# Comparison of the Cellular Composition and Cytokine-Release Kinetics of Various Platelet-Rich Plasma Preparations

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**Background:** Variations in formulations used to prepare platelet-rich plasmas (PRPs) result in differences in the cellular composition and biomolecular characteristics.

**Purpose:** To evaluate the cellular composition and the cytokine-release kinetics of PRP according to differences in the preparation protocols.

**Study Design:** Controlled laboratory study.

**Methods:** Five preparation procedures were performed for 14 healthy subjects, including 2 manual procedures (single-spin [SS] at 900*g* for 5 minutes; double-spin [DS] at 900*g* for 5 minutes and then 1500*g* for 15 minutes) and 3 methods with commercial kits (Arthrex ACP, Biomet GPS, and Prodizen Prosys). After evaluation of cellular composition, each preparation was divided into 4 aliquots and incubated for 1 hour, 24 hours, 72 hours, and 7 days for the assessment of cytokine release over time. The cytokine-release kinetics were evaluated by assessing platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), interleukin-1 (IL-1), and matrix metalloproteinase–9 (MMP-9) concentrations of each aliquot with bead-based sandwich immunoassay.

**Results:** The DS PRP had a higher concentration of platelets and leukocytes than did the SS PRP. Every PRP preparation exhibited an increase in PDGF, TGF, VEGF, and FGF release when compared with whole blood samples. The FGF and TGF release occurred quickly and decreased over time, while the PDGF and VEGF release was constant and sustained over 7 days. The PDGF and VEGF concentrations were higher in the DS PRP than in the SS PRP, whereas the TGF and FGF concentrations were higher in the SS PRP than in the DS PRP. Biomet GPS had the highest VEGF and MMP-9 concentrations but the lowest TGF concentration. Arthrex ACP had the highest FGF concentration but the lowest PDGF concentration. Prodizen Prosys had the highest IL-1 concentration and higher PDGF concentration than Arthrex ACP.

**Conclusion:** The DS method generally led to a higher concentration of platelet relative to the SS method. However, the cytokine content was not necessarily proportional to the cellular composition of the PRPs, as the greater content could be different between the SS or DS method depending on the type of cytokine.

**Clinical Relevance:** Physicians should select proper PRP preparations after considering their biomolecular characteristics and patient indications.

**Keywords:** platelet-rich plasma; preparations; cell composition; cytokine-release kinetics

The basis for platelet-rich plasma (PRP) treatment has historically centered on maximizing the growth factor (GF) found in platelet alpha granules, such as platelet-derived growth factor (PDGF), transforming growth factor–β (TGF-β), and vascular endothelial growth factor (VEGF). The goal is to regulate wound-healing processes and to promote an anabolic environment at the injury site,[43] and the use of PRP to enhance tissue healing and regeneration has remained a topic of great interest. However, evidence of its long-term clinical benefit has been controversial. Some studies have shown clinical and radiologic improvement in patients with muscle or tendon injuries,[16,33] but numerous other clinical trials have demonstrated the outcome of PRP usage as equivocal.[34,37] Many in vitro and in vivo studies using PRP preparations[8,19,20,30] have produced results that are difficult to interpret in terms of the dose-dependent relationship between the load of the platelets/GFs delivered to the injury site and actual healing[40] because sample variability poses some methodological challenges to investigators. Inconsistencies in results of clinical or laboratory tests are attributed to the lack of standardization in preparation protocols of PRPs.

PRP preparations have been conducted from various formulations and have resulted in differences in the cellular composition and biomolecular characteristics of the

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PRPs.[6,27,28] According to previous studies, PRP can be classified according to 3 components[10]: (1) the absolute number of platelets, (2) the manner in which platelet activation occurs, and (3) the presence or absence of white blood cells (WBCs). However, the cell count cannot comprehensively predict the GF content for some individual preparations, and variability in the isolation methods, centrifuge speed/time, and activation level of the PRP preparation can produce plasma preparations with differences in the quantity or quality of cytokine. Several studies have demonstrated differences in the platelet and cytokine concentrations in PRP systems,[24,27,38] but these have focused only on comparing the platelet or cytokine concentration after an uncertain incubation period.

Although the platelet concentration or the cellular composition of PRP is widely used to assess PRP preparations,[3,6] a description of the biomolecular characteristics of PRP may also be necessary to interpret study outcomes according to the preparation method. The present study hypothesizes that PRP preparations are different in terms of their cellular composition and quality of bioactive molecules as a result of the differences in their preparation protocols. This study (1) determined the cytokine release kinetics of PRP between single-spin (SS) and double-spin (DS) methods and (2) compared the results against those obtained with commercially available PRP kits.

## METHODS

### Subjects

Blood samples were obtained from 14 healthy subjects (7 women, 7 men; mean age ± SD, 34.6 ± 6.6 years; range, 26-50 years). An institutional review board approved this study, and all patients signed an informed consent form. The inclusion criteria included healthy subjects between the ages of 18 and 65 years without any known blood dyscrasia, and the exclusion criteria included medical history of any blood-derived illness or medication known to affect platelet or bone marrow function for a minimum of 2 weeks before testing.

