

# Plasma-Based Autologous Blood Systems: Arthrex ACP®, MTF Cascade®, and Orthovita® CellPaker® and Vitagel™ Systems

Arthrex Research

## INTRODUCTION

Platelets respond to vascular injury by adhering, aggregating, activating, and degranulating at the injury site. Alpha granules within the platelets release a host of growth factors—such as platelet-derived growth factor AB (PDGF-AB)—which act as chemoattractants and mitogens. During activation, P-selectin becomes rapidly expressed on the platelet surface membrane, facilitating the formation of fibrin-based complexes that enhance platelet aggregation. Study 1 compares the cellular concentrations of different plasma-based autologous blood systems in their nonactivated states. Study 2 compares the release profiles of PDGF-AB and P-selectin from the fibrin matrices of each system following activation.

## RESULTS

### Study 1: Nonactivated Samples

## METHODS AND MATERIALS

### Study 1: Nonactivated Samples

Whole blood samples from five donors (N = 5) were collected using the appropriate ratio of anticoagulant to blood. Each sample was processed according to the manufacturer’s specifications (Table 1). Complete blood count (CBC) analysis was performed immediately after centrifugation and processing (for the MTF Cascade system, CBC was conducted after the first centrifugation step), as shown in Figure 1 and Table 2. Growth factor analysis was performed via ELISA following a single freeze/thaw cycle at -81 °C (R&D Systems, Inc.), as shown in Table 2. Platelet capture efficiency was calculated using the following equation: 
$$\left[ \frac{\text{Volume}_{(\text{PRP})} \times \text{Platelet Concentration}_{(\text{PRP})}}{\text{Volume}_{(\text{whole blood})} \times \text{Platelet Concentration}_{(\text{whole blood})}} \right]$$

**Table 1.** Protocols for tested plasma-based autologous blood systems.

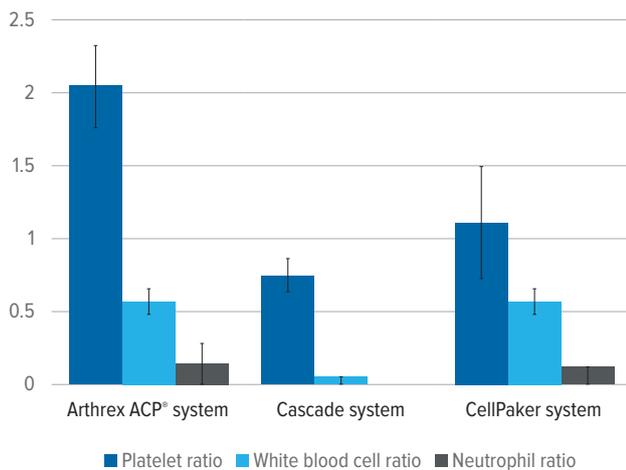
System, Company	Whole Blood Volume (mL)	First Spin Relative Centrifugal Force (RCF) (g)	Second Spin RCF (g)	First Spin Centrifugation Time (min)	Second Spin Centrifugation Time (min)
Arthrex ACP, Arthrex	11	350	-	5	-
Cascade, MTF	9	1100	1450	6	15
CellPaker, Orthovita	10	1530	-	2	-

**Table 2.** Platelet concentration, platelet ratios, PRP volume, platelet capture efficiency, PDGF-AB levels, and P-selectin levels of the plasma-based PRP systems (\* indicates statistically significant differences; one-way ANOVA, α = 0.05).

