Analysis of

EmCyte Corporation

Concentrating Systems

An independent review of pre-clinical performance data

REVISION 2

Principle Investigator(s): Dr. Robert Mandle Ph.D.

BioSciences Research Associates, Inc.
767c Concord Avenue
Cambridge, MA 02138

All Studies Presented in this Document has been independently conducted and validated by Dr. Robert Mandle Ph.D. at BioSciences Research Associates, Inc.

CONTENTS:

Analysis of GenesisCS For Concentration of Human Bone Marrow Aspirate 60mL

IN VITRO TESTING

RESEARCH STUDY PLAN:

Title: **Evaluation of GenesisCS with Bone Marrow Aspirate**	
Revision: 2	Revision Date: January 12, 2012

TEST OBJECTIVE:

Preliminary evaluation of GenesisCS for concentration of human bone marrow aspirate. Preclinical and clinical studies have suggested the benefit of using concentrated autologous bone marrow aspirate in bone repair, myocardial infarct and peripheral vascular disease. Bone marrow aspirate is often not sufficient for clinical efficacy in the absence of concentration1,2. This report represents results from an evaluation of GenesisCS device for the concentration of human bone marrow-derived stem cells. Sixty mL of human bone marrow aspirate were concentrated to approximately 6 ml with the GenesisCS. Samples of the bone marrow aspirate (BMA) and resulting bone marrow concentrate (BMC) were analyzed for Total Nucleated Cells (TNC), Platelets (PLT), and CD34 positive Hematopoietic Stem Cells (HSC). Yield calculation were done for TNC, PLT and HCS.

EXPERIMENTAL DESIGN:

Donor bone marrow samples, approximately 120mL, collected from two sites of the iliac crest, were obtained from Poetics (Cambrex). Bone marrow samples were collected in 30-50 units/mL of heparin. Processing and all testing were initiated within 24 hr of collection. After obtaining a 1mL start sample from a well mixed transfer pack of BMA, two 60 mL syringes were filled with approximately 60 ml of marrow aspirate and the volumes recorded. GenesisCS disposables were filled from these syringes through the luer-lock fitting at a fill rate of approximately 1 mL/sec. Disposables were centrifuged at 2400 rpm (1020 x g) for 12 min. Two independent centrifuge runs were performed for each donor BMA from two separate donors collected on separate days for a total of four runs. Following centrifugation, the plasma layer was removed, by lowering the collection head to within 2-5mm above the buffy coat layer which contained the concentrated nucleated cells and platelets. Next, 2 mL of the remaining plasma and an additional 4 mL of the buffy coat was removed (4 mL following the first flash of RBC observed in the suction tubing above the collection device) for a total of 6mL of BMC.

Analysis of BMA and BMC consisted of:

- Complete blood counts utilizing a Medtronic 620 -16 parameter hematology analyzer with extended platelet range.
- Cytometric analysis of CD34 positive hematopoietic stem/progenitor cells
- Manual differential counts on BMA and BMC samples.
- Yield of nucleated cells, platelets and CD34 positive HSCs were calculated for bone marrow concentrates

RESULTS:

Characterization of GenesisCS BMC:

The TNC values from the hematology analyzer for pre-sample (BMA) and for product (BMC) and the calculated concentration over baseline values are shown in Table I.

Table I: Total nucleated cells (2 donors with duplicate runs)

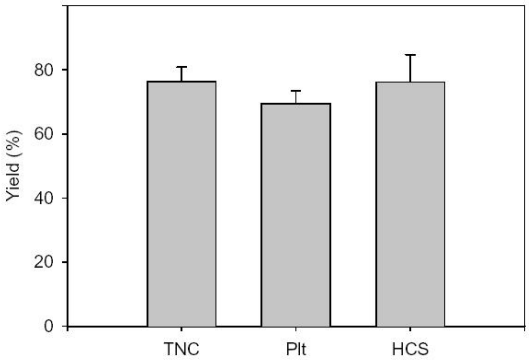
	Volume	Total Nuclear Cells x $10^3/\mu L$	Total Concentration Above Baseline
Bone Marrow Aspirate	60mL	16-23	1.0x
Bone Marrow Concentrate	4mL	170-271	11.5x
Bone Marrow Concentrate	6mL	178-286	11.9x

Table II lists the calculated total number of cells (volume x concentration) in BMA and BMC. TNC and PLT counts represent the values from the hematology analyzer times the volumes of BMA or BMC. HSC numbers are calculated from the percent of CD34+ cells gated with CD45+ events times the number of WBC (TNC minus nucleated red blood cells).

