ACCELLERATED BIOLOGICS

CATALOGUE 2015

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START HEALING AND GET BACK TO LIVING





1-800-367-0844
www.ACCELLERATEDBIOLOGICS.COM
801 Maplewood Drive Suite 15 Jupiter, FL 33458

ABOUT:

ACCELLERATED BIOLOGICS is uniquely positioned across the globe within the field of biologics. We are an independent medical distribution company focused on consulting and providing the physician, their staff and community with quality information and products related to Platelet Rich Plasma, Stem Cells, Bone Marrow Concentrate and other products that utilize biologics.

Our customers receive our ongoing commitment of supplying them with the most up to date information, the newest technologies and equipment because we firmly believe that not all PRP is created equal.

We have a commitment to all of our business partners. We want to provide cutting edge technology that ensures the practitioner has the knowledge, skill, and equipment to provide the best treatment options to their patients. That commitment leads us to all parts of the globe to find, secure, and distribute quality products that complement the use of autologous biologics.

PRP:

PLATELET RICH PLAMA (PRP)

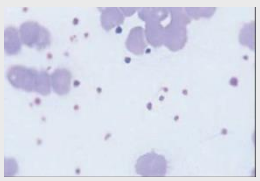
Platelet Rich Plasma (PRP) is concentrated from your own blood which contains healing factors, such as white blood cells and bioactive proteins, called growth factors and stem cell markers. These cells are vital for tissue regeneration and repair. Platelets, once thought of being responsible for only clotting, have been scientifically proven to be a reservoir of these vital healing components. With advanced techniques we are able to concentrate these regenerative healing cells in a simple outpatient setting.

PRP is from your own blood, autologous, so there is little to no risk when conducted by a trained professional. Since the cells are autologous there is no risk for an allergic or immune reaction. Side effects or complications with PRP are extremely rare.

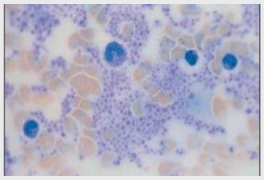
Patients can expect to see significant improvement in symptoms over the course of healing time. This procedure may eliminate the need for further invasive treatments, such as surgery or prolonged use of medications. While other treatments such as corticosteroid injections may provide temporary relief and stop inflammation, PRP injections stimulate healing of the injury over a shorter time period with less side effects. Patients usually report a gradual improvement in symptoms and return of function. Many patients require two to three treatments to obtain optimal results and may even experience a dramatic return of function and relief within 2-3 months.

NOT ALL PRP IS THE SAME

PRP products differ both qualitatively & quantitatively. It is well documented that not all PRP is the same. Patients may experience varying outcomes with PRP applications. This can be attributed to the system used to prepare the PRP. To get the best results, the PRP system must significantly concentrate the platelet growth factors in the treatment sample. The better the concentration, the better the chances for recovery.



Normal Platelet Count



Concentrated Platelet Count

PRP:

ACCELLERATED BIOLOGICS

GenesisCS Component Concentrating System by EmCyte

ACTIVE DISPLACEMENT DISC TECHNOLOGY (ADDT)

Direct access to the platelet and bone marrow aspirate buffycoat layer in a closed system environment. This is done in only 2 sterile barrier entries designed to reduce handling and improve sterility maintenance.

FULL SWINGING BUCKET CENTRIFUGATION

Optimizes separation & enhances the buffycoat concentrate. The Executive Series Centrifuge II provides full vertical to horizontal separation in a smooth and unhindered motion. There is no platelet slip phenomenon.

SOFT BRAKING TECHNOLOGY

With soft braking technology the acceleration and deceleration are both controlled to prevent the buffycoat from re-suspending into the plasma after separation.

CONSISTENT OUTCOME REGARDLESS OF HEMATOCRIT

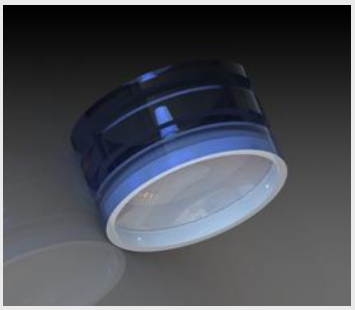
With Active Displacement Disc Technology the operator has the flexibility to access the buffycoat concentrate at any point of separation. The GenesisCS System is not volume dependent or hematocrit dependent. Physicians adore the versatility of GenesisCS and enjoy using a system that can respond to their specific needs.

CLOSED SYSTEM PROCESSING

GenesisCS is a closed system throughout. Trapping blood within a watertight sealed environment maximizes the safety of the contents and ensures sterility throughout processing.

PRP Concentrating Systems:

GS30 - Platelet Concentrating system 30mL
GS60 - Platelet Concentrating System 60mL
GS120 - Platelet Concentrating System 120mL



Pure PRP®:

The NEW Pure PRP® system is revolutionary. It is the only tabletop point of care system in its class to provide platelet concentrations of up to 16x baseline in 7ml of Pure PRP®. This results in a delivery of over 16 billion platelets to the treatment site, which is 3 to 4 times greater than the nearest competitor in the same volume of PRP. All delivered with the fewest amount of red blood cells.

The NEW Pure PRP® Concentrating System contains a Concentrating Accessory which is specially designed to improve the processing technique and performance outcomes of Pure PRP®. This PATENT PENDING & precedence setting process offers the highest concentrations of platelets and the largest volume of deliverable platelets in a 7ml treatment sample, when compared to other leading brands.

Pure PRP Concentrating Systems:

GS60-Pure II - Pure Platelet Concentrating system 60mL
GS120-Pure II - Pure Platelet Concentrating System 120mL

GS60-Pure II

GS120-Pure II

Pure PRP®

Lowest RBC content available (<0.2 109/mL)

Normal pH (7.5)

End user control over neutrophil content

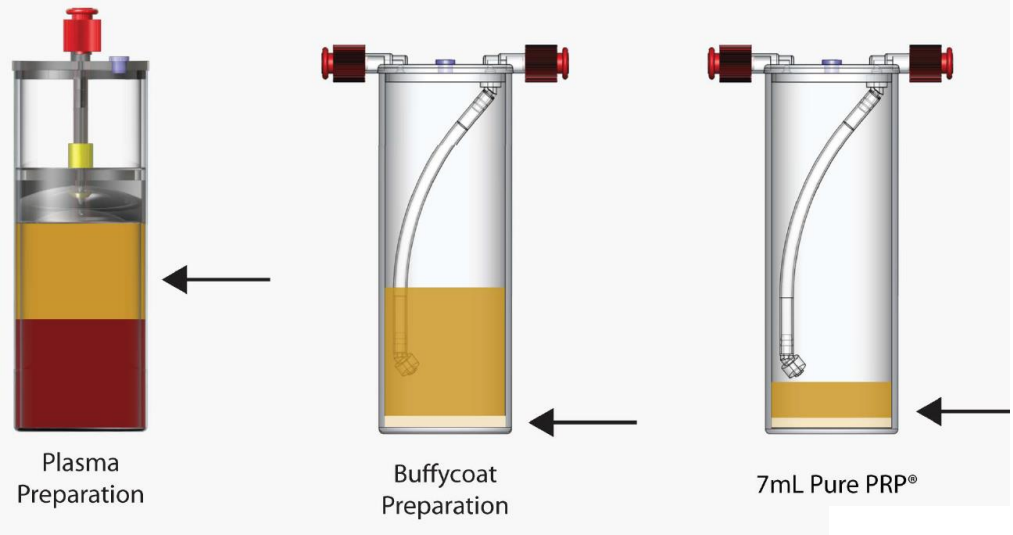
Lowest PRP viscosity available

The highest cell concentrations and volume of deliverable Platelets

Monocytes

Pure PRP®:

