Comparison of Growth Factor and Platelet Concentration From Commercial Platelet-Rich Plasma Separation Systems

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Background: Clinical studies claim that platelet-rich plasma (PRP) shortens recovery times because of its high concentration of growth factors that may enhance the tissue repair process. Most of these studies obtained PRP using different separation systems, and few analyzed the content of the PRP used as treatment.

Purpose: This study characterized the composition of single-donor PRP produced by 3 commercially available PRP separation systems.

Study Design: Controlled laboratory study.

Methods: Five healthy humans donated 100 mL of blood, which was processed to produce PRP using 3 PRP concentration systems (MTF Cascade, Arteriocyte Magellan, Biomet GPS III). Platelet, white blood cell (WBC), red blood cell, and fibrinogen concentrations were analyzed by automated systems in a clinical laboratory, whereas ELISA determined the concentrations of platelet-derived growth factor αβ and ββ (PDGF-αβ, PDGF-ββ), transforming growth factor β1 (TGF-β1), and vascular endothelial growth factor (VEGF).

Results: There was no significant difference in mean PRP platelet, red blood cell, active TGF-β1, or fibrinogen concentrations among PRP separation systems. There was a significant difference in platelet capture efficiency. The highest platelet capture efficiency was obtained with Cascade, which was comparable with Magellan but significantly higher than GPS III. There was a significant difference among all systems in the concentrations of WBC, PDGF-αβ, PDGF-ββ, and VEGF. The Cascade system concentrated leukocyte-poor PRP, compared with leukocyte-rich PRP from the GPS III and Magellan systems.

Conclusion: The GPS III and Magellan concentrate leukocyte-rich PRP, which results in increased concentrations of WBCs, PDGF-αβ, PDGF-ββ, and VEGF as compared with the leukocyte-poor PRP from Cascade. Overall, there was no significant difference among systems in the platelet concentration, red blood cell, active TGF-β1, or fibrinogen levels.

Clinical Relevance: Products from commercially available PRP separation systems produce differing concentrations of growth factors and WBCs. Further research is necessary to determine the clinical relevance of these findings.

Keywords: platelet-rich plasma; growth factors; platelet-rich plasma separation system

Platelet-rich plasma (PRP) has been recognized as a powerful adhesive and hemostatic agent since the 1970s and as a potent source of autologous growth factors since the 1990s. The concentrated levels of platelet-derived growth factor αβ and ββ (PDGF-αβ, PDGF-ββ), transforming growth factor β1 (TGF-β1), and vascular endothelial growth factor (VEGF) found in PRP are known to play a critical role in cell proliferation, chemotaxis, cell differentiation, and angiogenesis.

Consequently, there has been strong clinical interest in the use of PRP as a growth factor delivery medium to aid in tissue regeneration. Platelet-rich plasma was first recognized as an effective agent for bone and tissue repair within the field of dentistry and oral maxillofacial surgery. Its applicability then spread to the fields of plastic surgery by demonstrating evidence of improved skin graft wound healing.

Within the field of orthopaedic surgery, the potential role of PRP in enhancing the healing of bone, muscle, ligaments, and tendons has resulted in a number of studies within virtually all the orthopaedic subspecialties. Platelet-rich plasma has been reported to improve recovery from joint replacement, spine surgery, and fracture healing. However, the greatest interest in PRP now appears to lie within the field of sports medicine, where recovery time and return to play are often critical considerations in patient care. A number of studies have reported favorable clinical outcomes with the use of PRP in anterior cruciate ligament reconstruction, as well as in the treatment of acute and chronic tendinopathies and muscle strains.
involved in the present study performed the imaging.

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Sample Collection

All participants donated blood on the same day, and each sample was processed immediately after collection. A single technician collected 100 mL of blood from each participant using an 18-gauge arteriovenous fistula needle (JMS SysLoc, Hayward, California). Approximately 1 mL of whole blood was used for whole blood platelet analysis, 1.5 mL for fibrinogen analysis, 18 mL for processing through the MTF Cascade system, 26 mL for the Arteriocyte Magellan system, and 55 mL for the Biomet GPS III system.

