Report 515
Research Study

Comparisons of EmCyte *PurePRP® II 2015,* Harvest/Terumo *APC60,/Clear PRP,* and Arthrex *Angel PRP Products.*

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Executive Summary

There is market pressure for a PRP product with reduced red blood cell contamination, especially in aesthetic and cosmetic procedures and in sports medicine to reduce potential complications in joint treatment. Reduced granulocyte levels may also be desirable. While granulocytes are helpful in wound debridement and preventing infection, high granulocyte levels may be inflammatory.

This study evaluated the PRP products from three platforms: PurePRP® II 2015 (EmCyte Corporation), Clear PRP (Harvest/Terumo bct) and Angel system (Arthrex). PurePRP® II 2015 and Clear PRP are red cell reduction methods while Angel has a programmable setting to control RBC level in the product. The study is a paired sample design, with each donor tested on all three platforms.

Results: The PurePRP® II 2015 device produced a reduced Red Blood Cells PRP product with, on average, to 100 x 10^6 RCB/ml and an average hematocrit of 1.1%. Only 2% of the granulocytes were retained, a reduction of 84% from the baseline whole blood values. The PurePRP® II 2015 products had higher cell concentration and calculated cell metrics including platelet yield and concentration, RBC, mononuclear and granulocyte cell recoveries than either the Clear PRP or Angel products. The average concentrations for all growth factors measured were higher in PurePRP® II 2015 products compared to Clear PRP and Angel products.

Both red cell reduction platforms had similar processing times (24 min) and the number of aseptic entries (6). Only the PurePRP® II 2015 platform was capable of providing a PRP product with an optimum platelet concentration of > 1 x 10^6 platelets per µL (Giusti I, Rughetti A, D'Ascenzo S, et al. Identification of an optimal concentration of platelet gel for promoting angiogenesis in human endothelial cells. Transfusion 2009;49:771-8. Marx R, Garg A. Dental and craniofacial applications of platelet rich plasma. Carol Stream: Quintessence Publishing Co, Inc.; 2005

Table of Contents

1. Introduction

The objective of this study was to evaluate parameters associated with the platelet concentrates (PRP) produced by three commercially successful PRP systems. The Emcyte PurePRP® II 2015 system, Harvest/Terumo Clear PRP device, and the Arthrex Angel system were evaluated with paired samples from seven normal donors.

2. Study Design

This was a single center study conducted by BioSciences Research Associates, Inc. (BSR). BSR provides custom contract research and laboratory services for product development, medical device testing and clinical trials support to Pharmaceutical and Biotechnology companies. All studies were conducted within BSR's cGXP Quality Systems. BSR has extensive experience with development and evaluation of platelet concentration devices and product evaluation, including support for FDA CBER and CDRH filings.

Up to 160 ml of human whole blood was obtained from each of 7 donors following informed consent. The informed consent forms, as well as blood collection protocols were approved by the New England Institutional Review Board Protocol number 04-144 "The Collection of Whole Blood for Research Purposes". Donors met the requirements of the American Association of Blood Banks (AABB) and the FDA CBER. There were no specific exclusion specifications, other that the donor be healthy. There was no selection for age, sex or ethnicity. Donors were referenced only by assigned code numbers. Blood was drawn into a 60cc syringe that had been preloaded with anticoagulant according to Table I. An ETDA tube was drawn for baseline comparison.

Table I. Anticoagulant Protocol

Platform	Anticoagulant	Blood
Emcyte PurePRP® II 2015	10 ml Na Citrate	50 ml
Harvest Clear PRP	6 ml ACD-A	54 ml
Arthrex Angel	8 ml ACD-A	52 ml

PurePRP® II 2015 product was produced from 60 ml of Na Citrate anticoagulated blood samples according to manufacturer's instructions for use with a modified "Protocol A": Following the first centrifugation, the platelet plasma layer was withdrawn until the aspiration tubing filled with RBC. The recovered platelet plasma was transferred to the concentration disposable along with 5ml of ACD-A. After centrifugation, all but 7 ml of the plasma was removed, and approximately 7 ml of PRP recovered. For the Harvest/Terumo APC60 devices, 60 ml ACD-Blood samples were processed according to manufacturer's instructions for use to produce approximately 10 ml of platelet concentrate, which was further processed with the LP-10 Clear PRP Procedure Kit, to produce approximately 7 ml of product. The reduced red cell PRP was harvested without disturbing the RBC/Buffy interface. The Angel system processed 60 ml of anticoagulated blood with a Hct setting of 7% and the product adjusted with PPP to a volume of 7 ml.

