Research Study

Comparison of:
Purespin PRP® 30 REFINE USA
Purespin PRP® 60 REFINE USA
Arteriocyte MAGELLAN,
Stryker REGENKIT® THT,
and ECLIPSE PRP

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Executive Summary

This study evaluated the PRP products from five centrifuged-based platforms:
Purespin PRP® 30, Purespin PRP® 60 (Refine USA), MAGELLAN, (Arteriocyte), REGENKIT®-THT (Stryker) and, ECLIPSE PRP (ECLIPSE Aesthetics) in a paired assay design with all five platforms tested with each donor. All systems were used according to manufacturer's instruction for use with the most commonly used clinical method employed where alternate protocols were included. The REGEN and ECLIPSE platforms are vacuum tube systems containing a plasma separator gel. The Arteriocyte MAGELLAN system is a buffy coat system that harvests platelets from the surface of the RBC layer using an optical sensor. The Purespin PRP® systems use a dual spin process: the first step separates platelets from red cell using a soft spin that leaves the platelets in the plasma; the second spin concentrates the platelets and removes platelet poor plasma.

Results: The Purespin PRP® 30, Purespin PRP® 60 systems had the highest platelet recoveries (80 and 78% respectively) and platelet concentration factor (6.6 times baseline) of the five systems tested. The MAGELLAN system average platelet yield was 62% and the platelet concentration factor was 5.7 times baseline. The REGEN and ECLIPSE platforms both had average platelet recoveries of < 35% and platelet concentrations that were less in the product than in the starting blood sample. There was no significant device-dependent platelet activation observed with any platform. Four growth factors were measured in a thrombin-generated releaseate. The Purespin PRP® and MAGELLAN PRP products all had similar growth factor release. Growth factor release from the REGEN and ECLIPSE systems, compared to the other three systems, varied from 6% (PDGF) to 55% (SDF-1α) of growth factor released in the PRP products from the other system.

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1.   Introduction

The objective of this study was to evaluate parameters associated with the platelet concentrates (PRP) produced by five commercially autologous PRP systems. Purespin PRP ® 30, and Purespin PRP ® 60 (Refine USA),   MAGELLAN, (Arteriocyte),   REGENKIT®-THT (Stryker) and, ECLIPSE PRP (ECLIPSE Aesthetics). Each system was evaluated with paired samples from four 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 200mL of human whole blood was obtained from each of 4 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
Purespin PRP ® 30	5mL Na Citrate	25mL
Purespin PRP ® 60	10mL Na Citrate	50mL
Arteriocyte MAGELLAN	8mL ACD-A	52mL
REGENKIT®THT Tube	1mL Citrate	8mL
ECLIPSE PRP	≈1mL	≈ 9mL

Purespin PRP ® produced approximately 4mL of product from 30mL of anticoagulated blood with a final concentration of 16.6% sodium citrate. The Purespin PRP ® 60 product was produced using "Protocol B" where 1mL of RBC was transferred along with the platelet plasma following the first centrifugation. Approximately 7mL of PRP was produced from 60mL of citrate anticoagulated blood. The Arteriocyte MAGELLAN device produced approximately 7mL of platelet concentrate from 60mL ACD-A anticoagulated blood (13.3% ACD-A). The Stryker REGEN vacuum tube contained 1mL of anticoagulant into which ~8mL of blood was collected. The volume of PRP product produced was dependent on the donor hematocrit and ranged from 5 to 7mL. Similarly, the ECLIPSE vacuum tube system was a single spin gel separation device, processing 9mL of blood plus 1mL anticoagulant. The PRP product volume ranged from 5 to 7mL.

Materials

Disposable/Reagent	Manufacturer	Lot#	Expiration Date
Sodium Citrate	Emcyte	ASC-0915-010S	09/2020
ACD-A	Harvest Terumo	7188022	2016/09
Purespin PRP ® 60	Refine USA	20160151	2019-01
Purespin PRP ® 30	Refine USA	20150096	2018-08
MAGELLAN	Arteriocyte	15050501	2017-05-05
REGEN	Stryker	083	2017-10
ECLIPSE	Aesthetics	7073-201215E	2017-02

3. Study Objectives and Outcome Measures

The analytical parameters chosen to identify differences or similarities among the five 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

CBCs 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-128 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)

4. Statistical Methods

Data tables and descriptive statistics are shown for each of the following parameter:

4.1 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.