### PRP Preparation

Each of the preparation procedures was performed for each subject to minimize the effect of interdonor variability. Approximately 290 mL of peripheral venous blood was drawn from each subject and was collected into acid-citrate dextrose A (ACD-A) anticoagulant with a ratio of 1 mL ACD-A to 9 mL of whole blood. ACD-A binds with calcium and prevents blood clotting, with no known interference to platelet function. The blood was then divided and transferred to different protocol systems (Table 1).

### SS or DS PRP Preparations

The optimal separating centrifugation condition (first spin) has been suggested to be 900*g* for 5 minutes with a recovery ratio of 92.0% ± 3.1%, and the optimal condensation centrifugation condition (second spin) is of 1500*g* for 15 minutes with a recovery ratio of 84.3% ± 10.0%.[21] In this study, the same protocols were used for the SS and the DS PRP preparations. For the SS PRP preparation, 30 mL of whole blood was centrifuged at 900*g* for 5 minutes. Then, the top plasma layer was separated, and the lower 3 mL of volume of the plasma were used as the SS PRP preparation (Figure 1A). For the DS PRP preparation, the top plasma layer after the single spin was separated and was centrifuged a second time at 1500*g* for 15 minutes, the superficial plasma layer was removed, and finally, the lower 3 mL of volume of the plasma was used as the DS PRP preparation (Figure 1A).

### Comparison With 3 Commercially Available Kits

Three commercially available kits were used according to the manufacturer's instructions: ACP Double Syringe (Arthrex; SS, plasma technique), GPS III Platelet Concentrate System (Biomet; SS, buffy coat method), and Prosys kit (Prodizen Inc; DS, plasma technique). None of these 3 commercial kits were exogenously activated by calcium or thrombin. The ACP Double Syringe uses the SS method, with a relatively lower concentration of platelets and WBCs. Here, 15 mL of blood was filled into a double syringe to produce 3 mL of PRP. The syringes were centrifuged at 1500 rpm (1012.5*g*) for 5 minutes to separate erythrocytes from the remaining plasma components. The top portion of the plasma was drawn up using an inner syringe without disrupting the erythrocyte layer (Figure 1B). The GPS III Platelet Concentrate System uses the SS buffy coat extraction method, and 54 mL of whole blood was required to produce approximately 6 mL of PRP. The tubes were centrifuged for 15 minutes at 3200 rpm (2011.42*g*) according to the manufacturer's protocols. After the specific construction of these tubes, it was possible to draw a portion of the PRP into a 6-mL syringe according to the manufacturer's instructions (Figure 1C). The Prosys kit uses the DS method, with relatively higher concentrations of platelets and WBCs. Here, 30 mL of citrated blood was filled in a tube and centrifuged for 3 minutes at 3000 rpm (1660*g*) in the case of the male samples and 2800 rpm (1446*g*) in the case of the female samples. The upper plasma and the buffy coat layer after the first spin were separated and

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TABLE 1
Preparation Protocols and Cellular Compositions of 5 Platelet-Rich Plasmas Preparations[a]

| Preparation | Centrifugation | | Isolation | Final vol/WB vol |
|---|---|---|---|---|
| | First Spin | Second Spin | | |
| SS preparation | 900*g*, 5 min | | Plasma layer | 3 mL/30 mL |
| DS preparation | 900*g*, 5 min | 1500*g*, 15 min | Plasma layer | 3 mL/30 mL |
| Arthrex ACP | 1012.5*g*, 5 min | | Plasma layer | 3 mL/15 mL |
| Biomet GPS | 2011*g*, 15 min | | Buffy coat layer | 6 mL/54 mL |
| Prodizen Prosys[b] | 1660*g*, 3 min or 1446*g*, 3 min | 2008*g*, 3 min | Plasma layer | 3 mL/30 mL |

[a]DS, double-spin method; SS, single-spin method; WB, whole blood.
[b]The first-spin protocol of the Prodizen Prosys samples was subdivided into male and female subjects.