Pure PRP® 60mL system



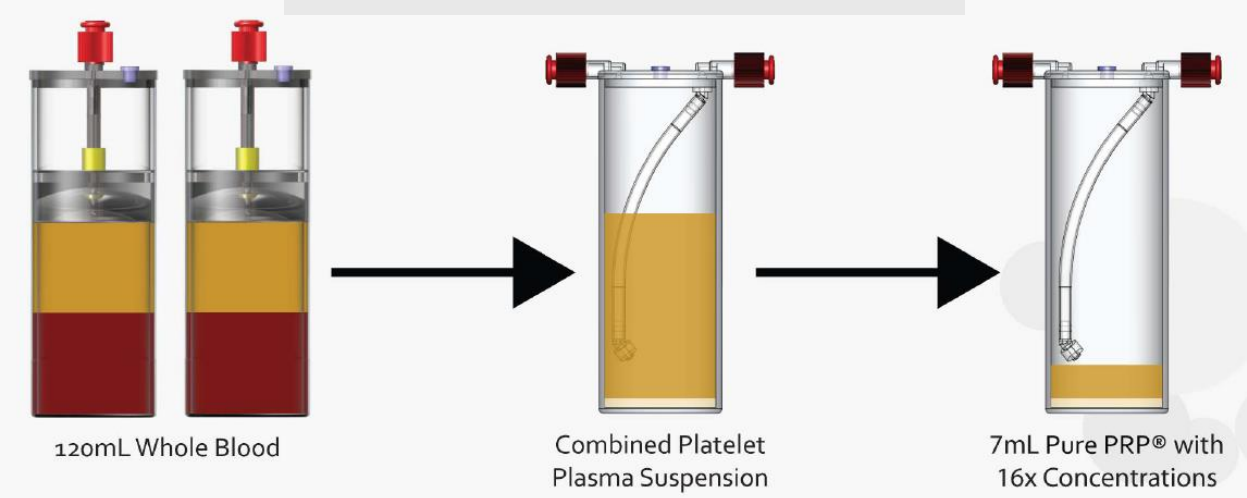
Pure PRP® 120mL system

16X Platelet Concentrations in 7mL
Over 16 billion Platelets Delivered in 7mL
No Red Blood Cells
Normal pH

The most powerful tabletop system available

A therapeutic treatment with resounding capabilities

Patent pending for your protection needs



Pure PRP®:

Lowest Red Blood Cells (RBCs) content available
The NEW Pure PRP® stays true to its technology and superior benefits, providing high concentrations of platelet & growth factors in a pure plasma suspension with less than .2 109/mL red blood cells. Red blood cells in PRP increases viscosity, making application more difficult for many physicians. Red blood cells in PRP has also been reported to cause pain and subsequent inflammation.

No pH Buffering Required. Pure PRP® has Normal pH
Pure PRP® is the ONLY PRP system that consistently provide PRP at a normal pH, without the need for buffering. Most PRP systems provide acidic PRP (pH <7-35) which requires buffering prior to application. Without buffering acid PRP can increase pain and discomfort at the application site. Pure PRP® eliminates the discomfort and improves the patient outcome making it the Perfect State-Of-The-Art .

An Independent Review of Pure PRP® vs other Leading Brands

Principle Investigator(s): Dr. Robert Mandle Ph.D.
BioSciences Research Associates, Inc.
Pure PRP® vs SmartPReP2®, March 12, 2013

Platform	Platelet x 10^6/ml	Recovery	Concentration	Hct (%)	pH in PRP	PRP volume (mL)
Pure PRP®	1128 (319)	76% (4)	6.7 (0.3)	1.1 (0.6)	7.5 (0.1)	6.6 (0.2)
SmartPREP2 APC60	1075 (262)	69% (11)	6.9 (0.9)	34.1 (12)	6.8 (0.1)	6.9 (0.2)

Principle Investigator(s): Dr. Robert Mandle Ph.D.
BioSciences Research Associates, Inc.
Pure PRP® vs Angel Whole Blood Separation System, March 7, 2014

Platform	Platelet x 10^6/ml	Recovery	Concentration	Hct (%)	pH in PRP	PRP volume (mL)
Pure PRP®	1067.6 (253)	68% (9.8)	6.9 (0.7)	2.8 (1.9)	7.44 (0.1)	7.4 (0.4)
Angel PRP	774 (324)	46% (6.3)	4.7 (0.7)	3.3 (0.7)	7.17 (0.7)	6.9 (0.2)

Distributed by Accellerated Biologics, 801 Maplewood Dr. Suite 15, Jupiter, FL 33458
A product of EmCyte Corporation, Fort Meyers, FL 33912

Platelet Sample Analysis:

ANALYZING PLATELET SAMPLES

Concentrated platelet samples prepared with the GenesisCS concentrating System tend to have higher platelet concentrations when compared to other systems. High concentrations of platelets suspended in low plasma volumes may clump together. When analyzing platelet samples attained with the GenesisCS Concentrating Systems the following procedures are recommended for accurate results.

Blood Analyzer

1. Platelet counts are best measured in the Beckman Coulter ACT 5, or any approved blood analyzer of similar or better quality.
2. The PRP test sample must be placed in an empty red top PLASTIC tube containing no anticoagulant. Glass tubes may activate platelets causing an inaccurate reading.

De-Clumping

3. To help remove platelet clumping prior to testing, the PRP sample **MUST** be placed on a rocker for a minimum of 1 hour. The PRP sample must mix with at least 1mL of air inside the sample tube or syringe. GenesisCS PRP samples are stable for up to 4 hours after collection.

Pre Testing Preparation

4. After the 1 hour de-clumping period is completed, the test sample **MUST** be diluted to 50% using an approved isotonic solution.

