concentration that was higher than whole blood baseline values, whereas the Selphyl System, Arthrex ACP, and the preparation developed in our laboratory delivered PRP with a WBC concentration that was lower than whole blood baseline values. ANOVA showed a significant difference among the different systems \( (P < .0001) \) (Table 3), and pairwise analysis showed that the Mini GPS III System provided a significantly higher WBC concentration \( (30.36 \pm 9.81 \times 10^{9}/L) \) compared with RegenPRP \( (10.61 \pm 3.64 \times 10^{9}/L) \), the preparation developed in our laboratory \( (2.27 \pm 2.01 \times 10^{9}/L) \), the Selphyl System \( (1.29 \pm 2.02 \times 10^{9}/L) \), and Arthrex ACP \( (0.39 \pm 0.33 \times 10^{9}/L) \). RegenPRP also had a higher WBC concentration than the preparation developed in our laboratory, the Selphyl System, and Arthrex ACP.

GF Concentrations
ANOVA showed a significant difference among the different systems for VEGF \( (P = .001) \) and EGF \( (P < .0001) \) concentrations of PRP. Pairwise analysis showed that the Mini GPS III System delivered PRP with significantly higher VEGF and EGF concentrations \( (1,204.58 \pm 785.89 \text{ pg/mL for VEGF and } 2,617.88 \pm 757.95 \text{ pg/mL for EGF}) \) than the preparation developed in our laboratory \( (484.81 \pm 438.35 \text{ pg/mL for VEGF and } 1,170.00 \pm 686.20 \text{ pg/mL for EGF}) \), RegenPRP \( (366.34 \pm 253.14 \text{ pg/mL for VEGF and } 993.92 \pm 411.15 \text{ pg/mL for EGF}) \), the Selphyl System \( (394.00 \pm 727.26 \text{ pg/mL for VEGF and } 898.63 \pm 213.66 \text{ pg/mL for EGF}) \), and Arthrex ACP \( (153.00 \pm 93.78 \text{ pg/mL for VEGF and } 606.39 \pm 423.05 \text{ pg/mL for EGF}) \) (Table 4).

Different Centrifugation Protocols Do Not Affect Percentage of Activated Platelets
Free of any activation protocol, the percentages of activated platelets ranged from 4.17% to 11.08%. ANOVA showed no significant difference among the different systems \( (P = .0801) \) (Table 3).

Platelet Dose
The platelet dose ranged from \( 1.25 \times 10^{9} \) to \( 3.61 \times 10^{9} \). ANOVA showed a significant difference among the different systems \( (P < .0001) \) (Table 3). Pairwise analysis showed that the Mini GPS III System delivered a significantly higher quantity of platelets \( (3.61 \times 10^{9} \pm 1.32 \times 10^{9}) \) than Arthrex ACP \( (1.49 \times 10^{9} \pm 2.86 \times 10^{8}) \), the Selphyl System \( (1.36 \times 10^{9} \pm 4.24 \times 10^{8}) \), and RegenPRP \( (1.25 \times 10^{9} \pm 4.91 \times 10^{8}) \). The preparation developed in our laboratory \( (2.62 \times 10^{9} \pm 1.20 \times 10^{9}) \) also provided a significantly higher quantity of platelets than the Selphyl System and RegenPRP.

GF Doses
ANOVA showed a significant difference among the different systems for VEGF \( (P = .0055) \) and EGF \( (P < .0001) \) doses in PRP. Pairwise analysis showed that the Mini GPS III System delivered PRP with significantly higher VEGF and EGF doses \( (3,831.35 \pm 2,459.67 \text{ pg for VEGF and } 8,429.44 \pm 2,583.17 \text{ pg for EGF}) \) than
RegenPRP (1,111.28 ± 746.35 pg for VEGF and 3,043.72 ± 1,267.67 pg for EGF) and the Selphyl System (1,606.19 ± 2,892.51 pg for VEGF and 3,670.08 ± 941.29 pg for EGF). The Mini GPS III System also delivered PRP with a significantly higher EGF dose than Arthrex ACP (2,450.59 ± 1,682.04 pg) (Table 4).

Positive Correlation Between Platelet Dose and All GF Doses

There was a significant correlation between platelet doses and the 4 GF doses calculated in the study. A correlation was found with EGF and VEGF, with Pearson r values equal to 0.79 and 0.46, respectively (P < .0001) (Figs 3A and 3C). Significant correlations between platelet doses with PDGF-AB and TGF-β1 were lower, with Pearson r values equal to 0.40 (P < .001) (Fig 3B) and 0.30 (P < .05), respectively (Fig 3D).

Positive Correlation Between WBC Dose and Certain GF Doses

There was a significant correlation between WBC dose and EGF and between WBC dose and VEGF, with Pearson r values equal to 0.72 and 0.40, respectively (P < .001 and P < .01, respectively) (Figs 4A and 4C). No significant correlation between WBC dose and PDGF-AB and between WBC dose and TGF-β1 was found, with Pearson r values equal to 0.19 and 0.14, respectively (Figs 4B and 4D).

