2023: High Dose Light Activated PRP the New Stem Cell Therapy

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Abstract: The Authors present a comparison of growth factors and cytokines found in Adipose Tissue, Stromal Vascular Fraction (SVF soluble fraction), Platelet Rich Plasma PRP, and Amniotic Fluid. It is proposed that since PRP contains nearly identical known growth factors and cytokines to SVF, that the same therapeutic effects that have been detailed for SVF can also be achieved with **High Dose Light Activated PRP** without the need for liposuction and at a much reduced cost.

Key Words: stem cell therapy, high dose light activated PRP, platelet rich plasma, growth factors and cytokines in blood and adipose, table of growth factors and cytokines, PRP for autoimmune disease

1. Introduction

Stem Cell Medicine has been developing for over 25 years. The term has been used to include all the modalities that relate to this “new medicine”, even though many researchers may be using “mixed cells”, **platelet rich plasma (PRP)**, conditioned media, amniotic fluid, exosomes and other methods that do not specifically use stem cells themselves. As **High Dose Light Activated PRP** is a derivative of our combined knowledge of stem cell medicine, we will continue to refer to the modality as a form of “stem cell medicine”.

The paired mice experiments, wherein the circulatory system of a young mouse and an old mouse were joined, demonstrated that it was serum factors from the young mouse and not stem cells themselves that caused the repair of damaged muscle in the old mouse (1). In fact, by 2011, that same lab had shown that the injection of young mouse plasma alone caused the reparative effect (2). Although somewhat buried in the lengthy research reports, we can thank scientific journalist Megan Scudellari for making things crystal clear:

“Wyss-Coray, who worked in the room next to Rando’s lab, had previously discovered prominent changes in levels of proteins and growth factors in the blood of aging humans and people with Alzheimer’s disease. Following up on Rando’s unpublished brain results, he used old–young

mouse pairs to show that old mice exposed to young blood did indeed have increased neuron growth, and that young mice exposed to old blood had reduced growth. **Plasma alone had the same effects. "We didn't have to exchange the whole blood," says Wyss-Coray. "It acts like a drug"** (3).

Since 2005, we have been teaching at the **Panama College of Cell Science** that serum factors and not stem cells themselves are what is required for the greatest therapeutic effect.

(A) Standard SVF Therapy Uses the Cellular Fraction of SVF

The **Stromal Vascular Fraction (SVF)** obtained from the liposuction of abdominal adipose tissue is the most widely used and accepted approach to stem cell treatments at this time (4). In the isolation and processing of the SVF, both a cellular fraction (5) and a liquid fraction (6) are obtained. However, although the SVF contains not only a wide variety of cells, but also a high concentration of growth factors, cytokines, and other messenger molecules, **only the cellular fraction is used in therapy.** The soluble fraction containing all the important biologicals are discarded in the rinsing procedure to remove collagenase. The therapeutic results obtained with stem cells from SVF are undoubtedly due to the required light activation indigenous to most protocols

Ramakrishnan and Boyd (5) summarized the cellular components used in therapy as including the following:

Adipose derived stem cells, including mesenchymal stem cells
Endothelial Cells
Smooth muscle cells
Fibroblasts
Pericytes and related cells
Macropages and other immune cells

In stem cell therapy using this cellular fraction, the cells are activated for 15-20 minutes at certain wavelengths which causes the injected cells, now excited, to produce immunomodulatory factors *in vivo*, leading to many and varied favorable therapeutic outcomes. There is a consensus that the cellular fraction contributes to *in vivo* secretion of various soluble factors, extracellular vesicles, and a whole array of paracrine activity that influences the therapeutic effect of the injected cellular component of SVF (6-7).

We have felt that this approach, while yielding excellent results, is flawed because of the discarding of the soluble cytokines and growth factors that could be useful in therapy following the paired mice observations. We have believed that, despite the successful therapeutic experiences with the activated cellular fraction, that therapeutic cures could be even more successfully achieved using the discarded adipose soluble factors.

