

Comparison of Angel® System and ART PRP and BMC Systems: Cellular Concentration

Arthrex Research

OBJECTIVE

This study analyzed the outputs of the Angel system (Arthrex) and ART systems (Celling Biosciences). Angel platelet-rich plasma (PRP) was compared to ART PRP, and Angel concentrated PRP (cPRP) from bone marrow aspirate (BMA) was compared to ART bone marrow concentrate (BMC). The differences in cellular concentration and fold change between the systems were evaluated.

MATERIALS AND METHODS

Blood Collection

Blood was collected from 6 donors (N = 6) and citrate dextrose solution A (ACD-A) was used as the anticoagulant according to the manufacturer's recommended ratio (13.3% vol/vol for the Angel system, 10% vol/vol for the Celling systems). A total of 120 mL of anticoagulated blood was drawn from each donor into 2 syringes preloaded with ACD-A via a standard arm venipuncture. A small aliquot of anticoagulated whole blood assay (WBA) from each syringe was reserved for baseline analysis. The WBA was then processed in both systems based on each manufacturer's instructions.

Bone Marrow

Heparinized fresh human BMA was obtained from the ilium of 6 donors (N = 6) through commercial vendors (AllCells or CGT Global) with volumes ranging from 92 to 110 mL. Samples were processed within 24 hours of harvest. A small aliquot was reserved for baseline analysis, and the remaining BMA was evenly divided and processed using each system according to manufacturer protocols.

Processing Times

For both blood and bone marrow, the Angel system used automated centrifugation settings with processing times ranging 16.5 to 17 minutes, depending on input volume. Hematocrit settings were set at 7% for blood and 15% for bone marrow. The ART system was spun at 3200 rpm for 15 minutes, and product collection was conducted based on the location of the buffy coat per manufacturer's standard operating procedure (SOP) (Figure 1).

Sample Analysis

Baseline, PRP, Angel cPRP from BMA, and ART BMC products were analyzed for specific cell concentrations using a Sysmex XE-5000 hematology analyzer. Concentrations of white blood cells (WBCs), red blood cells (RBCs), platelets (PLTs), neutrophils (NEs), lymphocytes (LYMPHS), monocytes (MONOs), and hematopoietic progenitor cells (HPCs; BMA samples only) were analyzed. To estimate mesenchymal stem cell levels for all BMA samples, 1 million total nucleated cells were cultured in triplicate for 10 days and fibroblast colony-forming units (CFU-Fs >50 cells) were stained with crystal violet and counted using standard methods.^{1,2} Statistical differences between devices were determined using paired t-tests with a significance level of $\alpha = .05$.



Figure 1: Process for extracting ART PRP (top) and BMC (bottom).



01
Inject WBA



02
Centrifuge device



03
Extract platelet-rich plasma (PPP)



04
Extract PRP



05
Inject BMA



06
Centrifuge device



07
Extract PPP



08
Extract BMC

RESULTS

For PRP preparation, the average input volume for both devices was 58.7 ± 1.6 mL WBA. The ART system produced an average true volume (TV) of 3.7 ± 0.4 mL, and the Angel® system produced an average TV of 2.9 ± 0.9 mL. This difference was not statistically significant ($P = .13$). A matched volume (MV) calculation of the cellular components was performed for each PRP device by matching all individual donor volumes to the highest volume obtained (4.2 mL) using PPP.

For bone marrow-derived products, the average input volume for both devices was 49.9 ± 3.5 mL BMA. The ART system produced an average TV of 5.3 ± 1.5 mL ART BMC, and the Angel system produced an average TV of 2.6 ± 0.7 mL cPRP from BMA. This difference was statistically significant ($P = .014$). An MV calculation was also performed by matching all individual donor volumes to the highest volume obtained (6.8 mL) using PPP.

The matched volume calculation was defined as follows:

$$[\text{PRP or cPRP}_{\text{BMA}} \text{ MV}] = \frac{\text{TV} \cdot [\text{PRP or cPRP}_{\text{BMA TV}}] + (\text{MV} - \text{TV}) \cdot [\text{PPP}]}{\text{MV}}$$

Note: In the calculation for the ART system, cPRP from BMA was replaced with ART BMC.

Tables 1 and 2 and Figure 2 depict the cellular fold changes of the true and matched PRP and cPRP from BMA products when compared to WBA and BMA inputs on a donor-by-donor basis. The fold change is calculated as: fold change = [PRP or cPRP_{BMA}]/[WBA or BMA].