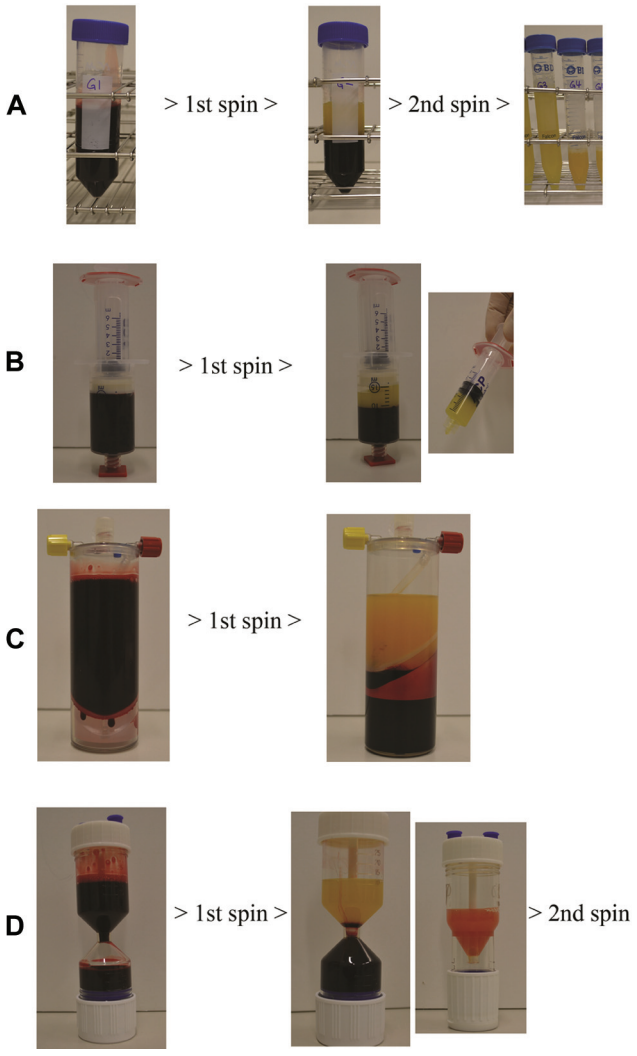


**Figure 1.** Images for 5 different platelet-rich plasma preparation procedures: (A) single-spin/double-spin manual methods, (B) Arthrex ACP, (C) Biomet GPS, and (D) Prodizen Prosys.

were centrifuged a second time at 3000 rpm (2008*g*) for 3 minutes, and the lower 3 mL of the plasma was used as the PRP (Figure 1D).

## Evaluation of the Cellular Composition and Kinetics of Cytokine Release

The platelet, WBC, and red blood cell (RBC) counts were performed before cytokine quantification by using a cell count machine (XE-2100; Sysmex). Most hematology analyzers are designed to operate within ranges found in whole blood, and PRP may exceed the upper limit of the linear range of platelets that can be counted. Therefore, PRP was suspended with normal saline at a ratio of 1:5, and the cellular content of PRP was analyzed.

Each PRP preparation or whole blood (control) sample was divided into 4 aliquots and was incubated for 1 hour, 24 hours, 72 hours, and 7 days at 37°C in 5% $CO_2$ (Figure 2). The samples were snap-frozen in individual aliquots after a given incubation period and were stored at –80°C for a cytokine assay. Then these samples were assayed in duplicate, and PDGF-BB, TGF-β1, VEGF, basic fibroblast growth factor (bFGF), interleukin-1β (IL-1β), and matrix metalloproteinase–9 (MMP-9) concentrations were evaluated using commercially available bead-based sandwich immunoassay kits (Bio-Rad Laboratories).

Previous studies have demonstrated that PRP concentrates platelets and therefore the GFs contained in alpha granules.[14] The PDGF, TGF-β, VEGF, and FGF, which play a crucial role in cell proliferation and differentiation in the wound-healing process,[35] are basic cytokines identified in platelets and were chosen for anabolic cytokines to be analyzed. Conversely, effects of PRP are attributable not only to concentrated platelets but also to the presence of leukocyte and catabolic cytokines.[38] Thus, IL-1β and MMP-9 were chosen for catabolic cytokines, which play roles in inflammation or matrix degradation.[38]

Four different incubation periods were chosen to assess the cytokine-release kinetics according to various PRP preparations. The average life span of circulating platelets is 8 to 9 days, but platelets that have been isolated from whole blood have a relatively short shelf life. Thus, a previous study recommended multiple injections 1 week apart for patellar tendinopathy.[8] The 1-hour and 1-day incubation period were chosen to detect a rapid release and an early depletion of some cytokines with short life span, and the 3-day period was considered as a check point for the intermediate-term sustainability of each cytokine release.

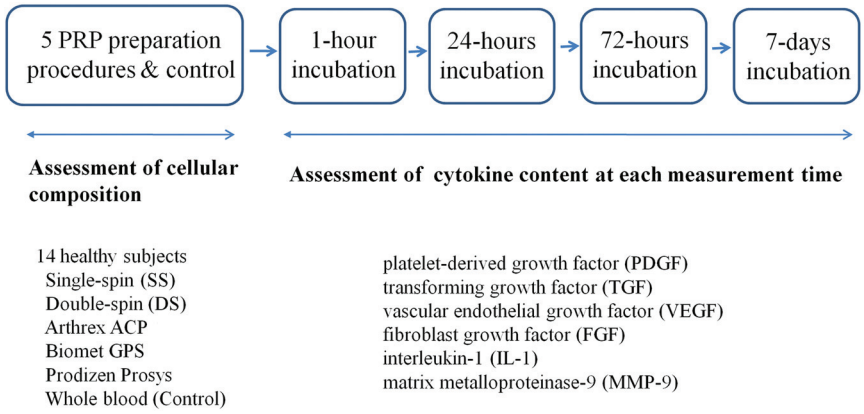


**Figure 2.** Flow diagram of study protocols. Four different incubation periods were chosen to assess the cytokine-release kinetics according to various platelet-rich plasma preparations.

TABLE 2
Mean Whole Blood Characteristics From 14 Healthy Donors

| Characteristic | Mean ± SD |
|---|---|
| Platelet concentration, $\times 10^3$ cells/μL | 147.8 ± 59.2 |
| Leukocyte concentration, $\times 10^3$ cells/μL | 6.27 ± 1.94 |
| Erythrocyte concentration, $\times 10^3$ cells/μL | 4269 ± 393 |
| Hematocrit, % | 42.6 ± 3.9 |

### Statistical Analysis

The measured data are presented here as the arithmetic mean and the standard deviation (SD). The concentrations of the cytokines in each of the incubation periods were analyzed using a Kruskall-Wallis test (nonparametric analysis of variance), a Bonferroni post hoc test was conducted to compare multiple values from each of the preparation conditions, and linear correlations between the cell count and cytokine content were analyzed via Pearson correlations. A $P$ value of .05 was considered significant.

