

Plasma-based Autologous Blood Systems: Arthrex ACP[®], MTF Cascade[®], and Orthovita[®] CellPaker[®]/Vitagel[™]

Arthrex Research and Development

Introduction

Platelets respond to vascular injury by aggregating, adhering, activating and degranulating at the injury site. Alpha granules within the platelets release a host of growth factors (ie PDGF-AB) which act as chemoattractants and mitogenic agents. During activation, P-Selectin becomes rapidly expressed on the platelet surface membrane and, in turn, fibrin-based complexes form which can enhance platelet aggregation. The following studies not only compare the cellular concentrations of different plasma-based autologous blood systems (Study 1, nonactivated), but also compare the PDGF-AB and P-Selectin release profiles of the fibrin matrices of each respective system (Study 2, activated).

Methods and Materials (Study 1, Nonactivated Samples)

Whole blood samples from five donors (n=5) were collected using the appropriate ratio of anticoagulant. Each sample was processed per the manufacturer's specifications, Table 1. CBC Analysis was performed immediately after centrifugation and processing (for MTF Cascade, the CBC was taken after the first centrifugation step), Figure 1 and Table 2. Growth factor analysis was performed after a single -81°C freeze/thaw cycle via ELISA analysis, Table 2 (R&D Systems, Inc.). Platelet capture efficiency was determined from the following equation: $[\text{Volume}_{(\text{PRP})} \times \text{Platelet Concentration}_{(\text{PRP})}] / [\text{Volume}_{(\text{whole blood})} \times \text{Platelet Concentration}_{(\text{whole blood})}]$.

Results (Study 1, Nonactivated Samples)

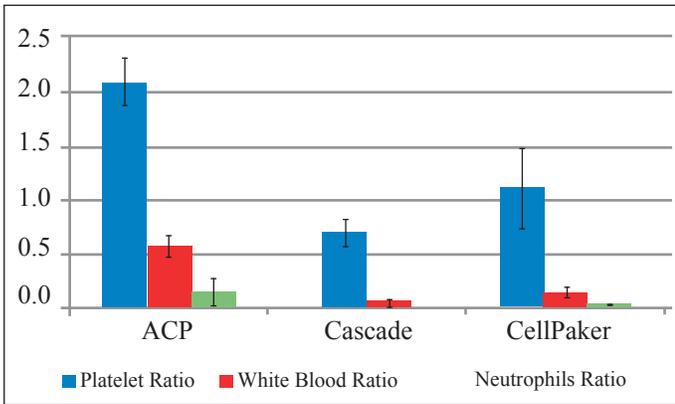
Table 1: Protocols for Tested Plasma-Based Autologous Blood Systems

System, Company	Whole Blood Volume (mL)	Centrifugal Force (RCF, g) First Spin	Centrifugal Force (RCF, g) Second Spin	Centrifugation Time (min) First Spin	Centrifugation Time (min) Second Spin
ACP, Arthrex	11	350	-	5	-
Cascade, MTF	9	1,100	1,450	6	15
CellPaker, Orthovita	10	1,530	-	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 (* denotes significantly different, one-way ANOVA, $\alpha=0.05$)

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 \pm 38	-	-	-	-	-
ACP, Arthrex	470 \pm 45*	2.1 \pm 0.2	3.7 \pm 0.8	0.60 \pm 0.10	26,259 \pm 3,061*	421 \pm 52*
Cascade, MTF	136 \pm 61	0.7 \pm 0.1	4.1 \pm 0.5	0.26 \pm 0.12	5,307 \pm 3,170	186 \pm 66
CellPaker, Orthovita	221 \pm 105	1.1 \pm 0.4	4.9 \pm 0.7	0.45 \pm 0.20	8,361 \pm 5,078	246 \pm 114

Figure 1: CBC Analysis of the plasma-based PRP Systems



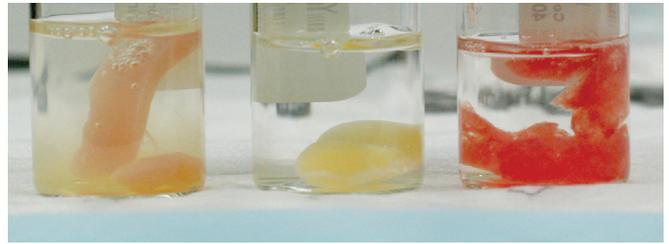
Discussion (Study 1, Nonactivated Samples)

The Arthrex ACP® system had significantly higher amounts of platelets, PDGF-AB, and P-Selectin in the non-activated state ($p < 0.05$ for all groups). While the CellPaker system had the shortest centrifugation time and the Cascade system had the longest, these two systems had an RCF that was almost three times higher than the ACP system. The elevated g-force causes platelets to become more tightly packed and thus more difficult to isolate. Cascade is FDA cleared as a PRP system, but a platelet concentration above baseline was not obtained using a Cascade-specific centrifuge and spin regime. Orthovita Vitagel is not FDA cleared as a PRP system and is instead cleared as a surgical hemostat. Vitagel utilizes an elevated g-force to facilitate separation of plasma with fibrinogen; by doing this, it decreases potential yield of platelets. ACP utilizes a g-force that allows for maximum collection of platelets with a plasma layer, while removing most of the RBCs and WBCs. The significantly higher level of PDGF-AB within ACP correlates to the significantly higher number of platelets when compared to baseline and the other groups tested.