Table II: The recovery of TNC, Plt and CD34+ HCS. Total cell numbers ± SD (yield percentages)

BONE MARROW ASPIRATE			**BONE MARROW CONCENTRATE**		
TNC x 10^6	**PLT x 10^6**	**HSC x 10^6**	**TNC x 10^6**	**PLT x 10^6**	**HSC x 10^6**
1161 ± 239 (954-1368)	10,830 ± 1836 (9240-12420)	8.8± 1.3 (8-10)	894 ± 232 (678-1105)	7,623 ± 1432 (6363-9341)	6.8 ± 1.5 (5-7)
Yield (%)			76 ± 4 (71-81)	70 ± 4 (67-75)	76 ± 8 (63-83)

Figure 1. Recovery of TNC, Plt and CD34+ HCS



DISCUSSION:

The percent of TNC, Plt, and CD34+ HSC were calculated by dividing the total number of cells recovered in the BMC by the total number present in 60ml of BMA and are represented as mean plus standard deviation for 2 donors with duplicate runs.

CONCLUSION:

The product (BMC) yields were 76% for TNCs and CD34+ HSC. These yields are consistent with other point of care bone marrow concentrating devices. Platelet yields in the BMC averaged 70% and the product Hematocrit averaged 31.6% with a range of 31-40% (data not shown). Hematocrit can be adjusted by including more or less of the plasma layer during the collection of BMC. Variation within donor samples appears to less than between donors. Between donor variation will need to be determined in a larger study. However, the data from this preliminary evaluation with two donors run in duplicate, is very encouraging.

Point of Care Preparation of Autologous Platelet Products for Regenerative Medicine: Comparison of Four Market Leading Commercial Methods

IN VITRO TESTING

TEST OBJECTIVE:

Platelet Rich Plasma (PRP) provides an autologous, complex mixture of blood cells and platelets that are able to mediate healing by supplying growth factors, cytokines, chemokines and other bioactive compounds. PRP technology that was initially used in dentistry and maxillofacial surgery to improve bone healing, is safe and capable of promoting and of accelerating the healing processes. PRP is now widely used in regenerative medicine including orthopedic surgery involving shoulder, hip and knee anterior cruciate ligament (ACL) reconstruction and meniscus repair. More recently, injectable forms of PRP have been helpful in the management of muscle, tendon and cartilage injuries.

PRP products differ both qualitatively, e.g. the presence of absence of leukocytes, and quantitatively, including platelet concentration leukocyte differential and the concentration of bioactive compounds. The purpose of this study was to compare key parameters of the PRP product from four commercial point-of-care technologies using paired samples from 3 normal donors.

EXPERIMENTAL DESIGN:

Donor Selection:

Blood was obtained from 3 normal donors following informed consent. All blood collection protocols and donors met requirements of the American Association of Blood Banks (AABB) and the United States Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER). The phlebotomy protocol, including informed consent was approved by the New England Institutional Review Board and was conducted in accordance with the Helsinki Declaration of 1975 as revised in 2000. Blood was drawn from the Median-cubital vein using a 16g apheresis needle and sliconized cannula (Reference Number 4R2441, Fenwal). Blood was drawn into transfer packs with the required ACD-A anticoagulant to blood ratio as suggested by each device manufacturer (See Table I).

Point of Care PRP Systems:

Four of the leading commercial point-of-care systems for autologous PRP production were tested with paired samples such that blood from each of three donors was tested in duplicate runs with each system. Table I lists the test device names, distributers, blood volume processed and the amount of anticoagulant used.

Device Name	Manufacturer	Process Volume	ACD-A: mL Blood	Lot Number
GenesisCS PRP	EmCyte Corporation	60 mL	5:55	2011031490
SmartPReP®2 APC+™	Harvest Technologies, Corp.	60 mL	6:54	863502-0008
GPSIII® Platelet Concentrating System	Biomet Biologics	60 mL	5:55	011011
Arthrex ACP	Arthrex Orthobiologics	10 mL	1:10	11012898

Arthrex ACP was filled directly from the transfer pack; all others were loaded form a 60 mL syringe that was drawn from the transfer pack. Baseline samples were drawn from each transfer pack. Two device disposables were processed for each donor. Complete blood count (CBC) analysis was done on a Medonic CA 620 Hematology Analyzer. Platelet relative concentration and platelet yield were calculated by comparison to baseline unprocessed whole blood samples. Growth factors PDGF A/B, VEGF, SDF-α and TGF-β1 were measured by quantitative ELSAs (R&D Systems Quantikine kits) in platelet releasates prepared from PRP by addition of 1 part thrombin (1000U/ml in 10% $CaCL_2$) per 10 parts PRP.

RESULTS:

The baseline WBC, Platelet and hematocrit values for three donors are shown in Tables II & III for samples collected in 8.3% (5:55 ratio) ACD-A anticoagulant and 10% (6:45 ratio) ACD-A.

Table II. Baseline hematology data for ACD-A: Blood ratio 5:55

Donor	WBC x 10^6/mL	PLT x 10^6/mL	HTC %
Donor 1	9.8	202	35.7
Donor 2	5.2	115	36.3
Donor 3	5.5	137	37.5

Table III. Baseline hematology data for ACD-A: Blood ratio 6:54

Donor	WBC x 10^6/mL	PLT x 10^6/mL	HTC %
Donor 1	9.9	186	34.9
Donor 2	5.2	122	35.3
Donor 3	5.1	157	37.8

Duplicate PRP samples were produced, for each donor, on each of the four systems tested. The average WBC, platelet and hematocrit values are shown in Table IV for 6 runs on each system.