## RESULTS

### Cellular Concentrations and Compositions

The mean platelet concentration of the whole blood (control) samples was 147 ± 59 × $10^3$ cells/μL. Mean whole blood characteristics from 14 healthy donors, which were all within the ranges of normal biological value, are demonstrated in Table 2. The platelet concentration of the SS PRP at 311 ± 72 × $10^3$ cells/μL was significantly higher than that of the control ($P < .01$) and that of the DS PRP was even higher at 1145 ± 244 × $10^3$ cells/μL ($P < .01$). The WBC concentration of the DS PRP was about 10 times higher than that of the SS PRP ($P < .01$), but both concentrations were lower than that of the control samples. Of the 3 commercial kits, Biomet GPS had the highest platelet and WBC concentrations at 1076 ± 333 and 32 ± 15 × $10^3$ cells/μL, respectively, and Prodizen Prosys (DS method) had higher concentrations of platelets and leukocytes than did Arthrex ACP (SS method; $P < .01$ for both). The mean RBC concentration of the control samples was significantly higher than that of any PRP system ($P < .01$ for all systems), and among all PRP systems, the Biomet GPS produced a PRP preparation with the highest mean RBC concentration in comparison with Prodizen Prosys ($P = .03$) and Arthrex ACP ($P < .01$; Figure 3).

### Time-Sequential Cytokine Release

*PDGF-BB.* The PDGF release was constant and was sustained over 7 days (Figure 4). The PDGF concentrations of PRP were correlated with platelet concentrations ($r = 0.71$, $P = .02$), and the PDGF concentrations of the DS PRP were 2 to 3 times higher than those of the SS PRP over a 7-day period ($P < .01$ for all concentrations). The PDGF concentrations of Prodizen Prosys exceeded those of Arthrex ACP at 1 hour, 24 hours, 72 hours, and 7 days ($P = .04$, .01, .04, and .02, respectively), and those of Biomet GPS exceeded those of Arthrex ACP at 1 hour, 24 hours, and 72 hours ($P = .04$, $< .01$, and .07, respectively). However, no differences in PDGF concentration were observed between Biomet GPS and Prodizen Prosys over a 7-day period.

*TGF-β1.* The TGF release was rapidly induced, and a maximum concentration was detected within 1 hour, with no significant differences in the TGF concentration between the SS and DS PRP preparations over a 7-day period (Figure 5). The TGF concentration of Arthrex ACP exceeded that of Biomet GPS at 1 hour, 24 hours, 72 hours, and 7 days ($P < .01$, $< .01$, .02, and $< .01$, respectively), and there was no significant difference between Arthrex ACP and Prodizen Prosys. The TGF-β1 concentration was not correlated with the platelet concentration of the PRP.

*VEGF.* The VEGF concentration of the PRP was correlated with the platelet concentration ($r = 0.71$, $P = .02$) and was constant and sustained over 7 days. The VEGF concentration of the DS PRP exceeded that of the SS PRP at 72 hours and after 7 days ($P = .06$ and $< .01$, respectively; Figure 6). The VEGF concentration of Biomet