System, Company	Platelet Concentration ( $\times 10^3/\mu\text{L}$ )	Platelet Increase Over Whole Blood	Plasma Volume (mL)	Platelet Capture Efficiency	PDGF-AB (pg/mL)	P-Selectin (ng/mL)
Whole Blood	238 ± 38	-	-	-	-	-
Arthrex ACP, Arthrex	470 ± 45*	2.1 ± 0.2	3.7 ± 0.8	0.60 ± 0.10	26,259 ± 3061*	421 ± 52*
Cascade, MTF	136 ± 61	0.7 ± 0.1	4.1 ± 0.5	0.26 ± 0.12	5307 ± 3170	186 ± 66
CellPaker, Orthovita	221 ± 105	1.1 ± 0.4	4.9 ± 0.7	0.45 ± 0.20	8361 ± 5078	246 ± 114



**Figure 1.** CBC analysis of the plasma-based PRP systems.



## DISCUSSION

### Study 1: Nonactivated Samples

The Arthrex ACP system had significantly higher amounts of platelets, PDGF-AB, and P-selectin in the nonactivated state ( $P < .05$  for all comparisons). While the CellPaker system had the shortest centrifugation time and the Cascade system had the longest, both employed RFGs nearly three times greater than that of the Arthrex ACP system. This elevated g-force causes platelets to become more tightly packed and thus more difficult to isolate.

Although the Cascade system is FDA-cleared as a PRP system, it did not yield a platelet concentration above baseline when processed using its specific centrifuge and spin regime. The Orthovita Vitagel system, which is FDA-cleared as a surgical hemostat rather than a PRP system, uses high g-force to separate plasma containing fibrinogen, thus reducing platelet yield. The Arthrex ACP system uses a g-force optimized for maximum platelet collection within the plasma layer, while removing most red and white blood cells. The significantly higher PDGF-AB level obtained with the Arthrex ACP system correlates with the significantly higher platelet count relative to both baseline and the other systems tested.

## METHODS AND MATERIALS

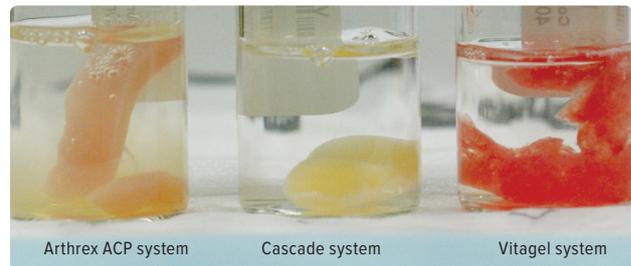
### Study 2: Activated Samples

The PRP samples described above ( $N = 5$ ) were activated according to each manufacturer’s specifications: the Cascade system was activated using calcium chloride ( $\text{CaCl}_2$ ), while the Orthovita system used its Vitagel kit, which contains collagen and thrombin. The Arthrex ACP system was activated using a  $\text{CaCl}_2$ /thrombin mixture based on a ratio published in peer-reviewed literature.<sup>1</sup>

The activated fibrin matrix was placed into a sterile 40 mL vial, and 10 mL of sterile phosphate-buffered saline (PBS) was added (Figure 2). After incubation at 37 °C for the specified durations, 2 mL aliquots of PBS were collected and cryopreserved at the following time points: 0 hours, 1 hour, 24 hours, 3 days, and 7 days.

Samples were frozen at  $-81\text{ }^\circ\text{C}$ , then thawed and analyzed via ELISA (R&D Systems, Inc.) (Figures 3 and 4). A one-way ANOVA was performed for all statistical comparisons, with a significance level of  $\alpha = 0.05$ .