Table IV. Hematology data for PRP products

System	WBC x 10^6/mL	PLT x 10^6/mL	Concentration factor	HTC %
GenesisCS PRP	49.7	1355	9.7	46.3
SmartPReP®2 APC+™	28.5	1105	7.1	27.4
GPSIII® Platelet Conc. System	37.7	624	4.4	11.9
Arthrex ACP	2.8	261	1.7	2.4

The average volume of PRP and the platelet yield were calculated for each PRP system. The platelet yields were measured by:

$$Yield = \frac{PLT_{PRP}\ x\ PRP\ volume}{PLTstart\ x\ Process\ volume}$$

Where PLT_{PRP} and PLT_{start} are the platelet counts in the PRP sample and baseline sample respectively. Platelet yields are the average of 6 PRP production runs with three donors. Four statistics of platelet yield are shown in Table V: mean value, plus the coefficient of variation about the mean, median value, and minimum and maximum values in the range of yield values.

Table V. PRP product volumes and PLT yields

System	PRP vol. (mL)	Platelet Yield
		Range
GenesisCS Platelet Concentrating System	5	70%-96%
SmartPReP®2 APC+™	7	75%-89%
GPSIII® Platelet Concentrating System	6.6	24%-82%
Arthrex ACP	4.0	58%-85%

Thrombin-generated releasates prepared from the PRP product of each system were analyzed with ELISA for PDGF-A/B, TGF-β1, VEGF, and SDF-1α. The relative concentrations of these growth factors and chemokine are shown in Figure1, 2, 3, and 4 respectively.

Figure 1. PDGF-A/B Comparison in PRP releasate

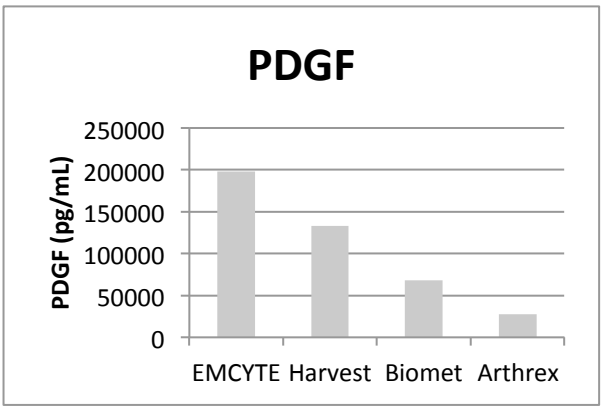


Figure 2. TGF-beta 1 Comparison in PRP releasate

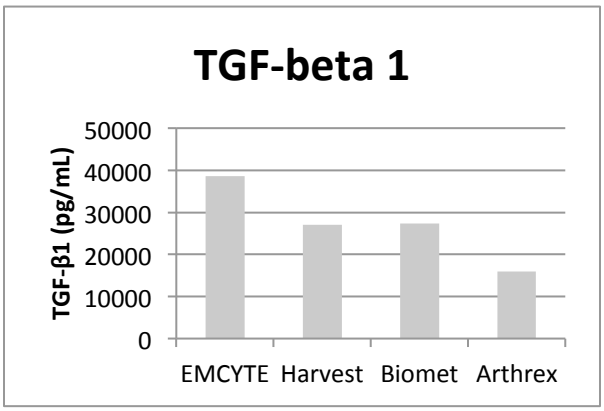


Figure 3. VEGF Comparison in PRP releasate

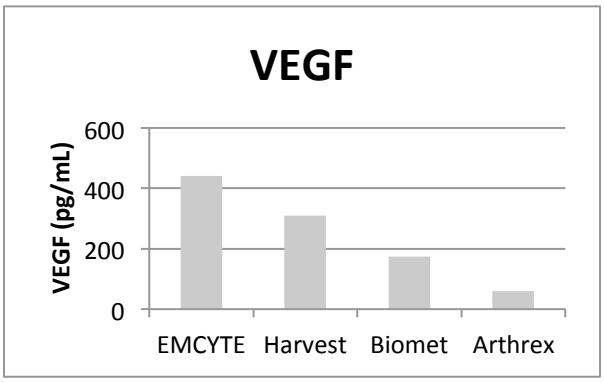
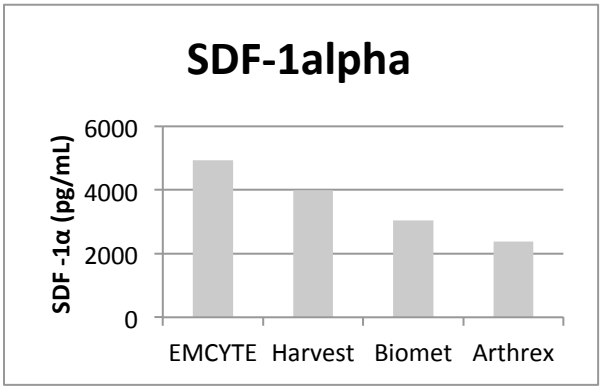