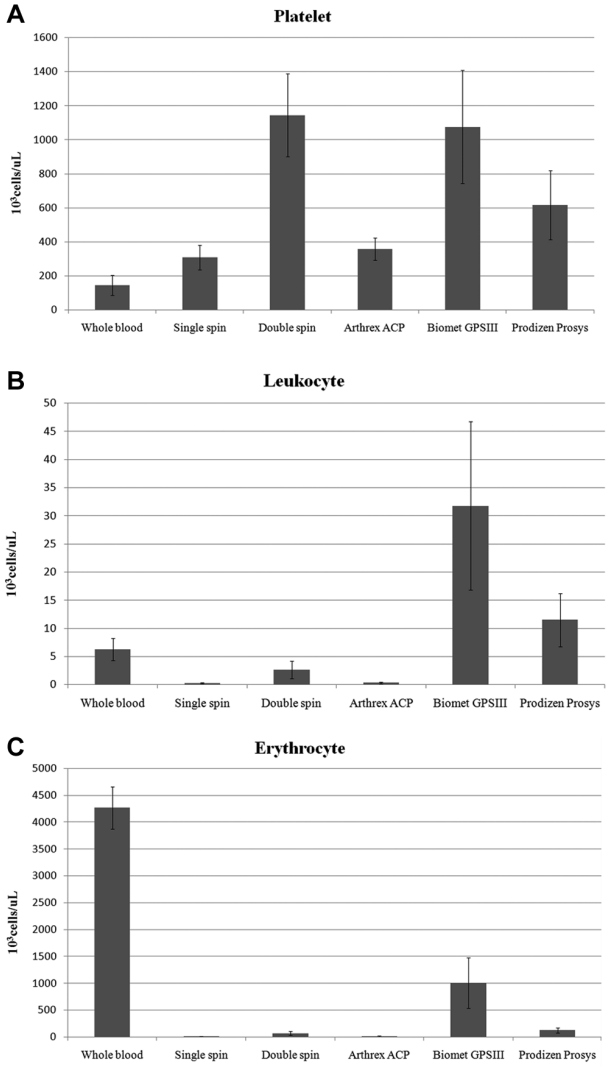


**Figure 3.** (A) Platelet, (B) leukocyte, and (C) red blood cell concentrations for the 5 different platelet-rich plasma (PRP) preparations. The platelet concentration of the single-spin (SS) PRP at 311 ± 72 × $10^3$ cells/μL was significantly higher than that of the control ($P < .01$) and that of the double-spin (DS) PRP was even higher at 1145 ± 244 × $10^3$ cells/μL ($P < .01$). The white blood cell concentration of the DS PRP was of about 10 times higher than that of the SS PRP ($P < .01$), but both concentrations were lower than that of the control samples. Biomet GPS had the highest platelet and white blood cell concentrations at 1076 ± 333 and 32 ± 15 × $10^3$ cells/μL, respectively, and Prodizen Prosys had higher concentrations of platelets and leukocytes than did Arthrex ACP. The mean red blood cell concentration of whole blood was significantly higher than that of any PRP system ($P < .01$ for all systems).

GPS exceeded that of Arthrex ACP at 24 hours, 72 hours, and 7 days ($P = .04$, $< .01$, and $< .01$, respectively) and that of Prodizen Prosys at 24 hours and 72 hours ($P = .04$ for both time periods). On the other hand, there were no significant differences in VEGF concentration between

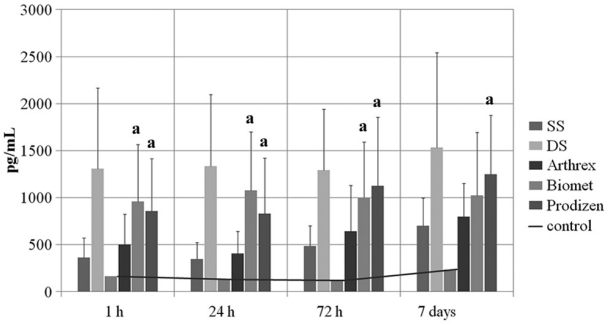


**Figure 4.** Platelet-derived growth factor (PDGF) release was sustained over 7 days. The PDGF concentrations of Prodizen Prosys exceeded those of Arthrex ACP at 1 hour, 24 hours, 72 hours, and 7 days, and those of Biomet GPS exceeded those of Arthrex ACP at 1 hour, 24 hours, and 72 hours. [a]$P < .05$ compared with the Arthrex ACP group. DS, double-spin method; SS, single-spin method.

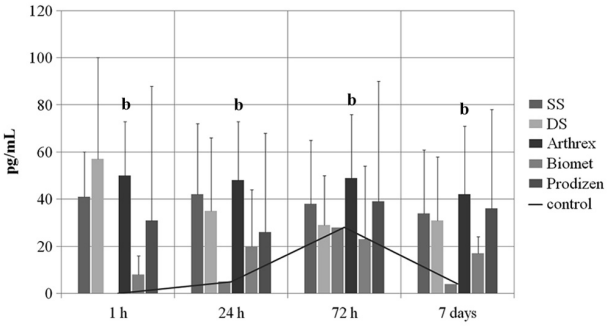


**Figure 5.** Transforming growth factor (TGF) release was rapidly induced, and a maximum concentration was detected within 1 hour. The TGF concentration of Arthrex ACP exceeded that of Biomet GPS at all measured times. [b]$P < .05$ compared with the Biomet GPS group. DS, double-spin method; SS, single-spin method.

Arthrex ACP and Prodizen Prosys at any of the measured times.

*FGF.* The FGF release was rapidly induced, and a maximum concentration was detected within 1 hour, but the FGF concentration was not correlated with the platelet concentration. The FGF concentration of the SS PRP exceeded that of the DS PRP at 24 hours, 72 hours, and 7 days ($P < .01$, $< .01$, and .04, respectively; Figure 7). The FGF concentration of Arthrex ACP exceeded that of Biomet GPS and Prodizen Prosys for all measured times ($P < .01$ for all).

*IL-1.* IL-1 release was constant and was sustained over 7 days. There were no significant differences in the IL-1 concentration between the SS and DS PRP preparations (Figure 8). The IL-1 concentration for Prodizen Prosys exceeded that of Arthrex ACP and Biomet GPS at 72 hours and 7 days ($P < .01$ for both times), but these were lower than the mean level of the control samples (Figure 6B). IL-1 concentration was not correlated with WBC or platelet concentration.

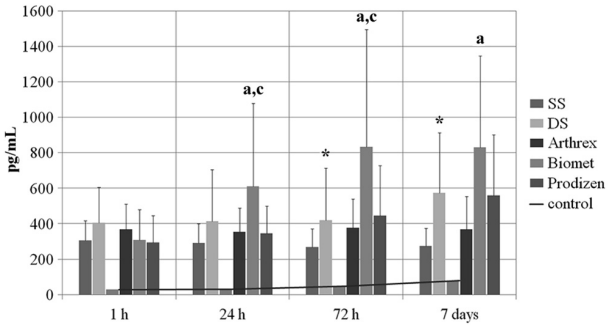


**Figure 6.** Vascular endothelial growth factor (VEGF) release was constant and sustained over 7 days. The VEGF concentration of Biomet GPS exceed that of Arthrex ACP at 24 hours, 72 hours, and 7 days and that of Prodizen Prosys at 24 hours and 72 hours. *$P < .05$ compared with the SS group; [a]$P < .05$ compared with the Arthrex ACP group; [c]$P < .05$ compared with the Prodizen Prosys group. DS, double-spin method; SS, single-spin method.

