Report 515 Research Study

EmCyte Corporation®
PurePRP® II 2015
Performance Evaluation

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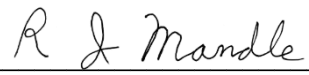

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Executive Summary

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 EmCyte PurePRP® II 2015 product as a red cell and granulocyte reduction PRP system. The study fully evaluated this platform.

Results: The PurePRP® II 2015 device produced a reduced red blood cells PRP product with an average hematocrit of 1.1% and 2% of the granulocytes were retained. The system had high average platelet cell and growth factor concentrations. The PurePRP® II 2015 platform was capable of providing a PRP product with an optimum platelet concentration of $> 1 \times 10^6$ 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

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1. Introduction

The objective of this study was to evaluate parameters associated with the platelet concentrates (PRP) produced in the Emcyte PurePRP® II 2015 system.

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 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

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.

Study Objectives and Outcome Measures

The analytical parameters chosen for the platforms were:

2.1. *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.

2.2. *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 the device.

2.3. *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.

2.4 *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)

3. Statistical Methods

Data tables and descriptive statistics are shown for each parameter.

3.1 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.

3.2 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.

3.3 Leukocyte, Erythrocyte and Platelet Counts

Results are summarized in tables showing data by donor, with calculated mean and standard deviation.

3.4 Growth Factors

Results are summarized in tables showing data by donor, with calculated mean and standard

deviation.

4. Acceptance Criteria

4.1 Mean platelet recovery ≥ 65%

4.2 Mean platelet concentration ≥ 3 times baseline

5. Conclusions

The PurePRP® II 2015 platform (EmCyte) showed the following results in 7mL PRP:

5.1 Mean platelet recovery of 81%.

5.2 The average platelet concentration factor was 7.0 times baseline

5.3 Mean hematocrit of 1.1%

5.4 Mean recovery of mononuclear cells was 70%

5.5 The granulocyte recovery was 2%

5.6 The average concentrations for all growth factors measured were high

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**

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**

Platelet Yield (% recovery)

Sample Number	EmCyte PurePRP® II 2015
603	82%
604	86%
605	82%
606	83%
607	67%
608	83%
609	82%
MEAN	**81%**
STDEV	**6%**

Mononuclear Cell Yield (% recovery)

Sample Number	EmCyte PurePRP® II 2015
603	65%
604	83%
605	116%
606	53%
607	59%
608	76%
609	37%
MEAN	**70%**
STDEV	**25%**

Granulocyte Yield (% recovery)

Sample Number	EmCyte PurePRP® II 2015
603	2%
604	2%
605	6%
606	2%
607	2%
608	1%
609	3%
MEAN	**2%**
STDEV	**1%**

Platelet Concentration (times baseline)

Sample Number	EmCyte PurePRP® II 2015
603	7.1
604	6.9
605	7.6
606	7.7
607	6.7
608	6.9
609	6.4
MEAN	**7.0**
STDEV	**0.4**

Growth Factor: PDGF(pg/ml PLT Releaseate)

Sample Number	EmCyte PurePRP® II 2015
603	53,474
604	65,312
605	50,308
606	76,886
607	87,233
608	82,483
609	61,843
MEAN	**68,194**
STDEV	**12,398**

Growth Factor: VEGF(pg/ml PLT Releaseate)

Sample Number	EmCyte PurePRP® II 2015
603	609
604	210
605	633
606	1,725
607	918
608	251
609	2,529
MEAN	**813**
STDEV	**811**

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

Sample Number	EmCyte PurePRP® II 2015
603	3,708
604	3,824
605	3,480
606	4,127
607	3,778
608	3,289
609	2,633
MEAN	**3,418**
STDEV	**537**

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

Sample Number	EmCyte PurePRP® II 2015
603	66,679
604	79,517
605	ND
606	56,745
607	124,924
608	77,057
609	60,490
MEAN	**75,546**
STDEV	**21,491**

6. Alpha 2 Macroglobulin Concentrator: Emcyte-FC120 PURE

November 2, 2015

Donors (N=2) PRP production

Date Drawn	**Donor ID**	**Emcyte 16.6% NA Citrate**	**Emcyte Process Volume/Run**
11/2/2015	**D1**- BSR-001	10ml sodium Citrate 50 ml whole blood	2 X 60 = **120 ml**
11/2/2015	**D2** -KP-44866	10ml sodium Citrate 50 ml whole blood	2 X 60 = **120 ml**

Production runs with PPP

Device	**Total volume PPP**	**Total Volume Concentrate**
Emcyte-FC120 PURE	60 ml	5 ml*

* With inclusion of the 8mL recoverable hold-up volume (not done) the total concentrate volume would be 13 mL, this volume is used for yield calculations listed below.

The PPP and PPP Concentrate were prepared according to the manufacturer's Instruction for Use (Emcyte FC120 PURE).

Average Results:

Alpha 2 Macroglobulin (normal value 1,500 – 1,800 µg/mL)

	PPP (µg/mL)	Concentrate (µg/mL)	Yield from PPP	Times baseline
EmCyte	1793	7021	84%	3.85

Fibrinogen (normal value 1,500 – 4,500 µg/mL)

	PPP (µg/mL)	Concentrate (µg/mL)	Yield from PPP	Times baseline
EmCyte	4,064	16,947	84%	3.89

Albumin (normal value 36 – 37.5 mg/mL)

	PPP (mg/mL)	Concentrate (mg/mL)	Yield from PPP	Times baseline
EmCyte	51	249	97%	3.89