Figure 4. SDF-1α Comparison in PRP releasate



DISCUSSION

Four of the most frequently used point-of-care autologous PRP systems were compared. All four systems are centrifuge based, and with the exception of loading the disposable with anticoagulated blood and harvesting the PRP product, the separations are automated. All systems concentrated platelets and WBC to varying degrees. Part of the variance was related to efficiency of platelet recovery and part was due to the volume of the PRP product produced. PRP volume collected can be adjusted during collection continuously on the Genesis system and in discrete increments of 10, 7 mL on the Harvest APC system. The Biomet GPSIII system is essentially fixed in PRP volume all though all PRP could be further diluted with the PRP fraction. The Arthrex ACP system contained the lowest concentration of WBC and platelets, with a mean platelet concentration of 70% greater than baseline levels. With respect to efficiency of platelet recovery, the GenesisCS and systems excelled with an average of 80% platelet yield across 3 donors. The highest yields were seen with the Genesis system; however the Smart PreP2 APC system was slightly more consistent between donors as reflected in the greater difference in sample median vs. sample mean in Table V.

All systems recovered viable platelets, with an average process dependent platelet activation of approximately 10%. The Biomet GPSIII system demonstrated the least process dependent activation, but only recovered approximately half of the platelets.

The measured concentration of growth factors, PDGF-A/B, TGF-β1, VEGF, and SDF-1α were all highest in the PRP produced with the GenesisCS system. The releasate concentrations of PDGF-A/B, TGF-β1 and to a lesser extent SDF-1α, correlate with the platelet count in the PRP. VEGF concentrations are influenced by both platelet and WBC concentrations. The efficiency of platelet and WBC recovery, the ability of the recovered platelets to retract the thrombin clot and ration of PRP volume to processed volume affect these results. The Arthrex ACP system despite only a 4mL PRP volume, only processed 9mL of blood vs. 54 or 56 mL for the other systems. In addition the PRP from the Arthrex ACP system did not have significant concentrations of platelets or WBC.

There was a large variation in number of RBC in the PRP products across the platforms with GenesisCS> Smart PreP2 APC> GPSIII>ACP. There has been no clinical data concerning adverse events due to RBC contamination in PRP and as the RBC are autologous, there are no antigen cross match or agglutination issues. Furthermore, a typical pooled buffy coat platelet concentrate for transfusion has a hematocrit of approximately 50%. In testing done in our laboratory, we have shown that contaminating RBCs do not activate platelets in PRP.

Point of Care Preparation of Autologous Platelet Products for Regenerative Medicine: Comparison of Harvest SmartPReP2 and EmCyte Pure PRP™

IN VITRO TESTING

INTRO:

Autologous platelet–rich therapy was introduced into maxillofacial and periodontal surgery just over a decade ago (1-3) and has found extensive clinical use in osseous regeneration, maxillary sinus augmentation, and consolidation of titanium implants (4-7). More recently it has proven to be an effective adjunctive therapy for general orthopedic surgery. In sports medicine, regenerative therapy, aesthetics, as well as soft and hard tissue wound healing; PRP has emerged as a first line treatment modality as a safe and effective alternative to surgery. Several automatic and semiautomatic devices have received device clearance from European and United States regulatory agencies for the generation of platelet-rich product (PRP) from small amounts of patient blood. Platelet Rich Plasma (PRP) and Platelet Concentrate (PC) are established terminology for blood components for transfusion. Unfortunately the continued use of the term PRP for autologous, topical platelet product, contributes to the misconception that all therapeutic autologous platelet products are equivalent. Platelet-rich therapy products contain mixture of bioactive compounds and formed elements, and differ quantitatively in the concentration of: a) platelets, b) mononuclear leukocytes, c) granulocytes and d) red cells, as well as, e) the potential to provide growth factors, cytokines, chemokines and other biologic mediators. The differences in PRP products may be a potential cause of conflicting clinical reports on the therapeutic efficacy of PRP. The quantitative and qualitative differences in platelet rich products may influence the biological effects and clinical therapeutic outcome of PRP treatment.

One obvious metric is the concentration of platelets in PRP. Current clinical practice targets a platelet concentration of approximately 1,000,000 platelets per ml of PRP, or a concentration of 5 times whole blood levels. The concentration of granulocytes and red blood cells that may contribute to inflammation, pain at the injection site and destruction of extracellular matrix proteins (reference RBC, WBC, and Elastase) should also be assessed.

This study is a preliminary evaluation of the GenesisCS Pure PRP™ system. The platelet concentration and yield, along with mononuclear leukocytes, granulocytes and red blood cell concentrations in the Pure PRP™ and SmartPReP2 systems both selectively concentrated the mononuclear cell fraction where the stem/progenitor cells reside, while eliminating the granulocytes that are pro-inflammatory. The PRP from the Pure PRP™ system had a granulocyte concentration less than that of whole blood and less than 20% granulocytes and greater than 80% mononuclear cells (Table 6). product are reported and compared with the product from Harvest/Terumo APC60 SmartPReP2 system on paired donor samples.