Platelet Separation Systems

Whole blood samples were collected with the appropriate ratio of anticoagulant, and each sample was simultaneously processed using the MTF Cascade, Arteriocyte Magellan, and Biomet GPS III PRP separation systems according to the manufacturer's protocol (Table 1) to produce PRP and, in the case of the Biomet system, platelet-poor plasma (PPP) as well. A representative from each company performed their respective sample processing under direct observation of the study personnel. All PRP and PPP samples were stored at -80°C for future analysis to determine the concentration of PDGF-α, PDGF-β, active TGF-β1, and VEGF.

Quantification of Platelet, WBC, Red Blood Cell, and Fibrinogen Concentration

All whole blood, PRP, and PPP samples were sent to the University Hospital Clinical Laboratory immediately after collection for platelet count, WBC, red blood cell (RBC), and fibrinogen concentration analysis. Platelet, WBC, and RBC counts were performed using a Coulter LH780 Hematology Analyzer (Beckman Coulter, Brea, California). Fibrinogen concentration was determined by an assay performed using a STA-R Evolution Analyzer (Diagnostica Stago, Parsippany, New Jersey).

Quantification of Growth Factors

The PRP and PPP growth factor (PDGF-α, PDGF-β, active TGF-β1, and VEGF) concentrations were determined by ELISA, performed according to the manufacturer's
Data were analyzed with SPSS 17.0 (SPSS Inc, Chicago, Illinois). Differences in mean platelet, WBC, RBC, fibrinogen, and growth factor concentration among PRP separation systems were analyzed by 1-way analysis of variance (ANOVA) and post hoc Bonferroni testing. Linear correlations between PC and growth factor concentration, as well as WBC and growth factor concentration, were analyzed with Pearson correlation. Significance was set at \( P < .05 \).

### RESULTS

Each system produced 6.0 to 7.5 mL of PRP per whole blood sample, even though the starting whole blood volume was different from each system (55 mL for the GPS III, 26 mL for the Magellan, and 18 mL for the Cascade).

#### Platelet Concentration

The overall average PRP PC (596.7 \( \times 10^{9} \mu L \)) was significantly higher than the baseline whole blood PC (273.8 \( \times 10^{9} \mu L \)) and PPP PC (45.2 \( \times 10^{9} \mu L \), \( P < .001 \); Table 2). Analysis of variance revealed no significant difference in mean PRP PC among systems (\( P = .09 \)). Pairwise analysis using Bonferroni post hoc testing also confirmed that there was no significant difference in mean PC between any of the systems. The mean PRP PC from the Magellan was the highest (780.2 \( \times 10^{9} \mu L \)), followed by the GPS III (566.2 \( \times 10^{9} \mu L \)) and the Cascade (443.8 \( \times 10^{9} \mu L \)). There was wide variability in the factor increase in PC among the PRP separation systems, which ranged from a 1.06- to 1.4-fold increase from baseline PC. Moreover there was a significant difference in platelet capture efficiency—\((\text{volume}_{\text{PRP}} \times \text{platelet}_{\text{PRP}})/\text{volume}_{\text{PPP}} \times \text{platelet}_{\text{PPP}}\)—among systems (\( P < .0001 \)). The highest platelet capture efficiency was obtained with the Cascade, which was comparable with the Magellan (\( P = .08 \)) but significantly higher than the GPS III (\( P < .0001 \)).