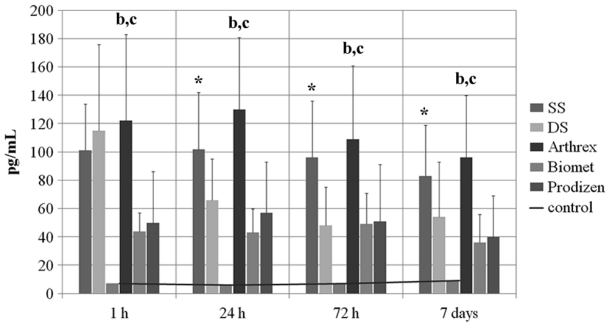


**Figure 7.** Fibroblast growth factor (FGF) release was rapidly induced, and a maximum concentration was detected within 1 hour. The FGF concentration of Arthrex ACP exceeded that of Biomet GPS and Prodizen Prosys at all measured times. *$P < .05$ compared with the SS group; [b]$P < .05$ compared with the Biomet GPS group; [c]$P < .05$ compared with the Prodizen Prosys group. DS, double-spin method; SS, single-spin method.

*MMP-9.* The MMP-9 concentration was lower than 50 pg/mL over 7 days for both the SS and DS PRP preparations, and these values were lower than that of the control (Figure 9). The MMP-9 concentration was strongly correlated with WBC concentration ($r = 0.938$, $P < .01$). There were no significant differences in MMP-9 concentration between the SS and the DS PRP preparations. The MMP-9 concentration for Biomet GPS exceeded that of Arthrex ACP for all measured times ($P < .01$ for all) and that of Prodizen Prosys at 1 hour, 24 hours, and 7 days ($P < .01$, .04, and $< .01$, respectively).

## Analysis of Variance for Each GF

The coefficient of variation (CV) represents the ratio of the standard deviation to the mean, and it is a useful statistic for comparing the degree of variation from one data series to another, even if the means are drastically different from each other. In terms of CV, the FGF showed the largest difference across donors, whereas the PDGF showed the smallest difference. The CVs of each cytokine are demonstrated in Table 3.

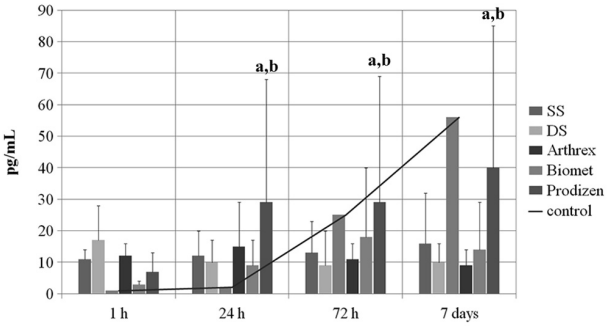


**Figure 8.** There were no significant differences in interleukin-1 (IL-1) concentration between the single-spin (SS) and double-spin (DS) platelet-rich plasma preparations. The IL-1 concentration for Prodizen Prosys exceeded that of Arthrex ACP and Biomet GPS at 72 hours and 7 days. [a]$P < .05$ compared with the Arthrex ACP group; [b]$P < .05$ compared with the Biomet GPS group.

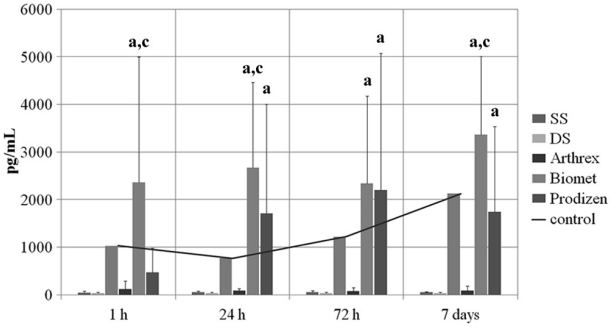


**Figure 9.** Matrix metalloproteinase–9 (MMP-9) concentration was lower than 50 pg/mL over 7 days for both the single-spin (SS) and double-spin (DS) platelet-rich plasma (PRP) preparations. There were no significant differences in MMP-9 concentration between the SS and the DS PRP preparations. The MMP-9 concentration for Biomet GPS exceeded that of Arthrex ACP at all measured times and that of Prodizen Prosys at 1 hour, 24 hours, and 7 days. [a]$P < .05$ compared with the Arthrex ACP group; [c]$P < .05$ compared with the Prodizen Prosys group.