EXPERIMENTAL DESIGN:

Blood was obtained for 7 normal donors following informed consent. All blood collection protocols met the requirements of the American Association of Blood Banks (AABB), the United States Food and Drug Administration Center for Biologics Evaluation and Research (CBER) were approved by an institutional review board and in accordance with the Helsinki Declaration of 1975 as revised in 2000. Blood was drawn from the Median-cubital vein using an 18g apheresis needle and sliconized cannula (Fenwall REF 4R2441). This was a crossover study design comparing the PRP products produced by EmCyte's GenesisCS Pure PRP™ System and the Harvest/Terumo SmartPReP® 2 System. Whole Blood Samples were collected in 60mL syringes preloaded with anticoagulant according to the manufactures instructions (see Table 1).

Table 1. Anticoagulated Whole Blood

Platform	Whole Blood (mL)	Anticoagulant	Volume of Anticoagulant (mL)
GenesisCS Pure PRP™	48	Na Citrate	12*
APC60 SmartPReP2	54	Acid Citrate Dextrose (ACD-A)	6

Donors 6 and 7 had 50mL of whole blood and 10mL of Na Citrate anticoagulant and 50 mL of whole blood.

Baseline anticoagulated whole blood samples were drawn in separate syringes with the same ratio of anticoagulant.

PRP PRODUCTION:

For each donor, 60ml of anticoagulated blood was processed on both the GenesisCS Pure PRP™ system and the SmartPReP2 system to prepare platelet concentrates according to manufactures' instructions. Complete blood count (CBC) analysis was done on a Beckman Coulter AcT diff2 Hematology Analyzer. Ph of samples was done on a Nova Biomedical Stat Profile blood gas analyzer.

Table 2. Centrifugation Protocols

Platform	Centrifuge	First Spin Time & Relative force	Second Spin
EmCyte Pure PRP™	Elite	1.5 min 2,500 x g	4 min 2,500 x g
APC60 SmartPReP2	SmartPReP2	4 min 1000 x g	10 min 900 x g

RESULTS:

Table 3. Anticoagulated Whole Blood Process Volumes; Mean and (SD) for Product volumes

Platform	Whole Blood Processed (mL)	Average Product volume (mL)
EmCyte Pure PRP™	60	6.6 (0.2)
APC60 SmartPReP2	60	6.9 (0.2)

Table 4. Platelet concentration and recovery in PRP Products

Platform	Platelet x 10^6/ml	Platelet Recovery	Platelet concentration over Baseline
EmCyte Pure PRP™	1128 (319)	76% (4)	6.7 (0.3)
APC60 SmartPReP2	1075 (262)	69% (11)	5.9 (0.9)

Table 5. Concentration of WBC and RBC in PRP Products

Sample	WBC x 10^6/ml	MN x 10^6/ml	Gran x 10^6/ml	PLT x 10^6/ml	RBC x 10^9/ml	Hct (%)
Baseline EDTA-Blood	5.9 (1.6)	2.3 (0.5)	3.7 (1.4)	185 (51)	4.2 (0.4)	37.5 (3.6)
EmCyte Pure PRP™	14.9 (4.9)	12.1 (3.7)	2.9 (2.5)	1128 (319)	0.2 (0.2)	1.1 (0.6)
SmartPReP2 PRP	20.6 (4.5)	15.3 (3.2)	5.3 (2.5)	1075 (262)	3.9 (1.4)	34.1 (12)

Table 6. Comparison of WBC Differential: PRP Products vs. Whole Blood Percent of total WBC

Sample	Mononuclear Cells	Granulocytes
Baseline EDTA-Blood	39.3 (8.8)	60.9 (8.9)
EmCyte Pure PRP™	81.4 (10.5)	18.6 (10.6)
SmartPReP2 PRP	74.7 (8.8)	25.3 (8.8)

Table 7. PRP Product pH

Platform	pH in PRP
EmCyte Pure PRP™	7.5 (0.1)
APC60 SmartPReP2	6.8 (0.1)

DISCUSSION:

Platelet concentration, inclusion or exclusion of mononuclear cells, granulocytes and red cells are hematologic parameters that define an autologous platelet product, and are likely to affect the clinical efficacy of the product. In this report we evaluated the platelet-rich product produced with two PRP systems: GenesisCS Pure PRP™ system and the SmartPReP®2 platelet concentrating system. Hematologic parameters, including WBC concentration, platelet concentration and hematocrit are reported. Both systems had excellent platelet concentration and recovery, with greater than 1,000,000 platelets per ml of PRP, yields of approximately 70% and greater than 6 fold concentrations over baseline (Table 4). The Pure PRP™ and SmartPReP2 systems both selectively concentrated the mononuclear cell fraction where the stem/progenitor cells reside, while eliminating the granulocytes that are pro-inflammatory. The PRP from the Pure PRP™ system had a granulocyte concentration less than that of whole blood and less than 20% granulocytes and greater than 80% mononuclear cells (Table 6).

