

# Angel<sup>®</sup> vs Magellan<sup>®</sup>: A Comparative Study on Platelet Concentration and Activation

Arthrex Research and Development

## Introduction

When processing whole blood to create a platelet rich plasma (PRP), platelets may become activated and degranulate. Once a platelet is activated by collagen exposure or inflammatory factors, alpha granules are stimulated and express growth factors (Bajorath). This degranulation and subsequent lysing of the alpha granules is synonymous with platelet activation. Generally it is believed that PRP systems should minimize the amount of platelet activation so platelets can “naturally” activate in vivo. The release of growth factors is the first step in beginning the healing process (Keast).

The Arthrex Angel System™ (Arthrex, Naples, Florida) and MAGELLAN<sup>®</sup> Autologous Platelet Separator System (Arteriocyte, Hopkinton, Massachusetts) are two systems that create platelet rich plasma from a whole blood sample. The following study compared the minimized red blood cell setting of the Magellan (Min RBC) to the 2% hematocrit setting of the Angel, and standard Magellan to the 7% hematocrit setting of the Angel<sup>®</sup>.

## Methods

A total of five donors were used for this study. Whole blood was drawn into 60 mL syringes with 8 mL of ACD-A then gently inverted to ensure thorough mixing of blood and anticoagulant. From each donor, 2 mL of anticoagulated whole blood (WBA) was aliquoted into labeled cryovials. The WBA sample was analyzed via Sysmex XE-5000 hematology analyzer and these values were used as baseline measurements.

### *Angel Procedure*

An Angel disposable set was loaded onto the Angel machine. For each donor, 58 mL of WBA was processed at a 2% Hematocrit setting and an additional 58 mL of WBA was processed at a 7% Hematocrit setting using the same disposable. After processing was complete, the raw volume output of PRP was observed and recorded. Samples were analyzed using Sysmex XE-5000 hematology analyzer and the PRP sample was allowed to gravimetrically separate at room temperature. After separation, a “cell free” sample of PRP was aliquotted for real time ELISA. The remaining portion of the sample was frozen for eventual growth factor analysis via ELISA.

### *Magellan Procedure*

A Magellan disposable was loaded onto the Magellan machine. For each donor, 58 mL of WBA was processed at the normal setting and an additional 58 mL of WBA was processed at MIN RBC setting using the same disposable for both. PRP output was programmed to 3 mL and the PPP button was selected for PPP output. After processing, the samples

were analyzed using Sysmex XE-5000 hematology analyzer then allowed to gravimetrically separate at room temperature. After separation, a “cell free” sample of PRP was aliquotted for real time ELISA. The remaining portion of the sample was frozen for growth factor analysis.

### *ELISA*

A sample of PRP from Angel 2%, Angel 7%, Magellan standard, and Magellan MIN RBC were pipetted into a separate cryovials and diluted 20-fold for real-time ELISA. The remaining portion of the samples was frozen at -81°C. After 24 hours, frozen samples were thawed and the ELISA preparation and testing process was repeated. The freeze/thaw process is assumed to fully activate all of the platelets due to lysing of the alpha granules. All ELISAs were performed according to R&D Systems Human sP-Selectin/CD62P Immunoassay kit. The real time sample values were compared to the freeze-thaw sample values in order to obtain a percentage of platelet activation that was present immediately after processing.

## Results

### *PRP*

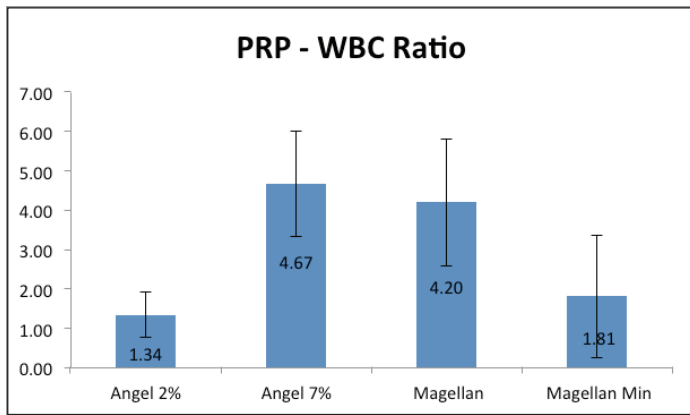
For all systems, the white blood cell concentration, red blood cell concentration, hematocrit percentage, platelet concentration, and neutrophil concentration were recorded for whole blood and the output PRP and PPP. The PRP concentrations were divided by the whole blood concentrations to obtain a ratio which will be referred to in the accompanying graphs.

The PRP output of the Magellan was set to 3 ml. The raw, undiluted PRP output for all Angel settings used averaged 3 mL for both settings.