Methods and Materials (Study 2, Activated Samples):

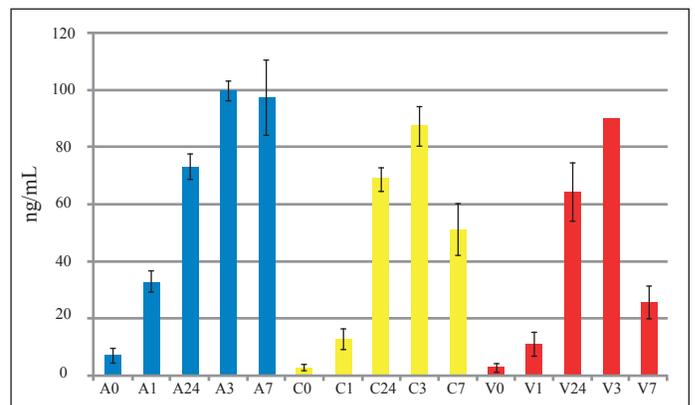
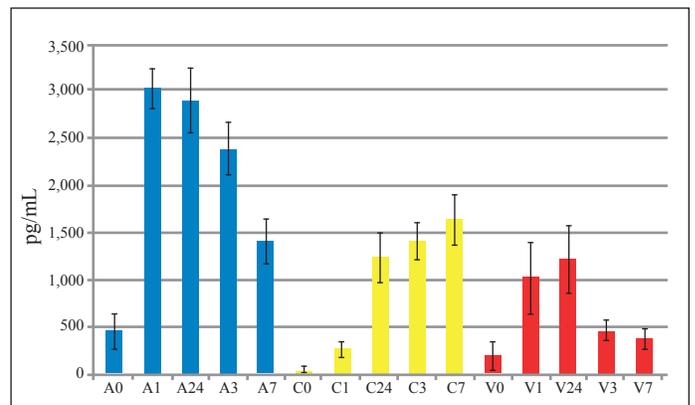
The PRP samples from above ($n=5$) were activated per each manufacturer’s specifications: Cascade uses CaCl_2 and Orthovita uses their Vitagel kit (collagen/thrombin). ACP was activated with CaCl_2 /thrombin according to a ratio published in peer reviewed literature¹. The activated fibrin matrix was then placed into a sterile 40 mL vial and 10 mLs of sterile phosphate buffered saline (PBS) was added, Figure 2. After the respective 37°C incubation times, 2 mL aliquots of PBS were withdrawn and cryofrozen at time zero, one hour, 24 hours, three days, and seven days. After freezing at -81°C, the samples were thawed and ELISA analysis was performed, Figure 3 and 4 (R&D Systems, Inc.). For statistical analysis, a one-way ANOVA, $\alpha=0.05$, was used for all comparisons.

Figure 2: Activated plasma-based PRP Systems *in vitro* (left to right: ACP, Cascade, Vitagel)



Results (Study 2, Activated Sample, Time Release)

Figure 3 and 4: PDGF-AB (left) and P-Selectin (right) *in vitro* release profile of the activated plasma-based PRP Systems (A=ACP, C=Cascade, V=Vitagel; 0=time zero, 1=1 hr, 24=24 hrs, 3=3 days, 7=7days)



Discussion (Study 2, Activated Sample, Time-Release)

The Arthrex ACP system had significantly higher amounts of PDGF-AB at every respective time point except at seven days ($p < 0.05$ for all time points). PDGF-AB concentrations trended toward increasing over time with Cascade, while it decreased over time in ACP. However, there was no difference in PDGF-AB concentrations at day seven ($p=0.370$). Vitagel reached a maximum release after 24 hours then dropped over time. P-Selectin concentrations, over time, indicate that all systems had continued platelet activation through seven days, all of the systems reaching a maximum at three days. After

seven days, it is intuitive to expect growth factor release and platelet activation to drop for all groups since the lifespan of a platelet, on average, is seven days². Within literature published for Cascade, it has been discussed that the use of CaCl₂ alone without thrombin is more optimal due to less initial activation of the platelets, and instead, a theoretical release over time occurs. This study illustrated how ACP still has increased PDGF release over time with the use of thrombin when compared to the other systems. One feature causing this statistical difference is the fact that ACP is able to concentrate more platelets initially than the other two systems. This study also indicated that many platelets remain unactivated in the fibrin matrix created by combining ACP with CaCl₂/thrombin. These platelets in turn become activated over time in a similar fashion to a fibrin matrix being created by CaCl₂ and centrifugation. These results illustrate that all systems show platelet viability, activation and growth factor release over a seven-day span of *in vitro* degradation regardless of the use of CaCl₂, thrombin, and/or collagen.

Summary

When choosing a PRP system, it is vitally important to pick a plasma-based system over a buffy-coat based system. The buffy-coat system will include increased levels of WBCs (specifically Neutrophils) and RBCs which have the potential of decreasing the healing potential of the tissue being treated³. Once choosing a plasma-based system, it is critical to understand what is being collected within the plasma being used for each treatment. Compared to the other plasma-based autologous blood systems, ACP has a higher platelet concentration with a correspondingly higher release of growth factors in the nonactivated state and after three days in the activated state. This work helps to identify why the Arthrex ACP[®] PRP system is the ideal plasma-based autologous blood system when compared to other systems available.⁴

References:

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2. Marx, RE. Platelet-rich plasma: Evidence to support its use. *J Oral Maxillofacial Surg.* 62 204:489-496.
3. Martin P., Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends Cell Biol.* 2005; 15(11):599-607.
4. Karakaplan, M., Elmalij, N., Mirel, E., Sahin, N., Ergen, E., Elmalij, C. Effect of microfracture and autologous-conditioned plasma application in the focal full-thickness chondral defect of the knee: an experimental study on rabbits. *J Orthop Surg Res.* 2015. doi: 10.1186/s13018-015-0254-0.