There were significant differences in the PRP products produced on the two platforms:

1. The concentrations of Red Blood Cells in the Pure PRP™ product was less than 5% of the red cell concentration in whole blood with an average hematocrit of 1%. The PRP product from the SmartPReP system had a RBC concentration and hematocrit closer to that of whole blood.

2. The pH of the Pure PRP™ product was 7.5 compared with pH 6.8 for the SmartPReP product. A pH closer to the normal blood pH of 7.35-7.45 alleviates the necessity of neutralizing with Sodium Bicarbonate to prevent pain at the injection site.

3. The Genesis CS Pure PRP™ retains a high percent of platelets while removing greater than 99% of the RBCs and 90% of the granulocytes. The two spin protocol is robust and reduces the effect of donor variability and technical skill to produce a reproducible PRP product.

4. The Pure PRP™ and SmartPReP2 systems both selectively concentrated the mononuclear cell fraction **where the stem/progenitor cells reside,** while eliminating the granulocytes that are pro-inflammatory. The PRP from the Pure PRP™ system had a lower granulocyte concentration and higher mononuclear cell concentration when compared to the SmartPReP product (Table 6).

Nuclear Cell Count Analysis of Human Adipose Tissue Concentrate Processed with the Secquire® 2 Concentrating Device

IN VITRO TESTING

TEST OBJECTIVE:

This study evaluated the product produced by the centrifuged-based Secquire-2 Cell Separator. Human adipose tissue was concentrated from lipoaspirate, and the nucleated cell concentration estimated by flow cytometry or fluorescent microscopy as a measure of product qualify.

BACKGROUND:

Adipose tissue provides a readily accessible source of autologous stem/progenitor cells and proangiogenic pericytes. Typically, the lipoaspirate contains 50% to 75% tumescent fluid. Centrifugation removes the fluid and condenses the buoyant adipose tissue. Concentrates of cellular and extracellular elements in the natural biological scaffolding of adipose tissue may promote wound healing and have applications in regenerative medicine.

EXPERIMENTAL DESIGN:

Lipoaspirate collection:

Harvest of adipose tissue from lower abdomen via lipoaspiration was performed using standard of care, closed syringe method. A multiport infiltrator sterile cannula attached to a 20-60 cc syringe was used to infiltrate tumescent solution (0.5gm Lidocaine with 1mg epinephrine per 1L of normal saline) into the subdermal fat plane. Adipose tissue suspended in the fluid media provided by the tumescent fluid was withdrawn by applying gentle suction with the syringe.

Adipose Concentrate Production:

The lipoaspirate was transferred immediately following harvest from the harvesting syringe into a Secquire-2 disposable and centrifuged for 3.5 min at approximately 140 x g according to manufacturer's instructions.

Study Outcome Measures:

An aliquot of the concentrated fat sample was shipped to BSR laboratories for analysis within 24hr of harvesting. A summary of the methods is listed below

- *Nuclear cell counts and cell viability:*
  The entire sample of adipose concentrate was digested with collagenase enzyme solution and the stromal vascular fraction (SVF) collected by centrifugation. The concentration of the nucleated cells was determined in the SVF by manual counting using a hemocytometer and fluorescent staining or by flow cytometer. Cell counts are reported as the concentration per ml of starting adipose concentrate.

- *Cell phenotype and estimate of adipose derived stem cell concentration:*
  The SVF cells were stained with fluorescent-labeled antibodies to CD45 a pan leukocyte marker) and CD31 a marker found on some white blood cells and on endothelial cells. Total nucleated cells were estimated by inclusion of the nuclear stain Syto-13. The fraction containing Adipose Derived Stem Cells (ASC) was determined by eliminating CD45 positive and CD31 positive cell populations from the nucleated cell population, as a maximum estimate of ASC. In separate experiments, 50% of cells in this fraction are positive for CD105, CD73 and CD90 ASC markers.

- Cell Viability:
  Viability was determined by dye exclusion (ethidium bromide homodimer) and with a viability stain (calcein AM) using a fluorescent microscope.

RESULTS:

Concentrated adipose samples from nine donors were analyzed. The nucleated cell counts expressed as per ml of starting concentrated adipose sample are shown in Table I.

Table I. Nucleated cell counts per ml of sample.

Harvest Date	Analysis Date	Sample ID	Cells x 10^5/ml sample
13 Dec 2011	14 Dec 2011	#3461436	8.0
27 Sep 2011	28 Sep 2011	#3463477	3.6
06 Sep 2011	07 Sep 2011	#3480166	9.8
30 Aug 2011	31 Aug 2011	#3459879	2.7
17 Aug 2011	18 Aug 2011	#7267895	4.6
17 Aug 2011	18 Aug 2011	#6634416	5.1
10 Aug 2011	11 Aug 2011	#3775709	4.7
04 Aug 2011	05 Aug 2011	#BSR 02	2.4
17 May 2011	18 May 2011	#BSR 01	5.0

The average cell count per ml of concentrated adipose sample was 5 x 105 with a range of 2.4 to 9.8 x 105. Sample # 341436 was also analyzed for cell viability. The percent viability of the nucleated cells was 81%.