4.2 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.

4.3 pH of Platelet Concentrate

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

4.4 Leukocyte, Erythrocyte and Platelet Counts

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

4.5 Growth Factors

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

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7.   Summary and Conclusions.

Five commercial PRP platforms were compared in a paired sample design with blood from each of the 4 donors processed on all platforms. The Purespin PRP ® 30, and Purespin PRP ® 60, from (Refine USA LLC), use a two spin protocol, and produced an average of 4.0 and 7.4mL of platelet concentrate from 25 and 50mL of blood respectively. MAGELLAN, (Arteriocyte) is a buffy coat protocol and collects the buffy from the top of the RBC interface using a RBC detector controlled syringe pumps and produced and average of 6.9mL of platelet concentrate from 52mL of blood. The REGENKIT®-THT (Stryker) and, ECLIPSE PRP (ECLIPSE Aesthetics) systems use vacuum tubes containing plasma separator gel, and produced an average of 5 and 7mL of platelet plasma from 8 and 9mL of blood respectively.

Mean platelet recoveries were 78% for Purespin PRP® 60, 81% for the Purespin PRP® 30, and 62% for the MAGELLAN platforms. Platelet recovery for the two gel systems each averaged 35% for REGEN and 30% for ECLIPSE. The average platelet concentration factor was 6.6 times baseline for the Purespin ® systems and 5.7 time baseline for the MAGELLAN platform, and approximately one-half the starting blood platelet concentration for the two gel-based systems. **There was no evidence of device-dependent platelet activation and platelet response to ADP agonist was normal for all PRP products.**

Mean hematocrit values were 9% for Purespin PRP® 60, 3% for the Purespin PRP® 30, and 8% for the MAGELLAN platform. The platelet products from the two gel systems had essentially no red cells. Retentions of mononucleated cells were 59% in the Purespin PRP® 60 product, 45% in Purespin PRP® 30 and 50% in the MAGELLAN product. Greater than 90% of the granulocytes were removed with the Purespin PRP® 60 and Purespin PRP® 30 systems. MAGELLAN product retained 16% of the granulocytes. The gel-based separator PRP products were essential free of all white blood cells. The REGEN product for Donor number 502 was atypical with high number of white blood cells in the product, probably due to a miss-match in gel formation and cell sedimentation. Donor number 505 demonstrated hemolysis and micro-blood clots were observed across all 5 platforms.

The mean pH of Platelet Concentrates was 6.7 from the Purespin PRP® 60, 7.0 for Purespin PRP® 30 and 7.2 for MAGELLAN PRP. The REGEN and ECLIPSE system products both averaged a pH of 7.7.

Three growth factors were measured in a thrombin generated releaseate: PDGF A/B, TGF-β and VEGF. The relative level of growth factors in the releaseate reflected the platelet concentration. The REGEN and ECLIPSE systems had significantly low platelet counts and growth factor concentrations.

Table 5.1. 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
501	14.6	3.3	11.3	312	41.7	4.55
502	6.8	1.6	5.1	161	47.3	4.82
504	5	1.8	3.2	221	48.3	5.8
505	6.9	2.2	4.7	115	39.2	4.41
MEAN	**8.3**	**2.2**	**6.1**	**202**	**44.1**	**4.9**
STDEV	**4.3**	**0.8**	**3.6**	**85**	**4.4**	**0.6**

Table 5.2. Hematology data: Purespin PRP® 60

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
501	27.2	21.4	5.8	1824	14.4	1.6
502	10.4	9	1.4	834	7.2	0.74
504	9.1	8.4	0.6	1052	5.9	0.74
505	20.3	11.5	8.8	940	8.8	4.41
MEAN	**16.8**	**12.6**	**4.2**	**1163**	**9.1**	**1.9**
STDEV	**8.6**	**6.0**	**3.9**	**450**	**3.7**	**1.7**

Table 5.3. Hematology data: Purespin PRP® 30

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
501	18.2	16.6	1.6	1552	5.2	1.6
502	8.2	7.6	0.4	861	2	0.2
504	5.8	5.4	0.3	757	2.2	0.28
505	12.8	6.2	0.3	1166	3.4	0.38
MEAN	**11.3**	**9.0**	**0.7**	**1084**	**3.2**	**0.6**
STDEV	**5.5**	**5.2**	**0.6**	**357**	**1.5**	**0.7**