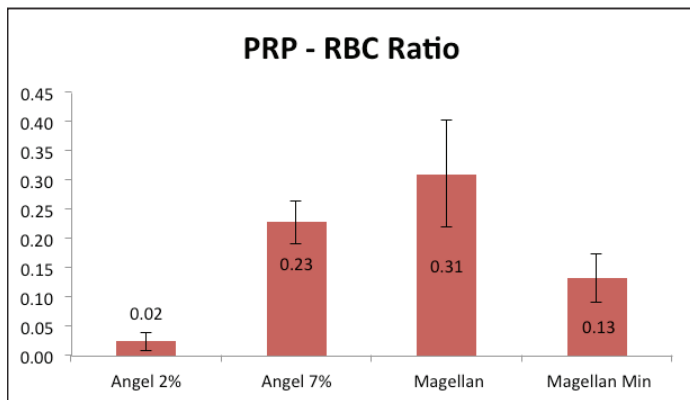
Angel 2% produced platelet rich plasma with a platelet ratio of approximately 9x over baseline. Angel 7% produced platelet rich plasma with a platelet ratio of approximately 13x over baseline. The Magellan standard regime produced a PRP with a platelet concentration 12x over baseline while the Magellan MIN RBC setting produced a PRP with a platelet concentration 4x over baseline. Angel 2% was compared to Magellan MIN RBC setting. Angel 2% had a significantly lower RBC ratio ( $p < 0.001$ ), a lower percentage of hematocrit ( $p = 0.008$ ), and a higher platelet ratio in the platelet rich plasma ( $p < 0.001$ ).

Angel 7% was compared to Magellan standard regime. There were no significant differences in white blood cell ratio ( $p = 0.628$ ), red blood cell ratio ( $p = 0.096$ ), percentage hematocrit ( $p = 0.097$ ), platelet ratio ( $p = 0.628$ ), or neutrophil ratio ( $p = 0.545$ ). See Figures 1 to 5 for all data.

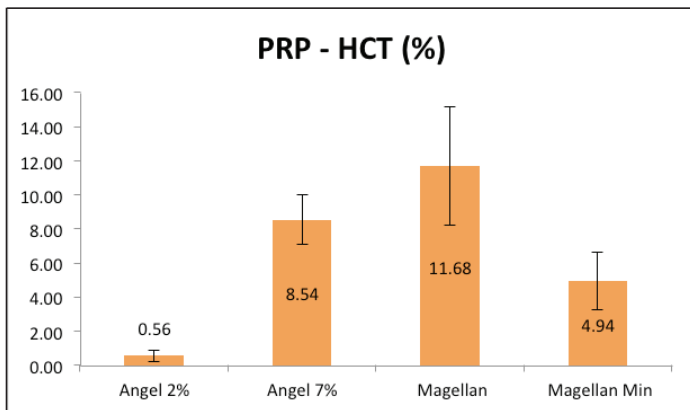
**Figure 1:** The ratio of white blood cells in PRP as compared to baseline.



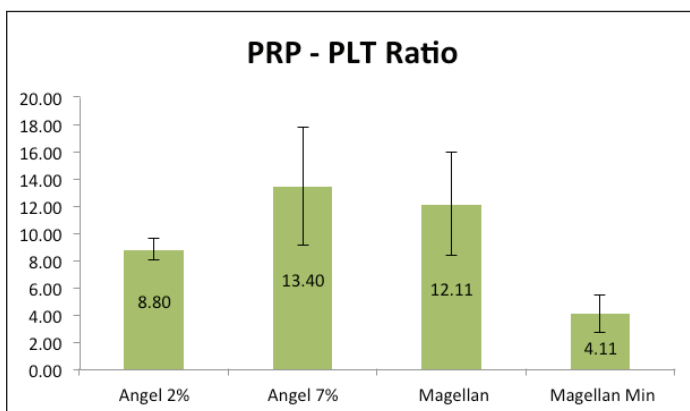
**Figure 2:** The ratio of red blood cells in PRP as compared to baseline.



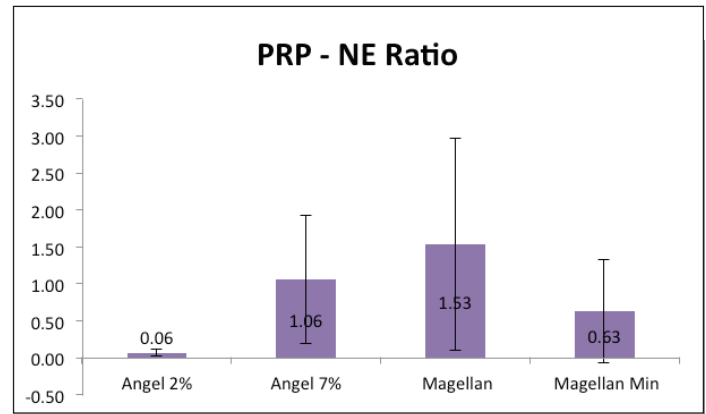
**Figure 3:** The ratio of hematocrit percentage in PRP as compared to baseline.