## DISCUSSION

The biomolecular characteristics of PRP in terms of the cytokine content are important to determine the method of local application as well as to evaluate the effectiveness of the procedure. This study evaluated the different qualities of bioactive molecules in PRP according to different preparation protocols. The cytokine content differed between the SS and the DS methods, while neither was

TABLE 3
Interindividual Variations of Each Cytokine[a]

| Type of Cytokine | CV, %, Mean ± SD |
|---|---|
| PDGF | 45 ± 11 |
| TGF | 65 ± 16 |
| VEGF | 57 ± 10 |
| FGF | 71 ± 22 |
| IL-1β | 78 ± 36 |
| MMP-9 | 73 ± 41 |

[a]CV, coefficient of variation; FGF, fibroblast growth factor; IL, interleukin; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

superior in terms of GF content. The commercial preparations exhibited wide variations in their ability to concentrate platelets and leukocytes or to release platelet-derived cytokines. Accordingly, proper PRP components should be selected by considering their biomolecular characteristics and patient indications.

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 $1000 \times 10^3$ cells/μL is widely considered to be an effective platelet concentration to induce an efficient local cellular response.[13,26,27] On the other hand, a platelet concentration of 6 times higher than that of whole blood has been reported to have an inhibitory effect on healing.[12] Conversely, the effect of highly concentrated WBCs in PRP preparation has been widely debated.[11] Leukocytes in PRP may play a valuable antimicrobial role[31] but may impede tissue healing by producing biologically active catabolic cytokines.[3] Leukocyte-rich preparations do not seem to be exclusively good or bad. For some specific indications such as chronic tendinopathy, leukocyte-rich PRP has been shown to be superior to leukocyte-poor PRP in clinical trials.[29] Importantly, the first phase of tendon healing is inflammation. This may explain why leukocyte-rich PRP is a better option for chronic tendinopathy compared with leukocyte-poor PRP. In other indications such as knee osteoarthritis, trials exist to support the use mainly of leukocyte-poor PRP.[3] The effect of the leukocyte may depend on the concentration of the leukocytes or the biological state of the injured tissue, indicating a need for further research about the role that leukocytes of PRPs play in different patient indications. In this study, the DS method generally produced higher concentrations of platelets and leukocytes, and similar outcomes were observed when using commercial kits. More specifically, Prodizen Prosys produced higher concentrations of platelets and leukocytes than did Arthrex ACP. It is noteworthy that Biomet GPS showed the highest concentrations of platelet and leukocytes, which may be due to direct PRP extraction from the buffy coat layer after a single round of centrifugation.

The PDGF, TGF-β, VEGF, and FGF concentrations in PRP are known to play a crucial role in cell proliferation, chemotaxis, cell differentiation, and angiogenesis.[36] PDGF is a powerful mitogen for fibroblasts and smooth muscle cells, and it is involved in all 3 phases of wound healing, including angiogenesis, the formation of fibrous tissue, and reepithelialization.[18] In this study, PDGF-BB, which is a prevalent circulating isoform, was evaluated, whereas normal unstimulated cells of the osteoblast lineage primarily synthesize PDGF-AA. TGF-β stimulates the proliferation of undifferentiated mesenchymal stem cells and the chemotaxis of endothelial cells and angiogenesis,[9] and it exists as 3 known subtypes in humans (TGF-β1, TGF-β2, and TGF-β3). VEGF was originally referred to as the vascular permeability factor, and it increases angiogenesis and vascular permeability.[17] In this study, VEGF-1, which is the most important member of the VEGF family that stimulates endothelial cell mitogenesis and cell migration, was evaluated. FGF plays a key role in the proliferation and differentiation of a wide variety of cells and tissues,[4] and bFGF mediates the formation of new blood vessels, which is a process known as angiogenesis, during the wound healing of normal tissue and tumor development. IL-1β and MMP-9 are catabolic cytokines that are known to play roles in inflammation or matrix degradation.[38] IL-1β is a primary cytokine during inflammation and matrix degradation, and it is a common target to reduce inflammation by manipulating IL-1ra.[39] Injured human rotator cuffs have been shown to result in an increase in the concentration of IL-1β in the tendon, along with other proinflammatory cytokines.[41] MMP-9 is known to degrade collagen and other extracellular matrix molecules and has been implicated as a predictor of poor healing.[7] Elevated MMP concentrations, which are found in acute injured and degenerative tissue and postoperative tissue, have been widely observed in the tens to hundreds of nanograms per milliliter. Several studies have shown these concentrations to be a component of nonhealing or poorly healing wounds.[23,41]

The results of this study indicate that the relationship between cytokine concentrations and cellular compositions was mainly dependent on the type of cytokine. The DS method generally resulted in higher concentrations of platelets and leukocytes and was supposed to produce more GFs than the SS method. However, the resulting GF or cytokine concentrations were not necessarily proportional to the cellular count. The PDGF and VEGF concentrations for PRP were correlated with the platelet count, but the TGF and FGF concentrations were higher in the SS method than in the DS method, despite the lower platelet count. In addition, the 3 commercial PRP kits provided similar results with higher TGF and FGF concentrations in Arthrex ACP than in Prodizen Prosys. Some studies have reported a correlation between the platelet concentration and the GF concentration in PRP[5,38] while others have not.[28,42] In addition, Lacoste et al[22] found no correlations between the concentrations of various GFs within the same donor with the highest bFGF concentration and the lowest VEGF concentration. Several factors, including interdonor variability of cytokine content[22] or the GF-absorbing proteins, manipulation-induced platelet stress, the variable susceptibility of platelets, and microaggregation in PRP, may contribute to differences in the correlations. These results are consistent with the findings reported by Mazzucco et al,[28] who demonstrated that

TGF-β1 and bFGF are promptly released within 24 hours after exogenous activation, whereas the GF release of the PDGF-BB and the VEGF are more dependent on the technique that is used. This suggests that the individual dynamics of the GF release depend exclusively not only on the preparation method but also on the type of GF.