The volumes of lipoaspirate processed and concentrated adipose tissue produced are shown in Table II.

Table II. Process volumes

Harvest Date	Analysis Date	Sample ID	LA Vol mL	Prod Vol mL
13 Dec 2011	14 Dec 2011	#3461436	44	20
27 Sep 2011	28 Sep 2011	#3463477	50	19
06 Sep 2011	07 Sep 2011	#3480166	25	17
30 Aug 2011	31 Aug 2011	#3459879	22	11
17 Aug 2011	18 Aug 2011	#7267895	26	12
17 Aug 2011	18 Aug 2011	#6634416	31	16
10 Aug 2011	11 Aug 2011	#3775709	26	12

The average aspirate volume was reduced by 2.1 fold (range 1.5 - 2.6) for seven samples.

Phenotypic analysis of SVF cells by flow cytometer is shown in Table III. The fraction containing the ASC is calculated by eliminating endothelial and white blood cells populations.

Table III. Percentage of total nuclear cells in the ASC fraction.

Harvest Date	Analysis Date	Sample ID	ASC%
30 Aug 2011	31 Aug 2011	#3459879	54%
17 Aug 2011	18 Aug 2011	#7267895	38%
17 Aug 2011	18 Aug 2011	#6634416	37%
10 Aug 2011	11 Aug 2011	#3775709	43%
04 Aug 2011	05 Aug 2011	#BSR 02	40%
17 May 2011	18 May 2011	#BSR 01	20%

The fraction containing the ASC constitutes on average 39% of the total nuclear cells in the SVF. The remainder is grouped as either CD45 positive, CD31 negative (lymphocytes); CD45 positive, CD31 positive (granulocytes); or CD 45 negative, CD31 positive (endothelial cells).

CONCLUSIONS:

Determining a concentration factor for the adipose product is difficult for the following reasons: a) Obtaining a representative sample is difficult because of the speed with which the sample separates into infranatant, fat and oil layers, and the great differences in viscosity between the various fractions. And b) The efficiency of digestion is difficult to assess and an assumption of consistent digestion between aspirate and concentrated product may not be valid. For these reasons, baseline aspirate samples are not included in the analysis.

The nuclear cell number per ml of adipose tissue is variable and highly sensitive to the harvest method. In our experience manual syringe methods produce higher average cell numbers compared to wall vacuum assisted aspiration. The average for these samples, 5.0 x 105 is consistent with our laboratory average of 4.8 x 105 cells per ml of decanted adipose tissue (tumescent fluid removed by allowing fluid to settle below the buoyant fat) from manual method aspiration (N=10) and is higher than our laboratory average of 1.7 x 105 cells per ml of decanted fat from vacuum assisted aspiration (N=20).

In Vitro Characteristics of Platelets Collected with the GenesisCS Concentrating System

IN VITRO TESTING

TEST RESULTS:

pH:

There were no pH values less than 6.6 for any CPP at Time 0 or + 4 hr. These values are within acceptable range for platelet concentrates. pH 6.2 correlates well with platelet survival and function3. While there was a statistically significant difference between the means for Time 0 CPP (6.74) and Time +4 hr CPP (6.70) the difference is not clinically significant.

P-selectin:

The in vitro p-selectin test is used to evaluate the quality of platelet products. Detection of p-selectin on platelet membranes correlates with platelet activation. High percentage of p-selectin positive platelets measured direct (unactivated) is associated with loss of viability. For comparison, the values of p-selectin for day 1 apheresis platelet concentrates collected on centrifugal equipment is approximately 8-23 percent4. The direct p-selectin values (averaging 14 percent, Table IV) observed for the Time 0 and +4 hr CPP from the GenesisCS were consistent with these values.

Functional reactivity of the platelets is demonstrated by adding an exogenous platelet agonist (ADP). The ADP-stimulated p-selectin values for Time 0 and +4 hr CPP were similar to ADP-stimulated values for paired whole blood samples. The low direct p-selectin values observed for the GenesisCS prepared CPP and the increase in p-selectin expression following exposure to ADP (averages greater than 60 percent) demonstrate the functional activity of the platelets is preserved.

Collagen-dependent Platelet Aggregation:

Platelet aggregation studies were performed using a collagen agonist. GenesisCS prepared CPP samples and their paired whole blood samples all had normal aggregation response (greater than 60 percent of maximum) with average values greater than or equal to 80 percent.

Hypotonic Stress Response:

The hypotonic stress response assay measures the ability of platelets to recover their resting volume after exposure to a hypotonic environment and demonstrates platelet membrane integrity5. The optical method used in this study is that of Valeri et al6 as modified by Farrugia et al7. The reported values are the percent of recovery of platelet volume (assessed by change in light transmission) in platelets diluted in water as compared to control platelets diluted in isotonic buffer. The observed hypotonic stress values for Accelerate-prepared CPP were similar for paired, whole blood samples.