### Blood Product

<table>
<thead>
<tr>
<th>Blood Product</th>
<th>Platelet Concentration ( \times 10^{9} \mu L )</th>
<th>Factor Increase in Platelet Concentration</th>
<th>Platelet Capture Efficiency (%)</th>
<th>White Blood Cells ( \times 10^{9} \mu L )</th>
<th>Red Blood Cells ( \times 10^{9} \mu L )</th>
<th>Fibrinogen ( \text{mg/dL} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>273.8 ± 7.4</td>
<td>6.4 ± 2.3</td>
<td>4.85 ± 0.4</td>
<td>238.2 ± 69.0</td>
<td>283.8 ± 34.2</td>
<td></td>
</tr>
<tr>
<td>Platelet-rich plasma</td>
<td>596.7 ± 250.4</td>
<td>51.9 ± 24.8</td>
<td>15.5 ± 16.8</td>
<td>0.7 ± 1.1</td>
<td>282.4 ± 33.7</td>
<td></td>
</tr>
<tr>
<td>Platelet-poor plasma</td>
<td>45.2 ± 6.5</td>
<td>0.2 ± 0.1</td>
<td>0.01 ± 0.02</td>
<td>278.1 ± 53.4</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>GPS III, Biomet</td>
<td>&lt; .0001</td>
<td>1.1 ± 0.2</td>
<td>0.1 ± 0.1</td>
<td>286.0 ± 42.7</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Cascade, MTF</td>
<td>443.8 ± 24.7</td>
<td>67.6 ± 4.1</td>
<td>1.5 ± 1.7</td>
<td>287.4 ± 30.5</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>GPS III, Biomet</td>
<td>566.2 ± 292.6</td>
<td>22.6 ± 11.3</td>
<td>1.6 ± 0.3</td>
<td>277.4 ± 30.5</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Magellan, Arterioxynet</td>
<td>780.2 ± 246.5</td>
<td>65.5 ± 19.6</td>
<td>0.5 ± 0.3</td>
<td>260.0 ± 42.7</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) From analysis of variance comparison of means among whole blood, platelet-rich plasma, and platelet-poor plasma.

\( b \) From analysis of variance comparison of means among platelet-rich plasma separation systems.

### Statistical Analysis

Data were analyzed with SPSS 17.0 (SPSS Inc, Chicago, Illinois). Differences in mean platelet, WBC, RBC, fibrinogen, and growth factor concentration among PRP separation systems was analyzed by 1-way analysis of variance (ANOVA) and post hoc Bonferroni testing. Linear
concentration in all PRP (15.5 × 10^6/μL) compared with PPP (0.2 × 10^6/μL, P = .008). In comparing the mean WBC concentration in PRP among all separation systems, there was a significant difference by ANOVA (P < .001) and pairwise analysis. The Cascade produced PRP with the lowest mean WBC concentration (1.1 × 10^6/μL), compared with that of the GPS III (P < .001) and the Magellan (P = .34). The GPS III produced PRP with highest mean WBC concentration (34.4 × 10^6/μL), which was significantly higher than that of the Magellan (11.0 × 10^6/μL, P = .005).

### RBC Concentration

The whole blood mean RBC concentration (4.35 × 10^6/μL) was significantly higher than the mean RBC concentration in PRP (0.7 × 10^6/μL, P < .001), which was also significantly higher than the mean RBC concentration in PPP (0.01 × 10^6/μL, P = .08; Table 2). All systems produced PRP with similarly low RBC concentrations (P = .10).

### Fibrinogen Concentration

Mean whole blood fibrinogen concentration (239.2 mg/dL) did not differ significantly from the PRP or PPP mean fibrinogen concentrations (282.4 mg/dL and 287.1 mg/dL, respectively, P = .18; Table 2). There was also no difference in mean fibrinogen concentration between PRP and PPP (P = .86) or mean PRP fibrinogen concentration among PRP systems (P = .93).

### Growth Factor Concentrations

Overall there was a significant difference among PRP systems in mean concentrations of PDGF-αβ (P = .006), PDGF-ββ (P = .009), and VEGF (P = .005) (Table 3). However, there was no significant difference in mean TGF-β1 concentration among any of the systems by ANOVA (P = .37) or pairwise analysis. The Magellan produced PRP with the highest mean PDGF-αβ and PDGF-ββ concentrations, which were significantly higher than the mean PDGF-αβ and PDGF-ββ concentrations produced by the Cascade

### DISCUSSION

Currently, there are more than 16 available platelet separation systems that produce differing types of platelet-rich concentrates. It is essential to better characterize the content of PRP produced by the various commercial systems to make more informed decisions regarding its use in the clinical setting. Although this study used a single-donor protocol in which only a few systems were concurrently tested, all available PRP separation systems should be subject to the same level of investigation.

The results of this study demonstrated no overall significant difference in PRP PC among the 3 studied PRP separation systems, which was a surprising finding given that they each started with a different whole blood volume (18 mL for the MTF Cascade system, 26 mL for the Arterioyte Magellan system, and 55 mL for the Biomet GPS III) and yet produced comparable volumes of PRP (6.0 to 7.5 mL). However, calculation of the platelet capture efficiency for each system explained this finding because the
Cascade and Magellan systems, which start with smaller whole blood volumes, have higher platelet capture efficiencies. Consequently, the volume of whole blood required to produce a desired volume of PRP and the ability to reliably predict platelet capture efficiency are practical considerations for surgeons to take into account when comparing PRP separation systems and counseling patients about their predicted PRP content with a given system.