Analyzing the Results

5. Once the sample results are attained, the CBC (WBC, RBC, HGB, HCT, PLT) results will represent 50% of the sample and then must be multiplied by 2 to attain the final results.
6. The WBC differentials (NE, LY, MO, EO, BA) percentages reflect the correct results without multiplying by 2 because the percentages remain constant throughout the dilution procedure.

Formulation

7. The Platelet yield is calculated using the following formulation

8. **Yield =** $$\frac{PLT_{PRP} \times PRP\ volume}{PLT_{start} \times Process\ volume}$$

   a. PLT_{PRP} = Platelet count in PRP sample
   b. PRP_{volume} = Total volume of the PRP collected (not just the volume used for testing
   c. PLT_{start} = Baseline platelet count of the blood sample with anticoagulant
   d. $Process_{volume}$ = Total volume of collected whole blood with anticoagulant

PureBMC®:

PureBMC® is the new standard and the flawless solution to Bone Marrow Cell Concentrate. PureBMC® is proven to concentrate viable platelets, hematopoietic stem cells (HSC), total nucleated cells (TNC) and mesenchymal stem cells (MSC) in a bath of plasma with a hematocrit of less than 15%. PureBMC® can be prepared without the use of Heparin, allowing it to provide viable platelet concentrates that further add to the strength of the composition. After years of development, EmCyte Corporation's PureBMC® delivers the excellence and reliability physicians can depend on.

An Independent Review of EmCyte PureBMC® vs Standard BMC

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

BioSciences Research Associates, Inc.

Table 1. BMC Total Nucleated Cell (TNC) Concentration Factor & Total Cell Count

System	BMA Baseline (TNC x 10^6/mL)	BMC (TNC x 10^6/mL)	Concentration Factor	BMC Total Cell Count (TNC/BMC Sample)
BMC	17	70.4	4.1	457,600,000
PureBMC®	17	155	9.1	1,162,500,000

Table 2. BMC Hematopoietic Stem/Progenitor Cells (HSC) Concentration Factor & Total Cell Count

System	BMA Baseline (HSC/mL)	BMC (HSC/mL)	Concentration Factor	HSC Total Cell Count (HSC/BMC Sample)
BMC	92,332	342,844	3.7	2,228,486
PureBMC®	92,332	1,230,172	13.3	9,226,290

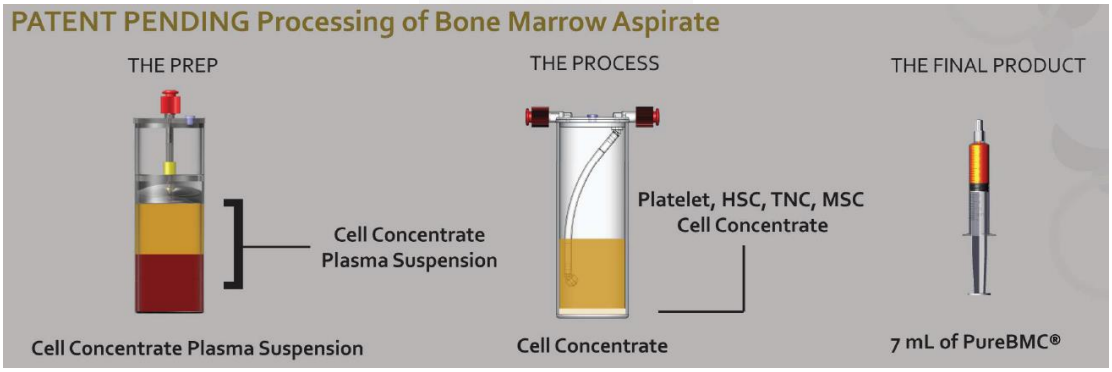
Table 3. BMC Platelet Concentration Factor & Total Cell Count

System	BMA Baseline (PLT x 10^6/mL)	BMC (PLT x 10^6/mL)	Concentration Factor	PLT Total Cell Count (PLT/BMC Sample)
BMC	96	326	3.4	2,119,000,000
PureBMC®	96	534	5.6	4,005,000,000

PureBMC®:

PureBMC®

Hematopoietic stem cells, total nucleated cell, mesenchymal stem cell and platelet isolation is perfected in the EmCyte PureBMC® system. Similar to the Pure PRP®, PureBMC® is designed to retain high concentrations of these multiple cell concentrates with the lowest concentrations of red blood cells in a bone marrow concentrate product. Using a specialized cell isolation technique, PureBMC® provide more than 9 times cell concentration in 7mL of PureBMC®. Preparation times ae less than 10 minutes at the point of care. With careful attention to the details of gradient cell isolation, PureBMC® has become the new standard for cell concentrates from a sample of bone marrow aspirate.



PureBMC® Sample Size: 7 mL
Total Nucleated Cell Concentrations: 9.1 X Baseline or Greater
Hematopoietic Stem/Progenitor Cells: 13.3 X Baseline or Greater
Platelet Concentrations: 5.6 X Baseline
Hemotocrit: Less than 15% on Average

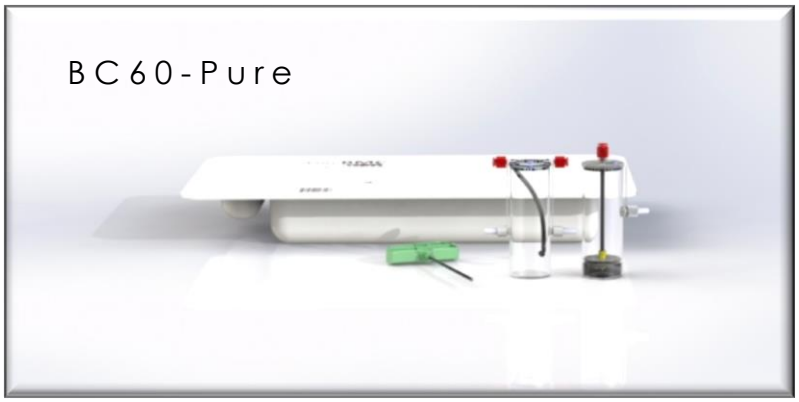
Independently Reviewed at the Bioscience Research Associates
Cambridge MA

PureBMC®:

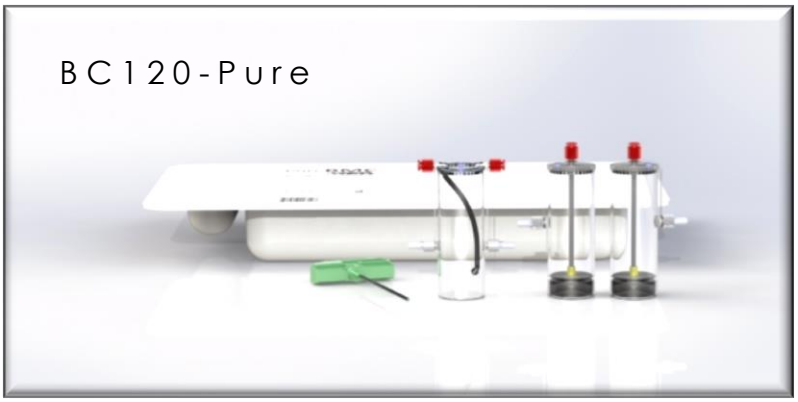
PureBMC® Concentrating Systems:

BC60-Pure - PureBMC® Concentrating System 60mL
BC120-Pure - PureBMC® Concentrating System 120mL

BC60-Pure



BC120-Pure



PureBMC®

HCT < 15% on Average

Normal pH (7.5)

Low viscosity

The highest cell concentrations and volume of deliverable

Hematopoietic Stem Cells

Total Nucleated Cells

Mesenchymal Stem Cells

Platelets

BMC:

BMC Concentrating Systems:

GSBMA-60 - Bone Marrow Concentrating System 60mL
GSBMA-120 - Bone Marrow Concentrating System 120mL

PRP Plus:

Pure PRP PLUS:

PL6050 - Pure PRP PLUS

Equipment:

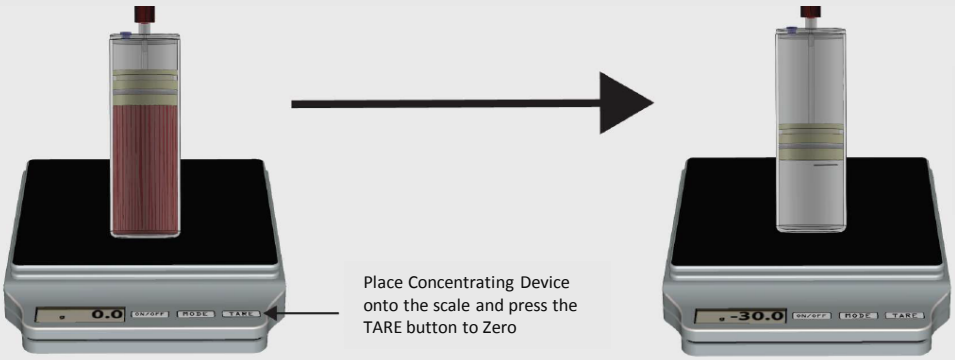
Executive Series Centrifuge II:

GS-022624340-ACB - AcCELLerated Biologics Executive Series Centrifuge II

Additional Equipment:

One Step Counterbalance with Tare Counterweight Scale

Eliminate the guess work and time spent counterbalancing Pure PRP® Concentrating Devices. With the new EmCyte Tare Counterweight Scale you are told exactly how much volume to add or remove from the counterbalance device, all in one easy step.



Place Concentrating Device onto the scale and press the TARE button to Zero

Place the counterbalance device onto the scale and get a digital read-out of exactly how much volume to add or remove for a perfect counterbalance.

For Example:

-30 g means add 30mL to balance

3 g means remove 3mL to balance

Aerosol Contained Lightweight Centrifugal Buckets

The new centrifugal buckets by EmCyte Corporation with aerosol containing caps to further improve the sterile handling of the concentrating devices. The buckets are also 30% lighter than its predecessor, causing a reduction in centrifugal load. This enhanced feature further improves cell separation during centrifugation and also promotes the longevity of the centrifuge machine.

PRP and Stem Cell Workstation

Easily store, move, and use the EmCyte PRP and Stem Cell kits with this workstation cart. The cart is rated to hold over 300lbs and the rubber wheels and braking system ensure a quiet and reliable spin for all kits.

ADI LIGHT 2:

PAPRP-PhotoActivated Platelet Rich Plasma AdiLight-2

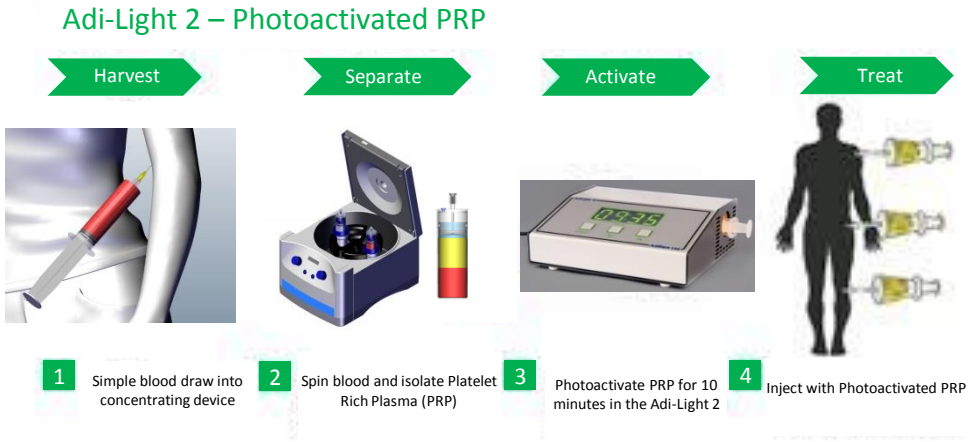
What is PhotoActivation?

The wavelength, or bandwidth of wavelengths, is one of the critical factors in selective photomodulation. Pulsed or continuous exposure, duration and frequency of pulses (and dark 'off' period) and energy are also factors as well as the presence, absence or deficiency of any or all cofactors, enzymes, catalysts, or other building blocks of the process being photomodulated. Different parameters with the same wavelength may have very diverse and even opposite effects. When different parameters of photomodulation are performed simultaneously, different effects may be produced. When different parameters are used serially or sequentially, the effects are also different.

The selection of wavelength photomodulation is critical as is the bandwidth selected as there may be a very narrow bandwidth for some applications —in essence these are biologically active spectral intervals. Generally the photomodulation will target flavins, cytochromes, iron-sulfur complexes, quinines, heme, enzymes, and other transition metal ligand bond structures but is not limited to these.

Using the patient's own blood, the specially prepared PRP platelets are taken and re-injected into the same patient's affected area. The whole simple process is performed in the physician's clinic on the same day —a 'point of service' treatment. These platelets release growth factors that lead to accelerated tissue healing. By using the concentration of platelets, the activated growth factors promote temporary relief and stop inflammation —creating a painless and faster healing treatment.

Note: PRP also signals the body to call in stem cells to repair any area of injury. Stem cells also encourage damaged cells to repair themselves.



ADI LIGHT 2:

How PhotoActivation Works
As a concentrated source of platelets, PRP contains several different growth factors and other cytokines that accelerate and enhance the healing of bone and soft tissue. The PRP is then activated under AdiLight-2 for 10 minutes since this has been shown to significantly reduce pain and further accelerate healing.