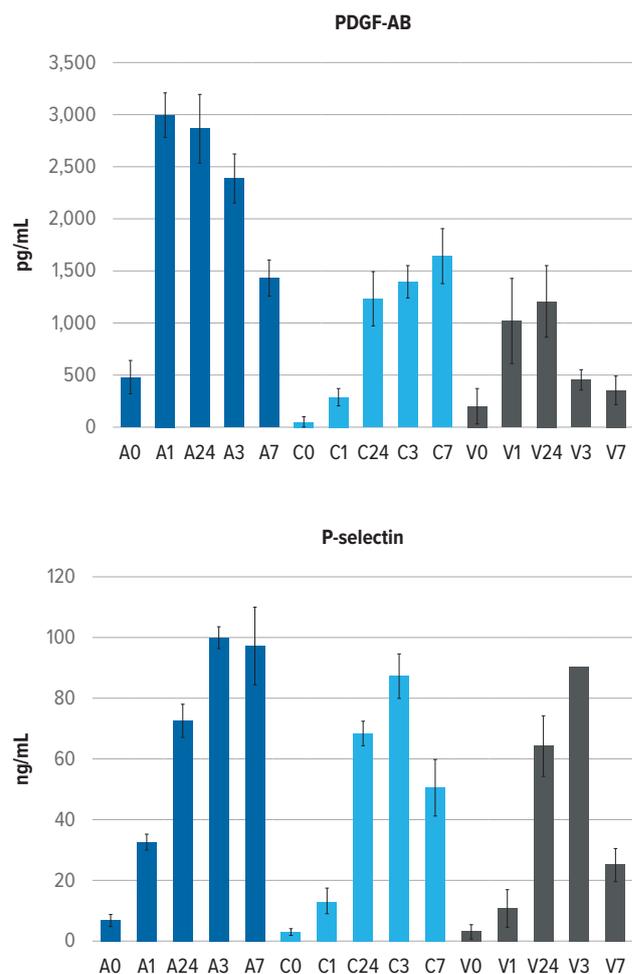
**Figure 2.** In vitro activated plasma-based PRP systems.



## RESULTS

### Study 2: Activated Sample—Time-Dependent Release

**Figure 3 and 4.** In vitro release profiles of PDGF-AB and P-selectin from activated plasma-based PRP systems over time. Systems include ACP (A), Cascade (C), and Vitagel (V). Time points are as follows: 0 = 0 hours, 1 = 1 hour, 24 = 24 hours, 3 = 3 days, and 7 = 7 days.



## DISCUSSION

### Study 2: Activated Sample—Time-Dependent Release

The Arthrex ACP® system demonstrated significantly higher levels of PDGF-AB at all measured time points except at day seven ( $P < .05$  for all earlier time points). PDGF-AB concentrations in the Cascade system showed an increase over time, whereas levels in the Arthrex ACP system decreased over time. By day seven, no statistically significant difference in PDGF-AB concentration was observed between the systems ( $P = .370$ ). The Vitagel system reached a maximum PDGF-AB release at 24 hours, followed by a decline. P-selectin concentrations over time indicated sustained platelet activation across all systems, with peak levels observed at three days. Due to the average platelet lifespan of approximately seven days, a decline in both growth factor release and platelet activation was expected after seven days.<sup>2</sup>

Previous literature on the Cascade system suggests that activation with  $\text{CaCl}_2$  alone (without thrombin) may be more favorable, as it may minimize initial platelet activation and promote a more gradual release. This study showed that the Arthrex ACP system, despite using thrombin in combination with  $\text{CaCl}_2$ , still achieved an increased PDGF-AB release compared to the other systems. This can in part be attributed to the Arthrex ACP system's superior initial platelet concentration. Additionally, this study indicates that many platelets within the fibrin matrix remain unactivated in the Arthrex ACP system used with  $\text{CaCl}_2$  and thrombin. These platelets undergo delayed activation over time, similar to the activation observed in matrices formed using  $\text{CaCl}_2$  and centrifugation alone. Overall, these findings demonstrate that all systems support platelet viability, activation, and growth factor release over a seven-day period of in vitro degradation, regardless of the activation method used ( $\text{CaCl}_2$ , thrombin, and/or collagen).

## SUMMARY

When choosing a PRP system, it is essential to pick a plasma-based system over a buffy coat-based system. Buffy coat systems typically contain elevated levels of WBCs (specifically neutrophils) and RBCs, which may negatively impact the healing potential of the treated tissue.<sup>3</sup> Once a plasma-based system is chosen, it is critical to understand what is being collected within the plasma used for each treatment. Compared to the other plasma-based autologous blood systems, the Arthrex ACP system demonstrated a higher platelet concentration and correspondingly greater release of growth factors, both in the nonactivated state and at three days post-activation. These findings support the conclusion that the Arthrex ACP PRP system is the ideal plasma-based autologous blood system when compared to other systems available.<sup>4</sup>

### References

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