The most noteworthy finding in the study was the significant difference among systems in PRP WBC concentration. The Cascade system decreased the PRP WBC concentration 5-fold when compared with whole blood, whereas the GPS III and Magellan systems increased the PRP WBC concentration 5-fold and 2-fold, respectively. This illustrates that the Cascade system produces leukocyte-poor PRP whereas the GPS III and Magellan systems produce leukocyte-rich PRP, which may explain the need for greater starting volumes of whole blood in the GPS III and Magellan systems. The clinical implications of these differences are unknown and merit further investigation.

Arguments can be made both for and against concentrating WBCs in PRP. It is possible that WBCs play a valuable antimicrobial role in PRP treatment. The concentrated presence of leukocytes can provide a local environment at the site of PRP injection with increased immunomodulatory capability that may aid in preventing or controlling infection at the identified site of injury. This would be of theoretic advantage in clinical scenarios where PRP is used as an adjunct treatment with invasive procedures that increase the chance of infection (eg, anterior cruciate ligament or Achilles repair). It is also possible that WBCs enhance PRP growth factor concentrations through their own release of growth factors or by stimulating platelet release of growth factors. Zimmermann et al found that WBC concentration in PRP accounted for one-third to one-half the variance of growth factor concentration. The leukocyte-rich PRP systems (GPS III and Magellan) demonstrated significant increases in the concentrations of PDGF-aa, PDGF-ββ, and VEGF, compared with leukocyte-poor PRP concentration (Cascade). The significant positive correlation between WBC concentration and VEGF and PDGF-ββ concentrations explains some of this observed difference in growth factor concentration between the leukocyte-rich and leukocyte-poor systems. This finding reinforces the observation by Zimmermann et al that WBC concentration may account for increased growth factor concentrations in leukocyte-rich PRP and thus be a reason to use leukocyte-rich PRP to optimize growth factor concentrations.

Conversely, it is possible that WBCs—namely, neutrophils—may impede tissue recovery by increasing local inflammation and therefore not be a desired component in PRP treatment. Currently, there are no controlled animal studies to discern whether leukocyte-rich PRP increases inflammation, compared with leukocyte-poor PRP. Additionally, clinical studies are necessary to differentiate soft tissue healing effects of leukocyte-rich versus leukocyte-poor PRP.

In general, our study had a lower mean PRP PC than that of some studies, although we used centrifuge separation processes. Although this may provide an interesting reference, the utility of these comparisons is limited given that these studies used different separation systems and different platelet counting methods. The same limitations hold true for comparing PRP growth factor concentrations among these studies.

Our PRP samples had markedly lower concentrations of TGF-β1 than those reported in other studies. Given that we used an ELISA for activated TGF-β1, the low TGF-β1 concentration provides evidence that there was little platelet activation. Because we did not use calcium chloride or thrombin to activate the platelets, the low level of activation was possibly secondary to platelet exposure to shear forces during processing. We chose not to activate the PRP, because we believe that it represents a more physiologic delivery of growth factors with platelet activation over time, as opposed to our causing release of all growth factors with artificial chemical platelet activation.

Interestingly, our samples contained greater PDGF-αα and VEGF levels than those of a previous report using unactivated PRP, which may again indicate that some platelets were activated by mechanical forces during processing.

The other unique finding in this study was that there was no significant difference in PRP fibrinogen concentration among systems, as well as no difference in fibrinogen concentration in PRP compared with whole blood or PPP. Thus, these systems seem to produce PRP with similar concentrations of adhesive proteins and are likely to produce similar fibrin matrixes, which is an important consideration in the application of PRP therapy. Because fibrinogen was the only studied protein present in a substantial concentration in PPP, the only indication to save and administer PPP would be to deliver additional fibrinogen.