While PRP treatment (without photoactivation) is fast becoming a popular new treatment for muscular and skeletal injuries, it is also known to cause aggravated pain in the affected area for 2-10 days after injection.

AdiStem Ltd. has researched the effect of different monochromatic light intensities and frequencies in the colored spectrum on various human and animal cell populations such as mesenchyme stem cells and white blood cells. The company has found that low-level light photoactivation or photomodulation can be utilized for significant benefit in stimulating the proliferation, differentiation, and inhibition/induction release of growth factors/cytokines of cells from any living organism.

Healing is Accelerated and Post-Treatment Pain for PRP Patients Reduced:
Once the PRP is prepared, it is activated briefly using AdiLight-2 before being injected back into the affected area. In most cases, photoactivation using AdiLight-2 increases Interleukin-1 Receptor Antagonist (IL-1RA) which decreases the pain and inflammation associated with PRP injections. In other cases, the duration of any pain is significantly reduced.

Benefits to Doctors Using PhotoActivation:
- The PhotoActivation Process Takes Only 10 Minutes.
- AdiLight-2 is Simple to Use. No Monitoring Required.
- No Training Necessary.
- Can be Used with Any High Quality PRP Kit.
- Used for both Orthopedic or Cosmetic PRP Applications
- Protocol: One Injection per Week for 3 Weeks.

ADI LIGHT 2:

How does AdiLight-2 work?
AdiStem Ltd. has researched the effect of different monochromatic light intensities and frequencies in the colored spectrum on various human and animal cell populations such as mesenchyme stem cells and white blood cells.

Low-level light photoactivation or photomodulation can be utilized for significant benefit in the stimulation of proliferation, differentiation, and inhibition/induction release of growth factors/cytokines of cells from any living organism.

The wavelength or bandwidth of wavelengths is one of the critical factors in selective photomodulation. Pulsed or continuous exposure, duration and frequency of pulses (and dark 'off' period) and energy are also factors as well as the presence, absence or deficiency of any or all cofactors, enzymes, catalysts, or other building blocks of the process being photomodulated.

Different parameters with the same wavelength may have very diverse and even opposite effects. When different parameters of photomodulation are performed simultaneously, different effects may be produced. When different parameters are used serially or sequentially, the effects are also different. The selection of wavelength photomodulation is critical as is the bandwidth selected as there may be a very narrow bandwidth for some applications —in essence these are biologically active spectral intervals.

AdiStem has ongoing international research projects looking at the effects of different frequencies of monochromatic lights on various cells including mesenchyme stem cells and white blood cells. It has now found five frequencies (three are present in AdiLight-2) that can activate stem cells, in vitro, and two frequencies that inhibit them. AdiStem has also found similar frequencies to modulate pro-inflammatory and anti-inflammatory cytokine release from peripheral blood white blood cells. AdiStem is also exploring the direct effect of different low-level frequencies of light on endogenous cells (in vivo).

ADI LIGHT 2:

AdiLight-2

AdiLight-2 is available from AcCELLerated Biologics for use in activating mesenchyme stem cells and modulating cytokine release by white blood cells.

Mesenchyme Stem Cells

When adipose-derived mesenchyme stem cells are taken out of a subject most of the cells are in a dormant state. In the body, stem cells and progenitor cells need to be activated by a physiological repair mechanism cascade, for example release of growth factor and chemokines by platelets. When the adipose-derived stem cells are photoactivated for 20 minutes with the AdiLight-2 device they show increased proliferation, increased production of integrins, vascular endothelial growth factor, thymosin beta 4 and interleukin 1 receptor antagonist. Hence, AdiLight-2 is of value in providing consistent clinical results, especially amongst age differences.

Peripheral Blood White Blood Cells

For many years internal medicine specialists in Eastern Europe and Korea have been using the photoactivation of blood, in vitro and in vivo, with various frequencies of light for immunomodulation in patients. When peripheral blood white blood cells (WBC) are photoactivated under AdiLight-2 for 10 minutes, an inhibition of pro-inflammatory cytokines (IL1, IL2, IL6and TNF alpha) and induction of anti-inflammatory cytokines (IL1Ra and IL10) and beta endorphins are observed.

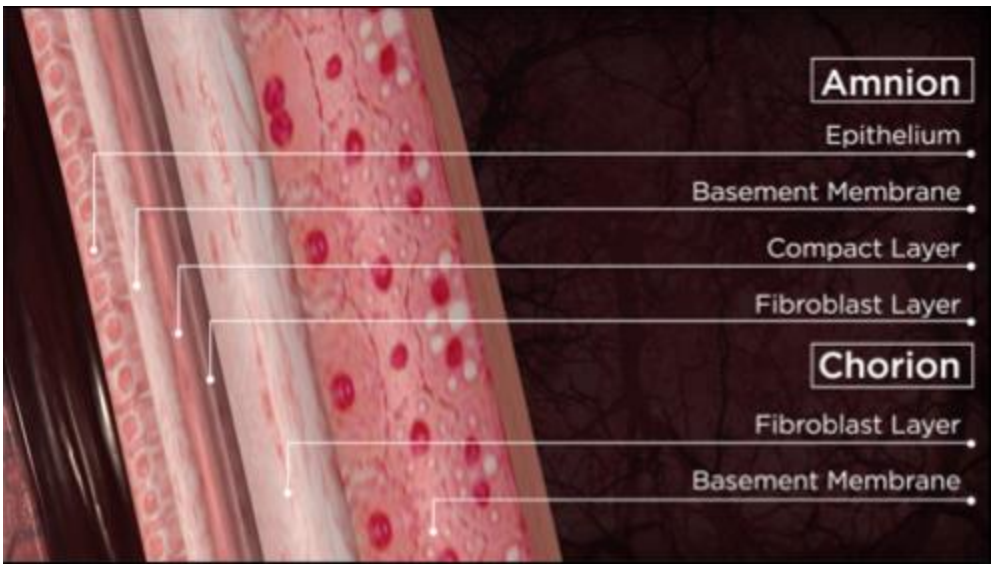
Reduces Pain and Accelerates Healing

Because of this property we have found AdiLight-2 to be a beneficial add-on to commonly used platelet rich plasma procedures in orthopedic and sports medicine procedures. One of the largest clinical drawbacks of the use of PRP in musculoskeletal healing is the aggravation of pain observed in the injected area post injection. Working with a group of Australian sports medicine specialists, we have deduced that a 10-minute exposure of WBC and platelets to AdiLight-2 prior to injection eliminates the aggravation of pain and potentiates the accelerated healing of PRP. It combines the benefit of autologous conditioned serum (ACS) with PRP in a simple 10-minute exercise.