Discussion

As expected, significant differences have been highlighted among the different PRP preparations, which could explain the large variability in the clinical benefit of PRP reported in the literature and support a minimal characterization of PRP before injection. Thus our study supports the results of the meta-analysis by Sheth et al.29 that showed a large variability in the results obtained from PRP applications in the orthopaedic domain.

However, our approach differs from comparable studies in that we included larger numbers of subjects and compared more preparations.18-20 Furthermore, the biological characteristics of these systems and protocols have not been described in this kind of study. We also included the relative composition of PRP obtained, the percentages of activated platelets, and the notion of platelet and GF doses, which has never been reported in this type of study.

All PRP preparations met Marx’s definition of providing preparations with platelet concentrations higher than whole blood baseline values in a small volume of plasma.3 Only the preparation developed in our laboratory had a lower platelet capture efficiency than the other preparations; this is explained by the very soft first spin used in order not to activate platelets that would result in significant platelet loss. However,
no differences were found in the platelet activation status of each preparation, suggesting that different centrifugation times and speeds do not affect it. PRP volumes were logically higher from preparations using protocols that did not require PPP removal (Selphyl System and Arthrex ACP). Two kinds of PRP were obtained according to the definition of Dohan Ehrenfest et al.\textsuperscript{30,31}: RegenPRP and the Mini GPS III System were leukocyte-rich PRP (LR-PRP), corresponding to leukocyte concentrations higher than whole blood baseline values, whereas the Selphyl System, Arthrex ACP, and the preparation developed in our laboratory were leukocyte-poor PRP (LP-PRP), corresponding to leukocyte concentrations lower than whole blood baseline values. However, it should be noted that the leukocyte concentration was at most only 1.37% in the

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**Fig 3.** Correlation between platelet dose and (A) VEGF dose, (B) PDGF-AB dose, (C) EGF dose, and (D) TGF-β1 dose.

**Fig 4.** Correlation between WBC dose and (A) VEGF dose, (B) PDGF-AB dose, (C) EGF dose, and (D) TGF-β1 dose. (NS, not significant.)
The final composition of the PRP obtained. As expected, LR-PRP was less pure than LP-PRP, principally because of a mean contamination rate of 53% of RBCs for the less pure PRP (RegenPRP). This result was reduced to 19% for the purest PRP (Arthrex ACP). LR-PRP also provided higher percentages of neutrophils than LP-PRP, which can be explained by their density, which is closer to that of RBCs than that of platelets. The best platelet concentrations, GF concentrations, and platelet and GF doses were logically obtained from PRP preparations requiring a large collection of whole blood (Mini GPS III System and preparation developed in our laboratory). Finally, we found a positive correlation for platelet doses with the 4 GFs studied in a large series (50 PRP preparations). The correlation between WBC doses and VEGF and EGF doses confirmed that platelets are not the unique source of GFs in PRP.

Our study found that among the various protocols that exist to obtain PRP, preparations defined as LR-PRP provide less pure PRP with a higher proportion of WBCs, neutrophils, and RBCs than LP-PRP. Clinicians should also know that platelet and GF concentrations and doses from preparations that collect larger amounts of whole blood should be logically higher.

The presence or absence of leukocytes in PRP, namely neutrophils, is hotly debated. Positive reports have shown that LR-PRP could play a valuable antimicrobial role in PRP treatment, whereas neutrophils, known to contain metalloproteases and to have a very short half-life, could impede healing. In vitro studies are being performed to compare LR-PRP and LP-PRP, correlating the presence of neutrophils with higher concentrations of catabolic and proinflammatory cytokines. Preclinical and clinical studies are now necessary to investigate this issue. However, leukocytes comprise only approximately 0.1% to 1% of the final content of PRP, and whether a higher proportion of RBCs in PRP could be clinically detrimental through the release of reactive oxygen species should also be considered.

After a peak of popularity and the high degree of enthusiasm that PRP has garnered, it is now clear that each PRP preparation injected should be well characterized in well-controlled trials. Future clinical trials should include volumes, doses, and concentrations of platelets, as well as the overall composition and the number of applications of PRP injected, to determine the clinical relevance of the huge variability in PRP composition. Whether PRP functions are optimal in the early phase of an acute injury and the duration of PRP’s effect should also be determined.

Limitations

No power analysis was performed to calculate the minimum sample size required to lead to significant differences among the different systems. The results were not correlated to specific characteristics of the systems and, thus, do not allow extension to systems other than the few specific systems studied. The study could have been strengthened if the differences in composition had been shown to lead to differences in biological effects, for either in vitro or in vivo systems. Finally, prior published works have already established that different systems will produce PRP with different platelet and GF concentrations.

Conclusions

In a single-donor model, significant biological variations in PRP obtained from different preparation systems were highlighted. The observed differences suggest different results for treated tissue and could explain the large variability in the clinical benefit of PRP reported in the literature. Our findings will help clinicians to choose a system that meets their specific needs for a given indication.

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