3. Study Objectives and Outcome Measures

The analytical parameters chosen to identify differences or similarities among the three platelet concentrating platforms were:

3.1. *Platelet Concentration Factor*

Complete blood counts (CBCs) were performed using a 3-part differential hematology analyzer to quantify the platelets contained within the start sample and platelet concentrates. The platelet concentration factor, which is the ratio of the concentration of platelets in the platelet concentrate product to the concentration of platelets in the start sample (adjusted for dilution with anticoagulant), was determined for each device. CBC was tested according to BSR TM-076 Coulter Ac-T diff 2 Hematology Analyzer.

3.2. *Platelet Yield*

CBC were performed using a hematology analyzer to quantify the platelets contained within start sample and platelet concentrates. The platelet yield, which is the ratio of the number of platelets in the platelet concentrate product to the number of platelets in the start sample, was determined for each device.

3.3. *pH*

Sample pH was measured in platelet concentrates. The testing was conducted on a blood gas analyzer according to SOP: TM-018 Blood pH.

3.4. *Leukocyte, Erythrocyte and Platelet Counts*

CBC was performed using a hematology analyzer for start sample and platelet concentrates. The Leukocyte, Platelet counts, Erythrocyte (RBC), and calculated hematocrit (hct) were recorded for each sample. CBC was tested according to BSR TM-076 Coulter Ac-T diff 2 Hematology Analyzer.

3.5 *Growth Factors*

PRP samples were treated with bovine thrombin reconstituted in 10% $CaCl_2$. The serum is collected by centrifugation. Growth factors (PDGF AB, TGF-β, SDF-1α, and VEGF) were measured by ELISA (R&D Systems)

5. Statistical Methods

Data tables and descriptive statistics are shown for each parameter.

5.1 Platelet Concentration Factor

The platelet concentration factor (PCF) was derived as the ratio of the platelet count in the platelet concentrate (PC) to the platelet count in baseline sample (adjusted for dilution with anticoagulant) (BL) :

$$PCF = PC/BL$$

Results are summarized in tables showing observations by donor, mean platelet concentration factor and standard deviation for each device.

5.2 Platelet Yield

The platelet yield (PY) was derived as the ratio of the platelet count in the platelet concentrate (PC) times the volume of the platelet concentrate (VPC) to the platelet count in the baseline sample (adjusted for dilution with anticoagulant) (BL) times the volume of the sample processed (VBL):

$$PY = (PC*VPC) / (BL*VBL)$$

Results are summarized in tables showing observations per donor, mean platelet yield and standard deviation for each device.
A two tailed, paired t-Test was used to compare the mean PLT yield for Clear PRP and PurePRP® II 2015.

5.3 pH of Platelet Concentrate

Product pH observations, per donor, from each device are shown in tables along with means and standard deviations.

5.4 Leukocyte, Erythrocyte and Platelet Counts

Results are summarized in tables showing data by donor, with calculated mean and standard deviation. A two tailed, paired t-Test was used to compare the for Clear PRP and PurePRP® II 2015 products mean yields for Mononuclear Cells, Granulocytes, and RBC.

5.5 Growth Factors

Results are summarized in tables showing data by donor, with calculated mean and standard deviation. A two tailed, paired t-Test was used to compare the Clear PRP and PurePRP® II 2015 products means.