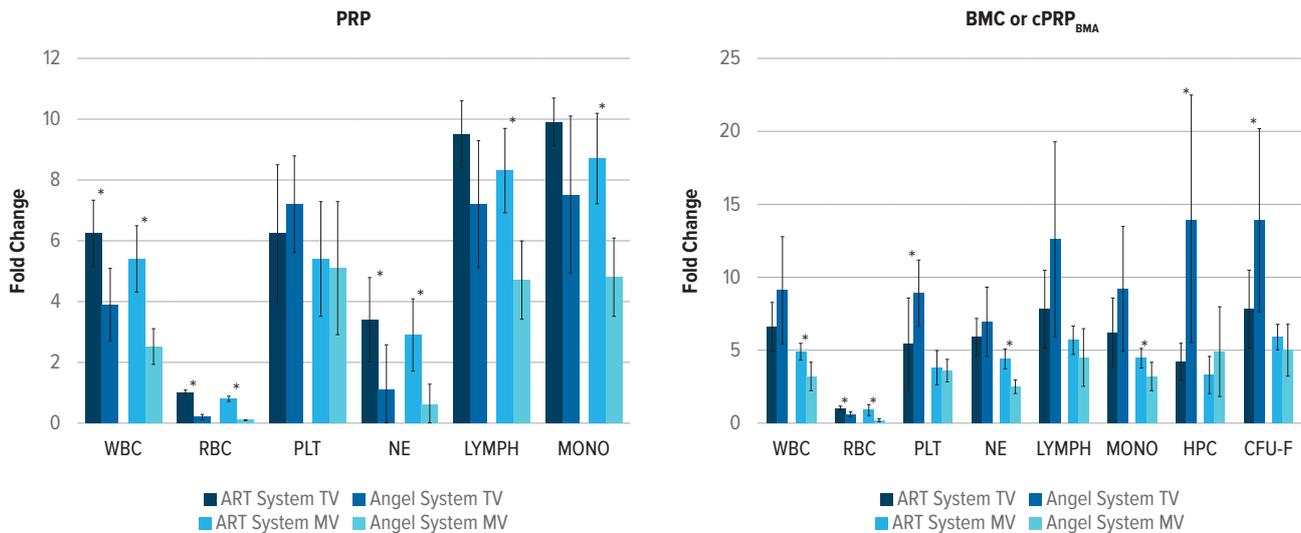
Table 1: Average cellular fold changes, with standard deviation, of PRP compared to baseline WBA.

		WBC	RBC	PLT	NE	LYMPH	MONO
True Volume	ART PRP	6.2 ± 1.1	1.0 ± 0.1	6.2 ± 2.3	3.4 ± 1.4	9.5 ± 1.1	9.9 ± 0.8
	Angel® PRP	3.9 ± 1.2	0.2 ± 0.1	7.2 ± 1.6	1.1 ± 1.5	7.2 ± 2.1	7.5 ± 2.6
Matched Volume	ART PRP	5.4 ± 1.1	0.8 ± 0.1	5.4 ± 1.9	2.9 ± 1.2	8.3 ± 1.4	8.7 ± 1.5
	Angel PRP	2.5 ± 0.6	0.1 ± 0.03	5.1 ± 2.2	0.6 ± 0.7	4.7 ± 1.3	4.8 ± 1.3

Table 2: Average cellular fold changes, with standard deviation, of ART BMC and cPRP from BMA compared to baseline BMA.

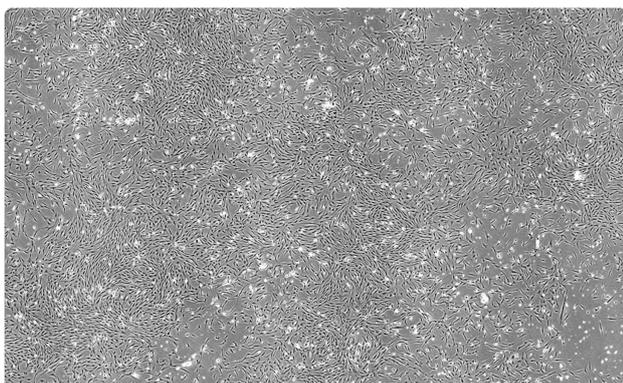
		WBC	RBC	PLT	NE	LYMPH	MONO	HPC	CFU-F
True Volume	ART BMC	6.6 ± 1.7	1.0 ± 0.2	5.4 ± 3.2	5.9 ± 1.3	7.8 ± 2.7	6.2 ± 2.4	4.2 ± 1.3	7.8 ± 2.7
	Angel cPRP _{BMA}	9.1 ± 3.7	0.6 ± 0.2	8.9 ± 2.3	6.9 ± 2.4	12.6 ± 6.7	9.2 ± 4.3	13.9 ± 8.5	13.9 ± 6.3
Matched Volume	ART BMC	4.9 ± 0.6	0.9 ± 0.4	3.8 ± 1.2	4.4 ± 0.7	5.7 ± 1.0	4.5 ± 0.7	3.3 ± 1.3	5.9 ± 0.9
	Angel cPRP _{BMA}	3.2 ± 1.0	0.2 ± 0.05	3.6 ± 0.8	2.5 ± 0.5	4.5 ± 2.0	3.2 ± 1.0	4.9 ± 3.1	5.0 ± 1.8