In this study, cytokine release kinetics were evaluated according to various preparation protocols, and there is a difference in processing for both the manual and the commercial procedures. Centrifugation spin is an important factor for PRP properties, and it could influence the results because the mechanical force can activate platelets. Furthermore, Prodizen Prosys has the subdivision of first-spin protocol for male and female subjects. The difference in centrifugation between sexes is related to previous findings that to include the maximum number of platelets, whole blood should be submitted to a lower centrifugation force if its hematocrit is less than 40%.[1] Low centrifugations provide a poor definition between the erythrocyte and platelet layer, optimizing their simultaneous harvest. There are some other commercial devices available for the preparation of PRP, and these protocols can differ in centrifuge conditions, methods of isolation, processes of activation, or methods of localization within a tissue. In terms of isolation technique, PRP can be manufactured in 2 basic formats: plasma-based and buffy coat–based techniques.[10] Plasma-based methods work to isolate plasma and platelet components from leukocyte and erythrocyte components. The buffy coat method isolates a buffy coat layer, which contains not only platelets but also leukocytes, and it seeks to capture all available platelets during the centrifugation and obtain a high concentration of leukocytes. In terms of activation process, some commercially available systems use thrombin to activate the clotting mechanism, resulting in rapid release of GFs from the platelets.[14] An alternative system to delay the release of GF was reported by addition of calcium chloride, which resulted in formation of a dense fibrin matrix.[14] Soluble type I collagen has also been found to be effective in stimulating the release of PDGF and VEGF.[15] Further studies to evaluate cytokine-release kinetics according to various activation processes or methods of localization within a tissue (such as collagen scaffold) are required for the proper application of PRP in various patient indications.

The DS method showed higher concentrations of IL-1 and MMP-9 than the SS method, but these concentrations were similar or lower than those of the control samples. However, the MMP-9 concentration was about 10 times higher in the Biomet GPS (direct buffy coat extraction) than in the control. The MMP-9 concentration was strongly correlated with the WBC count, while the IL-9 concentration was not, and the results indicate ng/mL-level concentrations of MMP-9 in Biomet GPS and Prodizen Prosys and pg/mL-level concentrations in the SS, DS, and Arthrex ACP preparations. (The latter were similar to or lower than those of the control samples.) These catabolic cytokines may impede tissue healing depending on their concentration,[3] and the DS or Biomet GPS PRPs should be selected by considering the biological state of the injured tissue with proper patient indications.

Most of the cytokine release assays exhibited rather large standard deviations,[25,35] which made the comparison studies challenging. In this study, high interindividual variations in GF content were observed depending on the GF analyzed. More specifically, FGF showed the largest differences across donors, whereas the PDGF showed the smallest difference. The results are consistent with previous findings by Lacoste et al,[22] who also compared concentrations of PDGF, TGF-β1, bFGF, and VEGF in platelet concentrates. Some previous studies have normalized the GF concentration to platelet count.[32] But, as reported by us and others, these interindividual variations do not necessarily correlate with platelet count.[35] The cytokine release may be normalized with the respective cytokine's whole blood levels, but there is a wide variation in cytokine levels not only between individuals but also within each individual over time.[2] Furthermore, some cytokine levels in the whole blood were undetectable (too low) in some individuals, which prohibited normalization of the cytokine level with the respective whole blood level. Nonetheless, a previous study revealed that GF release patterns were very similar among most of the donors, although significant variations in cytokine concentration were observed between individuals.[25]

The limitations of this study are a result of the in vitro observations of cytokine release of the platelets. First, tissue healing and regeneration generally occur via a multiplicity of cellular interactions, but these processes were absent in the in vitro assay in the current study. In this regard, future studies should investigate the relationship between GF kinetics and tissue healing/regeneration in vivo. Second, the cytokine content is influenced mainly by interindividual variability, which in turn may influence the release kinetics of the PRP GFs. Given that humans in a healthy state have variable hematocrits, it is difficult to appreciate differences in the concentration of GF. In addition, there is no information how the different concentrations of GF will react in humans. Finally, although the preparation methods that were considered in this study are representative techniques, various other methods are also commercially available. However, not all PRP kits could be considered because of patient recruitment and financial limitations.

In conclusion, the DS method generally leads to a higher concentration of platelet when compared with the SS method, although the commercial preparations exhibited wide variations in their ability to concentrate platelets and leukocytes. Cytokine content was not necessarily proportional to the cellular composition of the PRPs as, depending on the type of cytokine, there were differences in greater content between the SS or DS method. Although there are high variations in serum cytokine levels among individuals, these observed differences in cytokine content could explain inconsistencies in the outcome of PRP usage in clinical or laboratory tests. Further in vivo study is warranted to evaluate how the different concentrations of GF will react in the human body. In addition, proper PRP components should be considered in future studies by taking into account both the molecular characteristics of PRP and patient indications.

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