6. List of Tables: Data Analysis

7. List of Summary Tables

8. Conclusions.

Two red cell reduction platforms, PurePRP® II 2015 (EmCyte) and Clear PRP (Harvest/ Terumo) were compared along with the Angel (Arthrex) system, in a paired sample design. Mean platelet recoveries were 81% for PurePRP® II 2015, 62% for the Clear PRP platform and 49% for the Angel system. The average platelet concentration factor was 7.0 times baseline in an average product volume of 6.9 ml for PurePRP® II 2015, 5.0 times baseline in an average volume of 7.4 ml for the Clear PRP product and 4.1 times baseline in an average volume of 7.0 ml for Angel. The PurePRP® II 2015 had a mean hematocrit of 1.1% compared with 0.1% for the Clear PRP product and 2.8% for Angel. The mean recovery of mononuclear cells was 70% with the PurePRP® II 2015 system and 7% and 33% for Clear PRP and Angel platform, respectively. The granulocyte recoveries were low in all three platforms: 2%, 0% and 3% for PurePRP® II 2015, Clear PRP and Angel, respectively. The mean pH of Platelet Concentrates from the PurePRP® II 2015, Clear PRP and Angel products were 6.9, 7.0 and 7.1. The average concentrations for all growth factors measured were higher in PurePRP® II 2015 products compared to Clear PRP and Angel products. Samples collected in Na Citrate vs. ACD-A prior to processing were not clinically significant.

Table 6.1. Hematology data: EDTA Baseline anticoagulated blood

Sample Number	WBC x 10^6/ml	MC x 10^6/ml	Granulocytes x 10^6/ml	PLT x 10^6/ml	HCT %	RBC x 10^9/ml
603	5.6	1.4	4.2	192	38.1	12.40
604	7.5	2.1	5.3	210	37.4	3.98
605	4.5	1.4	3.0	170	37.5	4.26
606	8.0	1.7	6.3	240	37.6	3.95
607	11.3	2.9	8.5	335	35.8	3.98
608	7.2	1.8	5.4	261	35.8	4.25
609	10.4	3.0	7.4	142	36.1	4.19
MEAN	**7.8**	**2.0**	**5.7**	**221**	**36.9**	**5.3**
STDEV	**2.4**	**0.7**	**1.9**	**64**	**1.0**	**3.1**

Table 6.2. Hematology data: EmCyte PurePRP® II 2015

Sample Number	WBC x 10^6/ml	MC x 10^6/ml	Granulocytes x 10^6/ml	PLT x 10^6/ml	HCT %	RBC x 10^9/ml
603	7.1	6.6	0.5	1136	0.8	0.08
604	12.5	11.6	0.8	1202	1.1	0.14
605	13.7	12.4	1.3	1072	1.9	0.21
606	7.7	6.9	0.8	1524	0.9	0.10
607	15.3	14.1	1.2	1866	1.1	0.11
608	10.1	9.5	0.5	1494	1.2	0.14
609	8.5	7.3	1.3	760	0.8	0.10
MEAN	**10.7**	**9.8**	**0.9**	**1293**	**1.1**	**0.1**
STDEV	**3.2**	**3.0**	**0.4**	**362**	**0.4**	**0.0**

Table 6.3. Hematology data: Harvest Clear PRP

Sample Number	WBC x 10^6/ml	MC x 10^6/ml	Granulocytes x 10^6/ml	PLT x 10^6/ml	HCT %	RBC x 10^9/ml
603	3.0	2.7	0.3	741	0.3	0.08
604	0.8	0	0	914	0	0.02
605	0.7	0	0	810	0	0.02
606	0.2	0	0	1170	0	0.10
607	1.7	1.6	0	1548	0	0.02
608	0.2	0	0	1158	0	0.01
609	3.0	2.6	0.3	682	0.2	0.04
MEAN	**1.4**	**1.0**	**0.1**	**1003**	**0.1**	**0.0**
STDEV	**1.2**	**1.3**	**0.1**	**307**	**0.1**	**0.0**