**Figure 4:** The ratio of platelets in PRP as compared to baseline.



**Figure 5:** The ratio of neutrophils in PRP as compared to baseline.



### Platelet Activation

P-selectin is an adhesion molecule that can be found inside platelets. When a platelet is activated, by exposure to collagen or other inflammatory factors, P-selectin flips to the outside surface of the platelet and recruits more platelets for in-vivo coagulation (Bajorath). Soluble P-Selectin (sP-Selectin) that is present in plasma can be quantified by ELISA testing (Blann). It is believed that minimizing platelet activation allows for growth factors to be preserved until utilized at the point of care (Strelchik).

There was no statistically significant difference in platelet activation between the Angel 2% hematocrit setting group and the Magellan® minimum RBC group. The Angel® 7% hematocrit setting group and the Magellan standard setting also did not demonstrate a statistically significant difference in platelet activation. ( $p = 0.256$ ). See Table 2 for results.

**Table 1:** Cellular ratios of PRP. The cellular ratios of Angel and Magellan PRP were compared using t-tests with an  $\alpha$  of 0.05.

|           | Angel 7% vs Magellan     | Angel 2% vs Magellan MIN RBC |
|-----------|--------------------------|------------------------------|
| Type      | Significantly different? | Significantly different?     |
| WBC Ratio | No, $p = 0.628$          | No, $p = 0.540$              |
| RBC Ratio | No, $p = 0.096$          | Yes, $p < 0.001$             |
| HCT %     | No, $p = 0.097$          | Yes, $p = 0.008$             |
| PLT Ratio | No, $p = 0.628$          | Yes, $p < 0.001$             |
| NE Ratio  | No, $p = 0.545$          | No, $p = 0.105$              |

**Table 2:** Average platelet activation of PRP. Separate ELISAs were used to test the PRP samples immediately after processing and following 24 hours freezing at  $-81^{\circ}\text{C}$ . The values from immediate were compared to values after freezing, assumed to be 100% activation, to yield a percentage. The results below show the average amount of platelets that were activated in the PRP.

| Sample                | Average Platelet Activation |
|-----------------------|-----------------------------|
| Magellan Min RBC PRP  | 25% $\pm$ 6.4%              |
| Magellan Standard PRP | 19% $\pm$ 2.2%              |
| Angel 2% HCT PRP      | 25% $\pm$ 7.8%              |
| Angel 7% HCT PRP      | 21% $\pm$ 5.1%              |

\*None of the groups were statistically different.

## Discussion

### PRP

All systems were able to produce plasma that was rich in platelets. Angel® 2% was significantly higher than Magellan® Min RBC in platelet concentration. While not statistically significant the Angel 7% hematocrit group did average a higher platelet count than the Magellan standard setting group. In regards to other plasma constituents, Angel 2% PRP was also able to significantly decrease red blood cells in the final output as compared to Magellan Min RBC. An excess of red blood cells triggers a pro-inflammatory response which in turn attracts more leukocytes (Lundvig) and can also exacerbate, rather than ameliorate, disease and tissue damage (Larsen). High concentration of free heme may even lead to atherosclerosis (Lundvig).

The standard Magellan spin for PRP can produce an output of 3-10 mL. At the 7% Angel setting, average raw output was 3mL. It should be noted that the Angel hematocrit level can be adjusted to user specifications. In addition, Angel raw PRP output volume can be expanded to a higher volume by diluting with viable PPP by pulling directly on the PRP syringe while it is still attached to the machine.

### Platelet Activation

Platelet activation was not significantly different between any Magellan and Angel groups tested. Neither the Angel nor Magellan produced enough inflammatory parameters to fully activate platelets. This demonstrates that the Angel roller pump does not cause any more cell damage, degranulation of the alpha granules within the platelets, than the Magellan centrifugal separation kit.

A previous study using canine blood also confirmed that the Angel system produces a PRP with minimally activated platelets (Strelchik). Growth factors such as P-selectin have a short half-life; therefore, using a PRP with minimally activated platelets may preserve the total amount of growth factors delivered upon injection (Strelchik).

The question has been raised that roller pumps may cause cell damage due to mechanical force. Roller pumps are commonly used in adult and pediatric cardiopulmonary bypass procedures. Moreover, roller pumps are standard equipment in many life support devices (Wang). The Angel roller pump and disposable kit is non-occlusive which allows for blood to be pulled into the centrifuge cassette without causing hemolysis.

### Summary

The Arthrex Angel System™ and the Arteriocyte Magellan were both able to effectively concentrate platelets to produce a plasma rich in platelets. Angel 2% hematocrit setting group had significantly lower amount of red blood cells and a significantly higher amount of platelets in the raw PRP output as compared to Magellan Min RBC. The Angel 7% hematocrit setting group and Magellan standard group were not significantly different in any cellular comparisons. The Arthrex Angel groups and the Arteriocyte Magellan groups were not significantly different in terms of the percentage of

platelet activation found within the PRP produced. The findings of this comparative study support the following claims: that the Arthrex Angel produces a PRP with minimal platelet activation, that the Arthrex Angel System can produce a superior RBC, leukocyte reduced PRP than the Arteriocyte Magellan and that the Arthrex Angel can duplicate the output of other commercially available PRP systems if desired.

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