Table 5.4. Hematology data: Arteriocyte MAGELLAN

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
501	36.7	17.4	19.3	1208	9	0.98
502	10.4	7.8	2.4	812	7.2	0.74
504	14.5	12.4	2.1	939	6.6	0.8
505	22.3	12.6	9.6	971	8.5	0.96
MEAN	21.0	12.6	8.4	983	7.8	0.9
STDEV	11.6	3.9	8.1	165	1.1	0.1

Table 5.5. Hematology data: Stryker REGEN

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
501	0.7	0.0	0.0	25		
502	1.4	1.1	0.1	173	0	0.01
504	0.7	0.0	0.0	86	0	0.0
505	0.5	0.0	0.0	109	0	0.0
MEAN	0.8	0.4	0.0	98	0.0	0.0
STDEV	0.4	0.6	0.1	61	0.0	0.0

Table 5.6. Hematology data: Aesthetics ECLIPSE

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
501	0.1	0.0	0.0	66	0.0	0.0
502	0.3	0.0	0.0	40	0.0	0.0
504	0.3	0.0	0.0	109	0.0	0.0
505	0.3	0.0	0.0	101	0.0	0.0
MEAN	0.3	0.0	0.0	79	0.0	0.0
STDEV	0.1	0.0	0.0	32	0.0	0.0

Table 5.7. Platelet Yield (% recovery)

Sample Number	Purespin PRP® 60	Purespin PRP® 30	MAGELLAN	REGEN	ECLIPSE
501	87.7	89.5	52.1	5.4	16.7
502	88.1	79.1	67.9	65.8	17.9
504	70.5	61.7	55.6	21.9	35.6
505	65.9	93.8	74.8	45.1	50.8
MEAN	78.1	81.0	62.6	35	30.3
STDEV	11.5	14.3	10.6	26	16.2

Table 5.8. Lymphocyte Cell Yield (% recovery)

Sample Number	Purespin PRP® 60	Purespin PRP® 30	MAGELLAN	REGEN	ECLIPSE
501	103.3	96.0	73.3	0	0
502	96.8	75.1	68.3	51.8	0
504	72.0	57.6	95.9	0	0
505	64.2	43.5	73.2	0	0
MEAN	84.1	68.1	77.7	13	0.0
STDEV	18.9	22.7	12.4	26	0.0

Table 5.9. Monocyte Cell Yield (% recovery)

Sample Number	Purespin PRP® 60	Purespin PRP® 30	MAGELLAN	REGEN	ECLIPSE
501	70.0	66.0	60.6	0	0
502	90.7	49.3	53.8	20.4	0
504	54.3	36.0	61.0	0	0
505	61.0	14.0	77.3	0	0
MEAN	69.0	41.3	63.2	5.1	0.0
STDEV	15.8	22.0	10.0	10.2	0.0

Table 5.10. Granulocyte Yield (% recovery)

Sample Number	Purespin PRP® 60	Purespin PRP® 30	MAGELLAN	REGEN	ECLIPSE
501	7.7	2.5	23.0	0	0
502	4.7	1.2	6.3	1.2	0.0
504	2.8	1.7	8.6	0	0
505	22.8	0.9	26.3	0	0
MEAN	9.5	1.6	16.1	0.3	0.0
STDEV	9.1	0.7	10.1	0.6	0.0

Table 5.11. Platelet Concentration (times baseline)

Sample Number	Purespin PRP® 60	Purespin PRP® 30	MAGELLAN	REGEN	ECLIPSE
501	7.0	6.0	4.5	0.1	0.2
502	6.2	6.4	5.8	1.2	0.3
504	5.7	4.1	4.9	0.4	0.6
505	7.8	9.7	7.7	0.9	0.8
MEAN	6.7	6.6	5.7	0.7	0.5
STDEV	0.9	2.3	1.4	0.5	0.3