Amnio Fix:

Human amniotic membrane allografts have been used for a variety of reconstructive surgical procedures since the early 1900s. The use of the amniotic membrane as an allograft has accelerated due to the development of the PURION® Process, which among other things allows the tissue to be dehydrated and sterilized.
The proprietary PURION® Process safely and gently separates placental tissues, cleans and reassembles layers, and then dehydrates the tissue to preserve the key elements associated with healing. The PURION® Process removes blood components while protecting the delicate scaffold of the amniotic membrane, leaving an intact extracellular matrix. The result is a durable graft with natural barrier properties that offers clinicians a clear advantage in soft tissue applications. PURION® processed dehydrated human amnion/chorion allografts can be stored at ambient conditions for up to five years. The proprietary process has been specifically designed to deliver a clinically effective and minimally manipulated allograft tissue. All placental tissues are recovered under sterile conditions from patients who have been screened for underlying infectious disease. No chemicals are used in the PURION® Process that might result in chemical cross-linking or decellularization.

Human amniotic membrane is comprised of the innermost layer of the placenta and lines the amniotic cavity. The membrane is composed of multiple layers including a single layer of epithelial cells, a basement membrane and an avascular connective tissue matrix. The tissues of the placenta present a very complex interrelationship of materials that possess numerous physiologic characteristics, that can in turn change in importance with the appropriate stage of gestation. During pregnancy, the placenta permits the passage of nutrients, metabolites and metabolic gases, and provides physical and immunological protection to the developing fetus. In addition, it produces a variety of steroids and important metabolic hormones.8
Amniotic membrane is a unique material and its composition contains collagen types I, III, IV, V, and VII. Amniotic membrane is composed of structural extracellular matrix (ECM), that also contains specialized proteins fibronectin, laminins, proteoglycans and glycosaminoglycans. In addition, amniotic membrane contains essential, active, healing growth factors such as epidermal growth factor (EGF), transforming growth factor beta (TGF-b), fibroblast growth factor (FGF), and platelet derived growth factor (PDGF).8 Amniotic tissues have shown little to no HLA-A, B, C antigens and β2 microglobulin.3



AmnioFix Sizes:

20mg – single dose vial
40mg – single dose vial
100mg – single dose vial

MARROW CELLUTIONS™

"A QUANTUM LEAP IN BONE MARROW ASPIRATION"

- TO BE USED WITHOUT A CENTRIFUGE
- MARROW ASPIRATE OF STEM CELLS AND CFU'S COMPARABLE WITH LEADING CONCENTRATION SYSTEMS IN THE MARKETPLACE
- AS LITTLE AS 6-10 cc's OF BMA REQUIRED
- All THE COMPONENTS REQUIRED IN ONE SIMPLE KIT

*Independent Data available from 4 separate labs

"This is potentially a giant step in bone marrow processing. I am always suspicious that when we centrifuge bone marrow aspirate we might be throwing away significant aspects of the "bone marrow soup". With these new needles I see only positives and no negatives. This needle will usher in a new age in bone marrow aspirations. There is no stopping the advance of technology.

Dr. Joseph Purita M.D.
Orthopedic Surgeon
Boca Raton, FL

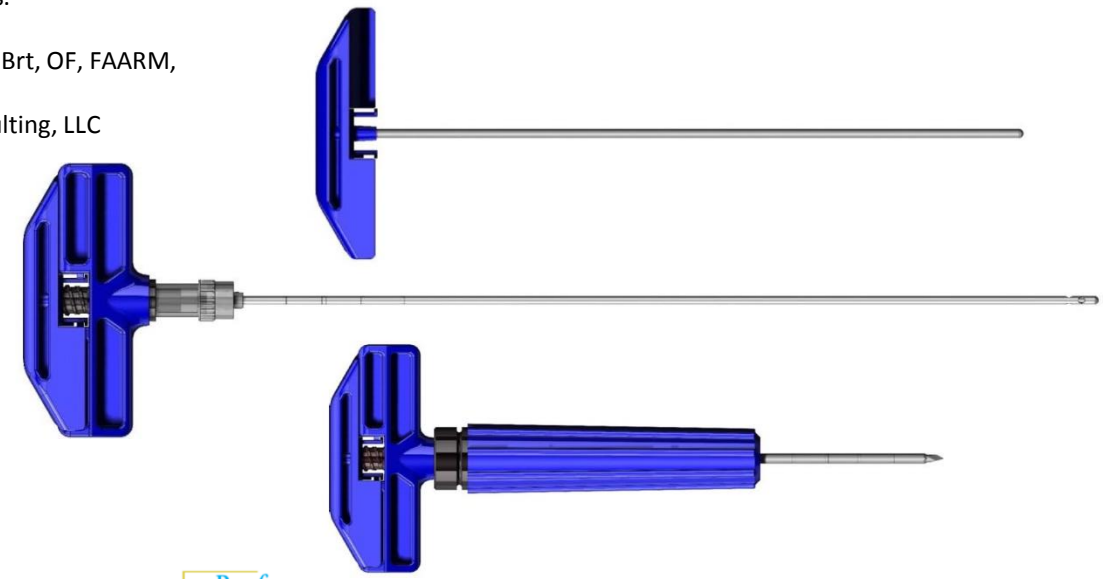
MARROW CELLUTIONS™, a breakthrough aspirating needle offering high concentrations of Stem Cells in an office setting in less than 10 minutes

Total nucleated cell (x10^6/mL) Marrow-Cellution, Traditional and Traditional Needle Centrifuged.

"In the initial pilot cases, Marrow Cellutions provided an extremely high quality cellular product as well as dramatically reduced the peripheral blood of traditional needle bone marrow aspiration. Moreover, the CFU-Fs and $CD34^+$ cell counts were at least as good as post-centrifugation with a traditional needle.
Combining the advantages of the new needle with centrifugation potentially allows for a "hyper-concentrate" to be produced. Such a combination could provide an ideal solution for even the most difficult-to-treat patients. "

Dr David B Harrell, PhD, Brt, OF, FAARM, FRIPH, DABRM
Harrell BioScience Consulting, LLC
Cambridge, MA

Patient ID	Marrow-Cellution	Traditional needle	SmartPrep concentrate Traditional aspirate centrifuged	Volume of concentrate after centrifugation
4	43.5	18.7	69.3	10
5	30.8	8.6	29.9	10
6	86.1	21.4	126.8	7
7	35.6	9.8	50	7
9	29.6	10.6	28.4	10
Average	45.12	13.8	60.9	8.8



USA pat pending and international patent pending
Manufactured by RANFAC, Avon, MA USA

PRPen:

For years health professionals have depended on surgery, chemical peels, microderm abrasions and fractional laser procedures as the only answers for patients wishing to remove signs of aging, rejuvenate skin tone, reduce scaring and make other cosmetic repairs. These treatments, while effective, are expensive, painful, cause bleeding, and require long recovery periods.