Table IV: In Vitro Characteristics of Platelets Collected with the GenesisCS System. Mean ± 1 SD (Range)

Parameter	Whole Blood	Time 0 hr	Time 4 hr	P-Value (0 hr vs. 4 hr)
pH	6.78±0.07 (6.61-6.84)	6.74±0.05 (6.64-6.85)	6.70±0.03 (6.63-6.74)	.03
p-Selectin (%) Direct Measurement	1±4 (-2-10)	14±8 (1-24)	16±11 (4-33)	NS*
ADP (20 μM) Activation	63±7 (51-76)	64±10 (54-83)	69±10 (50-82)	NS*
Platelet Aggregation (%) Collagen agonist (190 μg/mL)	80±7 (68-91)	84±9 (66-97)	81±6 (66-87)	NS*
Hypotonic Stress Response	85±17 (43-107)	90±12 (64-110)	77±13 (55-95)	NS*

**NS=Not significant, p>0.05 Student's t-Test (paired, 1 tail)*

CONCLUSION:

These data have established that the GenesisCS system is capable of preparing a platelet concentrate suitable for the purpose intended. Testing from in vitro studies, intended to evaluate the quality of the platelets have demonstrated that the functional characteristics are compatible to those using predicate devices or standard blood bank techniques. The GenesisCS system provided consistent concentrated platelet product with predictable platelet yields and concentration factors.

REFERENCES

1. Marx R, Carlson ER, Eichstaedt RM. Platelet rich plasma: Growth factor enhancement for bone grafts. Oral Surg 85:638, 1998

2. Monteleone K, Marx R. Healing enhancement of skin graft donor sites with platelet rich plasma. 82nd Annual American Academy of Oral and Maxillofacial surgery meeting. Sept 22, 2000,San Francisco

3. Weibrich G, Kleis W, Hafner G. Growth factor levels in the platelet rich plasma produced by 2 different methods: Curasan-type PRP kit versus PCCS PRP system. Oral Maxillofac Implants 2002;17:184-190

4. Kallianinen L, Hirshberg J. Role of platelet-derived growth factor as an adjunct to surgery in the management of pressure ulcers. Plast Reconstr Surg 106: 1243, 2000

5. Rosenberg E, Dent H, Torosian J. Sinus grafting using platelet rich plasma- initial case presentation. Pract Periodont Aesthet Dent 2000; 12(9):843-850

6. Anitua E. The use of plama-rich growth factors (PRGF) in oral surgery. Pract Proced Aesthet Dent 2001;13(6):487-493

7. Hood A, Hill A, Reeder G. Perioperative autologous sequestration lll: a new physiologic glue with wound healing properties. Proceedings of the American Academy of Cardiovascular Perfusion, volume 14 January 1993

8. Green D, Whitman D, Goldman C. Platelet gel as an intraoperatively procured platelet based alternative to fibrin glue: program implementation and uses in noncardiovascular procedures. 1997 Presented at the proactive hemostasis management: The emerging role of platelets symposium. Jan 23-24, 1997 Aspen Co.

9. Powell D, Chang E. Recovery from deep-plane rhytidectomy following unilateral wound treatment with autologous platelet gel. Arch Facial Plast Surg/Vol3, Oct-Dec 2001

10. Man D, Plosker H, Winland-Brown J. The use of autologous platelet-rich plasma (Platelet gel) and autologous platelet poor plasma (fibrin glue) in cosmetic surgery. Plast. Reconstr Surg 107:229,2001

11. Robiony M, Polini F. Costa F. Osteogenesis distraction and platelet-rich plasma for bone restoration of the severely atrophic mandible: preliminary result. J Oral Maxillofac Surg 60:630-635, 2002

12. Whitman D, Berry R, Green D. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg 55: 1294-1299, 1997 PRP Protocols Page 7 of 10

13. Slater M, Patava J, Kingham K, Mason R. Involvement of platelets in stimulating osteogenic activity. Journal of Orthopedic Research 13: 655-663, 1995

14. Krupski W, Reilly L, Perez S. Moss K, Crombleholme P, Rapp J. A prospective randomized trial of autologous platelet – derived healing factors for treatment of chronic nonhealing wounds: A preliminary report. J Vasc Surg 1991; 14; 526 – 236.

15. Clark RAF. The Molecular and Cellular Biology of Wound Repair, 2nd ed. New York, London: Penum, 1996.

16. Saigaw, T, et al, "Clinical Application of Bone Marrow Implantation in Patients with Arteriosclerosis Obliterans, and the Association between Efficacy and the Number of Implanted Bone Marrow Cells, Circulation Journal, 68(12):1189-1193, 2004

17. Hernigou, P.H., "Percutaneous Autologous Bone Marrow Grafting for Nonunions. Influence of the Number and Concentration of Progenitor Cells", Journal of Bone & Joint Surgery, 97-A:1430-1437, 2005