Table 6.4. Hematology data: Arthrex Angel

Sample Number	WBC x 10^6/ml	MC x 10^6/ml	Granulocytes x 10^6/ml	PLT x 10^6/ml	HCT %	RBC x 10^9/ml
603	4.0	3.2	0.8	673	2.7	0.29
604	5	4.5	0.6	755	3.0	0.33
605	5.9	3.4	2.5	691	2.8	0.32
606	7.2	6.4	0.8	964	2.8	0.31
607	7.8	7.4	0.4	1304	2.3	0.28
608	4.6	4.4	0.2	942	2.6	0.33
609	7.0	4.2	2.8	682	3.2	0.38
MEAN	**5.9**	**4.8**	**1.2**	**859**	**2.8**	**0.3**
STDEV	**1.4**	**1.6**	**1.0**	**231**	**0.3**	**0.0**

Table 6.5. Platelet Yield (% recovery)

Sample Number	EmCyte PurePRP® II 2015	Harvest Clear PRP	Arthrex Angel
603	82%	57%	47%
604	86%	60%	47%
605	82%	64%	55%
606	83%	68%	54%
607	67%	56%	56%
608	83%	62%	48%
609	82%	67%	34%
MEAN	**81%**	**62%**	**49%**
STDEV	**6%**	**5%**	**8%**

Table 6.6. Mononuclear Cell Yield (% recovery)

Sample Number	EmCyte PurePRP® II 2015	Harvest Clear PRP	Arthrex Angel
603	65%	29%	31%
604	83%	0%	28%
605	116%	0%	33%
606	53%	0%	50%
607	59%	7%	37%
608	76%	0%	33%
609	37%	12%	19%
MEAN	**70%**	**7%**	**33%**
STDEV	**25%**	**11%**	**10%**

Table 6.7. Granulocyte Yield (% recovery)

Sample Number	EmCyte PurePRP® II 2015	Harvest Clear PRP	Arthrex Angel
603	2%	1%	3%
604	2%	0%	1%
605	6%	0%	11%
606	2%	0%	2%
607	2%	0%	1%
608	1%	0%	0%
609	3%	1%	5%
MEAN	**2%**	**0%**	**3%**
STDEV	**1%**	**0%**	**4%**

Table 6.8. Platelet Concentration (times baseline)

Sample Number	EmCyte PurePRP® II 2015	Harvest Clear PRP	Arthrex Angel
603	7.1	4.3	4.0
604	6.9	4.8	4.1
605	7.6	5.3	4.7
606	7.7	5.4	4.6
607	6.7	5.1	4.5
608	6.9	4.9	4.1
609	6.4	5.3	2.9
MEAN	**7.0**	**5.0**	**4.1**
STDEV	**0.4**	**0.4**	**0.6**

Table 6.9. pH

Sample Number	EmCyte PurePRP® II 2015	Harvest Clear PRP	Arthrex Angel
603	6.8	7.0	7.1
604	6.8	6.9	7.2
605	6.7	7.0	7.1
606	6.9	7.0	7.2
607	7.0	7.1	7.1
608	6.9	7.1	7.2
609	6.9	7.1	7.2
MEAN	**6.9**	**7.0**	**7.1**
STDEV	**0.1**	**0.1**	**0.0**

Table 6.10 Platelet Activation:

Sample Number	Na Citrate	ACD-A
610	1.8%	0.8%
611	4.4%	1.7%

Two Blood samples from each of 2 donors were drawn, the differences observed were not clinically significant.

Table 6.11 Platelet Function

Sample Number	Na Citrate	ACD-A
610	95%	96%
611	92%	94%

Two Blood samples from each of 2 donors were drawn the differences observed were not clinically significant.

Table 6.12. Growth Factor: PDGF(pg/ml PLT Releaseate)

Sample Number	EmCyte PurePRP® II 2015	Harvest Clear PRP	Arthrex Angel
603	53,474	34,669	35,807
604	65,312	45,871	39,289
605	50,308	32,391	26,270
606	76,886	59,154	49,693
607	87,233	64,260	53,658
608	82,483	60,745	51,745
609	61,843	50,721	25,993
MEAN	**68,194**	**55,860**	**39,714**
STDEV	**12,398**	**18,013**	**11,248**

Table 6.13. Growth Factor: TGF-β (pg/ml PLT Releaseate)