Figure 2: Cellular fold changes of each system.

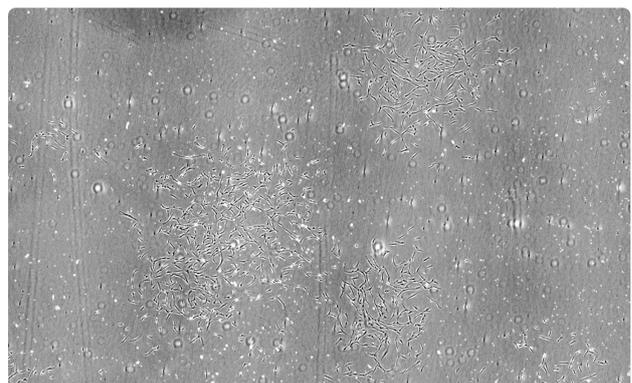


*Indicates a statistically significant difference between the devices.

Figure 3: Relative mesenchymal stem cell (MSC) density after 1 week of culture when equal volumes of Angel cPRP from BMA and ART BMC were cultured.



Angel System cPRP from BMA at 1 week



ART BMC at 1 week

DISCUSSION

Both systems require approximately the same processing time. The ART devices require manual manipulation to collect the product, whereas the Angel® system is fully automated, contributing to the ease of sample processing. The variability in the end product volume produced by the Angel system is based on the cell concentration detected by the sensor and the selected hematocrit setting. The Angel system produced more concentrated products than the ART devices. This high concentration allows the clinician to either deliver a concentrated end product or expand the treatment volume with PPP, which is beneficial when treating sites with volume limitations.

The PRP produced by the Angel system had a 7.2× increase in platelet concentration, compared to a 6.2× increase with the ART devices. Additionally, the Angel system PRP had significantly lower RBC, WBC, and NE fold changes than the ART system PRP, both in true and matched volumes. Matching the volume further reduced the WBC concentration in the Angel system PRP, which is significant because increased levels of WBCs (specifically NEs) and RBCs can potentially decrease healing potential.³

The cPRP from BMA prepared by the Angel system contained significantly higher concentrations of PLTs, HPCs, and CFU-Fs compared to the ART BMC system (Figure 3). The Angel system also had significantly lower RBCs in the final cPRP from BMA product compared to the ART system. The matched volume of the Angel system cPRP from BMA showed significantly decreased WBCs (specifically NE and MONO) compared to the ART BMC. MSCs were also more enriched, as demonstrated by the CFU-F frequency among total nucleated cells ($0.005\% \pm 0.002\%$ vs $0.004\% \pm 0.002\%$, $P = .008$) with the Angel system. This is because the ART device captures all WBCs, including heavier granulocytes and neutrophils, whereas the Angel system minimizes the collection of these cells. An ideal cPRP from BMA system would both concentrate and enrich progenitor cells (MSCs or HPCs) in the product.¹ The Angel system was found to be superior to the ART systems in concentrating and enriching whole blood and BMA samples.

References

1. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. *J Bone Joint Surg Am.* 2005;87(7):1430-1437. doi:10.2106/JBJS.D.02215.
2. Ciapetti G, Ambrosio L, Marletta G, Baldini N, Giunti A. Human bone marrow stromal cells: in vitro expansion and differentiation for bone engineering. *Biomaterials.* 2006;27(36):6150-6160. doi:10.1016/j.biomaterials.2006.08.025.
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. doi:10.1016/j.tcb.2005.09.002.