Finally an offering is available to physicians and technicians that provides similar or better results, without surgery, with less downtime, at a fraction of the cost.

With your new device, you will be able to visibly tighten the skin of the face, neck, décolleté and hands, for a more youthful appearance that your patients will love.

Helping healthcare professionals boost business results is why the popularity of the PRPen is skyrocketing. The procedure is affordable for both professionals and patients and the results are remarkable.

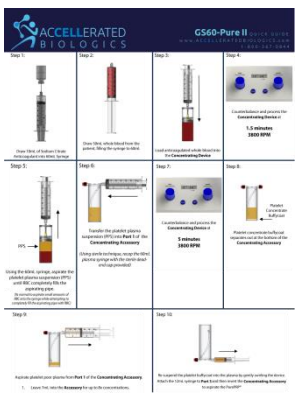
Because the ***PRPen*** allows you to administer bio-active agents you know and find effective along with the microneedle treatment, it is a safe new option patients will understand.

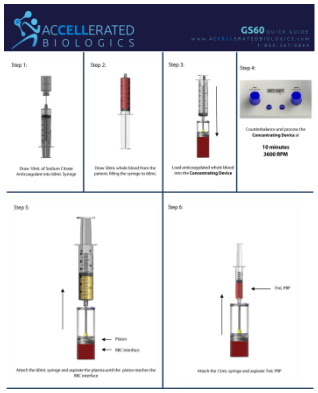
- **Best treatment for youthful skin – No surgery**
- **Excellent results — Micro-needle precision**
- **Fast recovery — Minimally invasive**

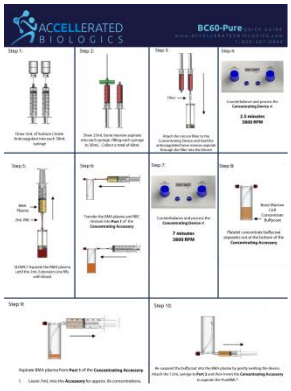
MARKETING SUPPORT:

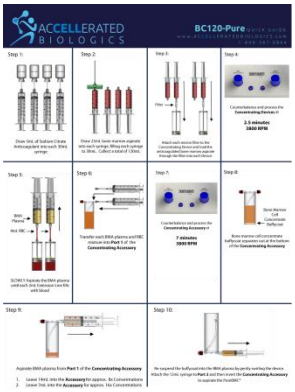
Patient Education Brochures:

Quick Guides:









MARKETING SUPPORT:

A revolutionary approach for patient care, outreach, surveys, ASAP Texts, E-Newsletters to patients and other customizable marketing concepts.

Ac**CELL**erated Biologics' Marketing programs include ways to enhance your online reputation, boost your search ranking, and educate existing and prospective patients about Stem Cells, PRP, or any other specialty you want to promote. These customized programs will increase your marketing and efficiency efforts and will show measurable results over the next 4 quarters.

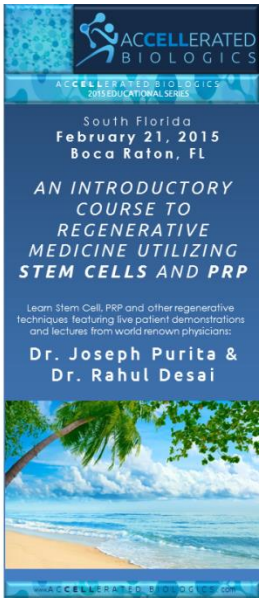
EDUCATIONAL SERIES:

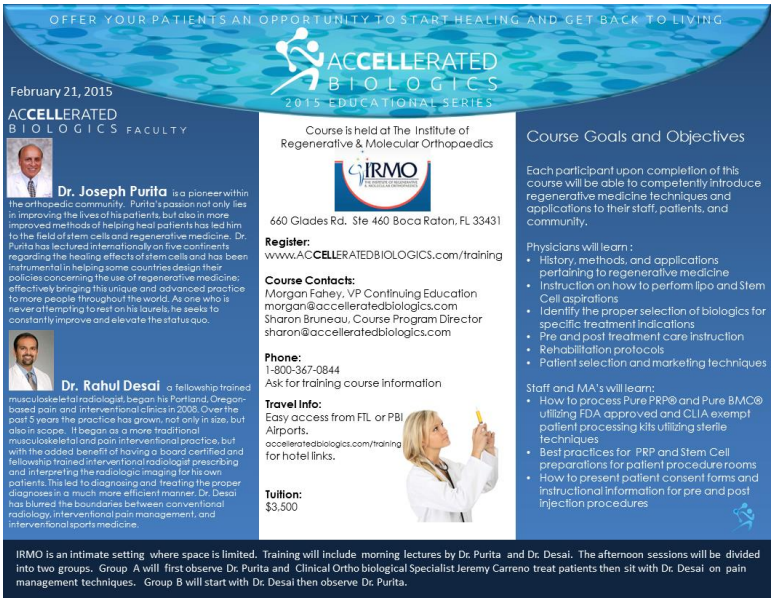
AcCELLerated Biologics is proud to offer a continuing educational series of workshops, courses, webinars, and training conferences to provide the practitioner comprehensive training for Stem Cell aspiration, PRP and other autologous biologic products.

Our Educational Series has courses all throughout the United States. The facilities and training centers we use are all first class medical centers with a wide array of options for the doctors to learn in.

Our staff physicians are leaders in regenerative medicine and other autologous biologic practices. These world renown physicians take great pride and passion in improving the methods of helping patients.

Educational Series courses vary depending on location and doctors but will all be focused on PRP and Stem Cell therapies.





REFERENCES:

- Foster, TE, Puskas, BL, Mandelbaum, BR, Gerhardt MB, Rodeo SA. **Platelet-rich plasma: from basic science to clinical applications.** *Am. J. Sports Med. 37, 2259–2272 (2009).*
- Gosens T, Peerbooms JC, van Laar W, den Oudsten BL. **Ongoing positive effect of platelet-rich plasma versus corticosteroidinjection in lateral epicondylitis: a double-blind randomized controlled trial with 2-year follow-up**. *Am. J. Sports Med. 39(6),1200–1208 (2011).*
- Mishra A, Woodall J Jr, Vieira A. **Treatment of tendon and muscle using platelet-rich plasma**. *Clin. Sports Med. 28(1), 113–125 (2009).*
- de Vos RJ, Weir A, van Schie HTM et al. **Platelet-rich plasma injection for chronic achilles tendinopathy: a randomized controlled trial**. *JAMA 303(2), 144–149 (2010).*
- Nguyen RT, Borg-Stein J, McInnis K. **Applications of platelet-rich plasma in musculoskeletal and sports medicine: an evidence-based approach**. *PM R. 3(3), 226–250 (2011)*
- 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 49(4), 771–778 (2009).*
- Lisa A. Fortier, Hollis G. Potter, Ellen J. Rickey, Lauren V. Schnabel, Li Foong Foo, Leroy R. Chong, Tracy Stokol, Jon Cheetham and Alan J. Nixon**Concentrated Bone Marrow Aspirate Improves Full-Thickness Cartilage Repair Compared with Microfracture in the Equine Model***J Bone Joint Surg Am. 2010;92:1927-1937. doi:10.2106/JBJS.I.01284*
- Kenneth Mautner, Gerard Malanga2 & Ricardo Colberg **Optimization of ingredients, procedures and rehabilitation for platelet-rich plasma injections for chronic tendinopathy***10.2217/PMT.11.56 © 2011 Future Medicine Ltd Pain Manage. (2011) 1(6), 523–532 ISSN 1758-1869*
- Donna D. Alderman, DO, Robert W. Alexander, MD, DMD, FICS, Gerald R. Harris, DO, Patrick C. Astourian, MS, PA-C **Stem Cell Prolotherapy in Regenerative Medicine Background, Theory and Protocols***JOURNAL of PROLOTHERAPY | VOLUME 3, ISSUE 3 | AUGUST 2011*
- Kevin S. Sadati, DO; Anthony C. Corrado, DO; Robert W. Alexander, MD, DMD **Platelet-Rich Plasma (PRP) Utilized To Promote Greater Graft Volume Retention in Autologous Fat Grafting** *The American Journal of Cosmetic Surgery Vol. 23, No. 4, 2006*
- Julien Ben Freitag,1 Adele Barnard **CASE REPORT To evaluate the effect of combining photo-activation therapy with platelet-rich plasma injections for the novel treatment of osteoarthritis***BMJ Case Rep 2013. doi:10.1136/bcr-2012-007463*
- Feng Lin, Steven F Josephs, Doru T Alexandrescu, Famela Ramos, Vladimir Bogin, Vincent Gammill, Constantin A Dasanu, Rosalia De Necochea-Campion, Amit N Patel, Ewa Carrier, David R Koos **Lasers, stem cells, and COPD** *Journal of Translational Medicine 2010, 8:16*
- Hernigou, et al, **"Percutaneous Autologous Bone-Marrow Grafting for Nonunions: Influence of the Number and Concentration of Progenitor Cells"**, *Journal of Bone and Joint Surgery, 87:1430-1437, 2005*
- Yaokai Gan a, b, Kerong Dai, Pu Zhang, Tingting Tang, Zhenan Zhu, Jianxi Lu a **The clinical use of enriched bone marrow stem cells combined with porous beta-tricalcium phosphate in posterior spinal fusion Biomaterials***29 (2008) 3973–3982*
- Witko-Sarsat, V; Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L (2000). **"Neutrophils: molecules, functions and pathophysiological aspects"**. *Lab Invest 80 (5): 617–53. doi:10.1038/labinvest.3780067. PMID 10830774.*
- Klebano , SJ; Clark, RA (1978). **The Neutrophil: Function and Clinical Disorders.** Elsevier/North-Holland Amsterdam. ISBN 0444800204.
- Nathan, C (Mar 2006). **"Neutrophils and immunity: challenges and opportunities".***Nature Reviews Immunology 6 (March): 173–82. doi:10.1038/nri1785. ISSN 1474-1733.PMID 16498448*
- Ear T, McDonald PP (2008). **"Cytokine generation, promoter activation, and oxidant-independent NF-kappaB activation in a transfectable human neutrophilic cellular model"**. *BMC Immunol. 9: 14. doi:10.1186/1471-2172-9-14. PMC 2322942. PMID 18405381*
- Adam W. Anz, MD, Joshua G. Hackel, MD, Erik C. Nilssen, MD, James R. Andrews, MD "**Application of Biologics in the Treatment of the Rotator Cuff, Meniscus, Cartilage, and Osteoarthritis**" J Am Acad Orthop Surg 2014;22:68-79

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 /μL	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

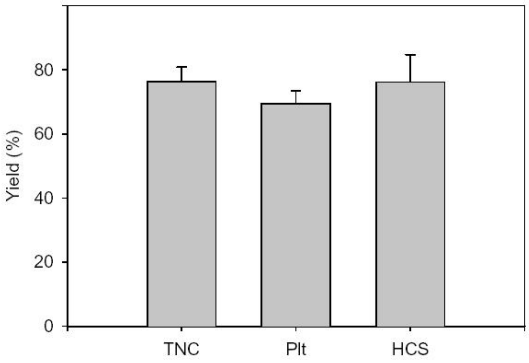
Table II lists the calculated total number of cells (volume x concentration) in BMA and BMC. TNC and PLT counts represent the values from the hematology analyzer times the volumes of BMA or BMC. HSC numbers are calculated from the percent of CD34+ cells gated with CD45+ events times the number of WBC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

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.

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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.

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	1±4 (-2-10)	14±8 (1-24)	16±11 (4-33)	NS*
Measurement 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 III: 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

NOTES:

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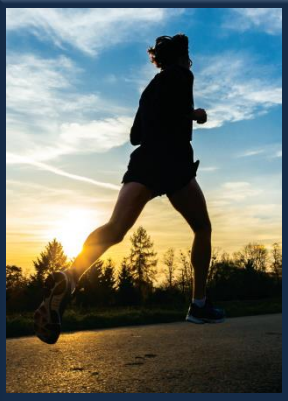


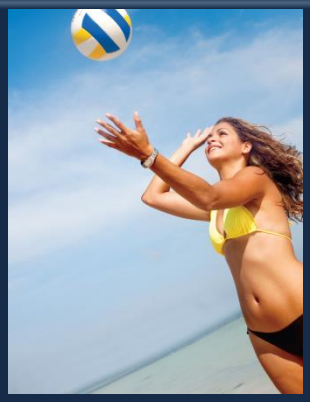
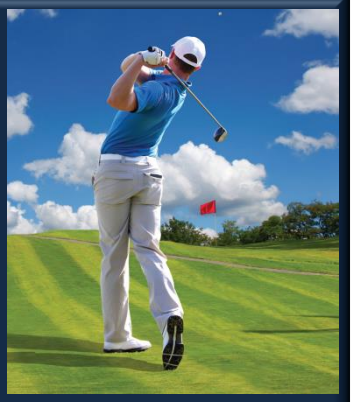

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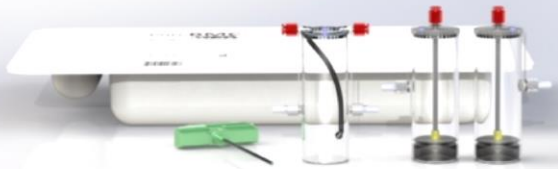

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