Sample Number	EmCyte PurePRP® II 2015	Harvest Clear PRP	Arthrex Angel
603	66,679	40,311	44,807
604	79,517	52,584	43,292
605	ND	38,759	29,661
606	56,745	78,611	55,254
607	124,924	69,838	57,448
608	77,057	51,209	45,886
609	60,490	42,608	22,274
MEAN	**75,546**	**58,505**	**41,886**
STDEV	**21,491**	**18,048**	**12,794**

Table 6.14. Growth Factor: VEGF(pg/ml PLT Releaseate)

Sample Number	EmCyte PurePRP® II 2015	Harvest Clear PRP	Arthrex Angel
603	609	386	374
604	210	151	119
605	633	504	300
606	1,725	1,408	808
607	918	702	562
608	251	313	183
609	2,529	2,186	861
MEAN	**813**	**689**	**387**
STDEV	**811**	**679**	**293**

Table 6.15. Growth Factor: SDF-1α (pg/ml PLT Releaseate)

Sample Number	EmCyte PurePRP® II 2015	Harvest Clear PRP	Arthrex Angel
603	3,708	2,941	3,184
604	3,824	3,590	3,380
605	3,480	3,204	2,475
606	4,127	4,162	3,981
607	3,778	3,367	2,862
608	3,289	2,528	2,207
609	2,633	2,354	2,027
MEAN	**3,418**	**3,113**	**2,771**
STDEV	**537**	**610**	**661**

Table 7.1. Process Time and Number of Aseptic Entries

	EmCyte PurePRP® II 2015	Harvest Clear PRP	Arthrex
Nominal Centrifuge Time 1st Spin	1.5 min.	4 min .	18 m in.
Nominal Centrifuge Time 2nd Spin	5 min.	10 min.	-
BSR Overall Process Time	19 min.	24 min.	23 m in.
Aseptic Entries	6	6	3

Table 7.2 Summary: Hematology of Products (Mean ± Standard Deviation)

Platform	WBC x 10^6/ml	MC x 10^6/ml	GRAN 10^6/ml	PLT x 10^6/ml	HCT %
EmCyte PurePRP® II 2015	**10.7** ±3.2	**9.8** ±3.0	**0.9** ±0.4	**1293** ±362	**1.1** ±0.4
Harvest Clear PRP	**1.4** ±1.2	**1.0** ±1.3	**0.1** ±0.1	**1003** ±307	**0.1** ±0.1
Arthrex Angel	**5.9** ±1.4	**4.8** ±1.6	**1.2** ±1.0	**859** ±231	**2.8** ±0.3

MC= Lymphocytes + Moncytes; GRAN = Granulocytes; PLT = Platelet
Statistical significance (α=0.05) between the means for PurePRP® II 2015 and Clear PRP products was demonstrated for all hematology parameters measured.

Table 7.3 Summary: Cell Yield (%) (Mean ± Standard Deviation)

Platform	PLT	MC	GRAN	RBC
EmCyte PurePRP® II	**81%** ±6	**61%** ±58	**2%** ±1	**0.4%** ±0.2
Harvest Clear	**62%** ±5	**7%** ±11	**0%** ±0	**0.1%** ±0.1
Arthrex Angel	**49%** ±8	**38%** ±24	**3%** ±4	**1.0%** ±0.3

Statistical significance (α=0.05) between the means for PurePRP® II 2015 and Clear PRP products was demonstrated for all cell yields calculated.

Table 7.4 Summary; Growth Factors (Mean and ± Standard Deviation)

Platform	PDGF AB (pg/ml)	TGF-β (pg/ml)	VEGF (pg/ml)	SDF-1α (pg/ml)
EmCyte PurePRP® II	**68,194** ±12,398	**75,546** ±21,491	**813** ±811	**3,418** ±537
Harvest Clear	**55,860** ±18,013	**58,505** ±18,048	**689** ±697	**3113** ±610
Arthrex Angel	**39,714** ±11,248	**41,886** ±12,794	**387** ±293	**2,771** ±661

Statistical significance (α=0.05) between the means for PurePRP® II 2015 and Clear PRP products was demonstrated for PDGF and SDF-1α.