The small sample size of this study limits its ability to detect differences among systems. Nonetheless, its strength is its use of single donors to test all systems concurrently, where participants serve as their own controls, which helps to minimize the potential confounding variables when making comparisons among the tested PRP systems. The lack of a baseline whole blood growth factor concentration limited our ability to demonstrate the magnitude of increase in growth factor concentration in the PRP; however, this was less important because the samples were drawn and processed from the same 5 donors simultaneously. Therefore, the relative concentration of the growth factors should be comparable among the different systems.

Larger future studies are necessary to further characterize the inter-system and intrasystem variability in platelet, WBC, and activated and unactivated growth factor concentrations in the PRP extracted from commercial PRP separation systems. Further studies are also necessary to distinguish the indications and clinical utility of leukocyte-rich versus leukocyte-poor PRP. Ultimately, randomized controlled clinical trials are needed to establish the optimal PRP dosing for the treatment of orthopaedic injuries that may benefit from PRP therapy. However, in the design of these studies, it is critical to characterize the platelet, WBC, and growth factor content of the PRP.
produced by the chosen commercial separation systems. This will provide an accurate context for physicians to interpret the results and thereby make informed decisions about when to use the various PRP separation systems to provide effective PRP treatment.

ACKNOWLEDGMENT

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REFERENCES

Comparison of In Situ Forces and Knee Kinematics in Anteromedial and High Anteromedial Bundle Augmentation for Partially Ruptured Anterior Cruciate Ligament

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Investigation performed at the University of Pittsburgh, Pittsburgh, Pennsylvania

Background: High tunnel placement is common in single- and double-bundle anterior cruciate ligament (ACL) reconstructions. Similar nonanatomic tunnel placement may also occur in ACL augmentation surgery.

Purpose: In this study, in situ forces and knee kinematics were compared between nonanatomic high anteromedial (AM) and anatomic AM augmentation in a knee with isolated AM bundle injury.

Study Design: Controlled laboratory study.

Methods: Seven fresh-frozen cadaver knees were used (age, 48 ± 12.5 years). First, intact knee kinematics was tested with a robotic–universal force sensor testing system under 2 loading conditions. An 89-N anterior load was applied, and an anterior tibial translation was measured at knee flexion angles of 0°, 30°, 60°, and 90°. Then, combined rotatory loads of 7-N-m valgus and 5-N-m internal tibial rotation were applied at 15° and 30° of knee flexion angles, which mimic the pivot shift. Afterward, only the AM bundle of the ACL was cut arthroscopically, keeping the posterolateral bundle intact. The knee was again tested using the intact knee kinematics to measure the in situ force of the AM bundle. Then, arthroscopic anatomic AM bundle reconstruction was performed with an allograft, and the knee was tested to give the in situ force of the reconstructed AM bundle. Knee kinematics under the 3 conditions (intact, anatomic AM augmentation, and nonanatomic high AM augmentation) and the in situ force were compared and analyzed.

Result: The high AM graft had significantly lower in situ force than the intact and anatomic reconstructed AM bundle at 0° of knee flexion (P < .05) and the intact AM bundle at 30° of knee flexion under anterior tibial loading. There were no differences between anatomic graft and intact AM bundle. The high AM graft also had a significantly lower in situ force than the intact and anatomic reconstructed AM with simulated pivot-shift loading at 15° and 30° of flexion (P < .05). Under anterior tibial and rotatory loading, there was a difference in tibial displacement between anatomic and high AM reconstructions and between the high AM graft and intact ACL under rotational loading with the knee at 15° of flexion.

Clinical Relevance: Anatomic AM augmentation can lead to biomechanical advantages at time zero when compared with the nonanatomic (high AM) augmentation. Anatomic AM augmentation better restores the knee kinematics to the intact ACL state.

Keywords: anterior cruciate ligament; augmentation; partial rupture; in situ force; biomechanics

The anterior cruciate ligament (ACL) consists of 2 functional bundles: the anteromedial (AM) bundle and the posterior lateral (PL) bundle. The 2 bundles have their distinct femoral and tibial insertion sites and are separated by a fine septum. Each bundle contributes to the overall biomechanical function of the ACL. Previous studies showed that the AM bundle has higher levels of in situ forces during knee flexion whereas the PL bundle supports high in situ forces at 0°, 15°, and 30° of flexion and decreases at greater angles of flexion. The PL bundle especially contributes to the rotational stability of the knee at 0° to 30° of flexion; both bundles contribute to