Table 5.12. pH

Sample Number	Na Citrate Baseline	Purespin PRP® 60	Purespin PRP® 30	MAGELLAN	REGEN	ECLIPSE
501	6.45	6.59	6.86	7.15	7.63	7.62
502	6.51	6.84	7.12	7.30	7.65	7.62
504	6.56	6.74	6.94	7.33	7.88	7.76
505	6.70	6.62	6.95	7.17	7.81	7.76
MEAN	6.6	6.7	7.0	7.2	7.7	7.7
STDEV	0.1	0.1	0.1	0.1	0.1	0.1

Table 5.14. Growth Factor: PDGF(pg/mL PLT Releaseate)

Sample Number	Na Citrate Baseline	Purespin PRP® 60	Purespin PRP® 30	MAGELLAN	REGEN	ECLIPSE
501	19339	145888	96999	97986	1467	3530
502	9118	41976	52887	42039	11151	3099
504	17635	97535	72675	96189	4295	6732
505	15564	67469	74429	84545	5730	4707
MEAN	15,414	88,217	74,248	80,190	5,661	4,517
STDEV	4,472	44,652	18,041	26,122	4,066	1,626

Table 5.15. Growth Factor: TGF-β (pg/mL PLT Releaseate)

Sample Number	Na Citrate Baseline	Purespin PRP® 60	Purespin PRP® 30	MAGELLAN	REGEN	ECLIPSE
501	15638	54755	43033	46976	1967	4250
502	9587	32501	31773	33298	12211	4381
504	18123	51628	39766	56119	8082	9539
505	16197	50323	50317	47361	8756	7718
MEAN	14,886	47,302	41,222	45,939	7,754	6,472
STDEV	3,690	10,041	7,690	9,426	4,261	2,599

Table 5.16. Growth Factor: VEGF(pg/mL PLT Releaseate)

Sample Number	Na Citrate Baseline	Purespin PRP® 60	Purespin PRP® 30	MAGELLAN	REGEN	ECLIPSE
501	172	1096	837	792	14	39
502	159	860	843	857	254	83
504	50	240	185	275	14	20
505	28	170	168	171	8	6
MEAN	102	592	508	524	73	37
STDEV	74	457	383	351	121	34

Table 6.1 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 %
Purespin PRP ® 60	16.8 ±8.6	12.6 ±6.0	4.2 ± 3.9	1163 ±450	9.1 ±3.7
Purespin PRP ® 30	11.3 ±5.5	9.0 ±5.2	0.7 ±0.6	1084 ±357	3.2 ±1.5
MAGELLAN	21.0 ±11.6	12.6 ±3.9	8.4 ±8.1	983 ±165	7.8 ±1.1
REGEN	0.8 ±0.4	0.4 ±0.6	0.0 ±0.1	98 ±61	0.0 ±0.0
ECLIPSE	0.3 ±0.1	0.0 ±0.0	0.0 ±0.0	79 ±32	0.0 ±0.0

MC= Lymphocytes + Monocytes; GRAN = Granulocytes; PLT = Platelet

Table 6.2 Summary: Cell Yield (%) (Mean ± Standard Deviation)

Platform	WBC x 10^6/mL	MC x 10^6/mL	GRAN 10^6/mL	PLT x 10^6/mL	RBC %
Purespin PRP ® 60	29.2 ±5	56 ±28	9.5 ±9.1	78±11	5 ±5
Purespin PRP ® 30	21.8 ±3	45 ±30	1.6 ±0.7	81 ±14	1±0.5
MAGELLAN	33 ±9	50 ±16	16.1 ±10.1	62 ±11	2±1
REGEN	7 ±4	6 ±11	0.3 ±0.6	35 ±26	0
ECLIPSE	3 ±2	0 ±0	0 ±0	30 ±16	0

Table 6.3 Summary; Growth Factors (Mean and ± Standard Deviation)

Platform	PDGF pg/mL	TGF-β ng/mL	VEGF Pg/mL
Purespin PRP ® 60	88.2 ±44.6	47.3 ±18.0	592 ±457
Purespin PRP ® 30	74.2 ±18.0	38.5 ±9.0	508 ±383
MAGELLAN	80.2 ±26.1	45.9 ±9.4	524 ±351
REGEN	5.7 ±4.1	7.7 ±4.3	73 ±121
ECLIPSE	4.5 ±1.6	6.5 ±2.3	37 ±34
Citrate Baseline	15.4±4.5	14.9 ±2.6	102 ±74