(B) The Discarded Non-Cellular Aqueous Portion of SVF Should Be Considered For Therapy

An aqueous, cell-free fraction can be obtained from adipose aspirates using non-enzymatic techniques such as mechanical shearing. The soluble factors in the adipose cellular matrix, which are discarded in the standard SVF protocol, include at least the following:

Acylation stimulating protein
Adiponectin
Agouti signaling protein
Angiotensin II
Apelin
Complement factor D (adipsin)
Heparin binding – epidermal growth factor
Hepatocyte growth factor (HGF)
Insulin growth factor-1
Interleukin-1
Interleukin-6
Interleukin-8
Interleukin-10
Leptin
Migration inhibitory factor (MIF)
Monocyte chemoattractant protein-1 (MCP-1) Nerve growth factor
Nitric oxide (NO)
Plasminogen activator inhibitor-1 (PAI-1)
Prostacyclin
Prostaglandin E (PGE)
Renin
Resistin
Tissue factor
Tumor necrosis factor- α (TNF-α)
Vascular endothelial growth factor (VEGF)
Visfatin

[From Jerzy Chudek and Andrzej Wiêcek (8)]

More recently, Chun et al. described the following most important growth factors in adipose extracellular matrix (9), from which abdominal lipoaspirates are collected:

Basic fibroblast growth factor (bFGF)
Transforming growth factor beta 1(TGF-b1)
Insulin like growth factor 1 (IGF-1)
Vascular endothelial growth factor (VEGF)
Platelet-derived growth factor (PDGF)
BMP4 (bone morphogenetic protein 4)
Nerve growth factor (NGF)

Hepatocyte growth factor (HGF)
Epithermal growth factor (EGF)

Some practitioners are now questioning whether the soluble fraction of SVF may be a more promising therapeutic than the cellular fractions being widely used today. For example, Semenzato et al. are pursuing protocols to isolate cell-free extracts enriched in growth factors and immunomodulatory cytokines by means of non-enzymatic shearing of the extracellular matrix(10), stating:

"We characterized the aqueous phase of the StemRewind isolates from lipoaspirates. This fraction contained key immunomodulatory factors. ***As the paracrine effects of this aqueous phase may be more therapeutically promising than the ability of ADSCs to regenerate tissues,*** *the extracted fraction containing multiple cytokines, chemokines, and growth factors might represent another biomedicine for regenerative medicine. Importantly, the liquid and the cell fractions from the obtained aqueous phase can be easily separated by a simple centrifugation step".*

A microlyzer can be also be used in lieu of collagenase (11).

Likewise, He et al. stated that "[aqueous liquid extract] *ALE is a novel growth-rich therapeutic agent that is cell-free and easy to produce. Besides, it is also able to induce angiogenesis and adipogenesis both in vitro and in vivo, thus indicating that it could be used for wound repair and soft tissue regeneration"* (12). They did a massive analysis of Angiogenesis-related proteins, Adipogenesis-related proteins, as well as the concentrations of basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and platelet-derived growth factor (PDGF) found in the aqueous liquid extract. They concluded that **the aqueous fraction is a *"growth-rich therapeutic agent…that could be used for wound repair and soft tissue regeneration"*.**

Following the results from the paired mice experiments, we have likewise believed that the soluble fraction of SVF should be considered for therapy.

(C) Cytokines in Adipose Tissue vs PRP

In addition to comparable growth factors and other cytokines, **PRP also contains the explosive Platelet Granules**. As summarized by Sharda and Flaumenhaft (13):

> "Platelet granule exocytosis is central to platelet function and participates in the full repertoire of platelet activities. Platelets contain at least three major types of granules—α-granules, dense granules, and lysosomes—which carry distinct cargos and vary in biogenesis, trafficking, and exocytosis. In addition, platelets have peroxisomes and recently described T granules…α-Granules contain mainly proteins, both membrane-associated receptors (for example, αIIbβ3 and P-selectin) and soluble cargo (for

example, platelet factor 4 [PF4] and fibrinogen). Proteomic studies have identified more than 300 soluble proteins that are involved in a wide variety of functions, including hemos- tasis (for example, von Willebrand factor [VWF] and factor V), inflammation (for example, chemokines such as CXCL and interleukin-8), and wound healing (for example, vascular endothelial growth factor [VEGF] and fibroblast growth factor [FGF])"

Here, we present a Table comparing the major growth factors and cytokines observed in Adipose Tissue, SVF cell free fraction (soluble fraction), PRP, and Amniotic Fluid. Numbers in parentheses refer to the Reference numbers below wherein the identification was demonstrated. The check-marks indicate that the named growth factor or cytokine was found.

Table Comparing Major Factors/Cytokines (Copyright Walter P. Drake June 27, 2023)

Growth Factors/ Cytokines	Adipose Tissue	SVF Cell Free Fraction	PRP	Amniotic Fluid
CTGF Connective Tissue Growth Factor	✓ (15)		✓ (22)	
ECGF Endothelial Cell Growth Factor			✓ (22)	
bFGF Basic Fibroblast Growth Factor	✓ (9)	✓ (8,9,10)	✓ (22)	✓ (26)
TGF β1 Transforming Growth Factor Beta 1	✓ (9)	✓ (8,9)	✓ (22)	✓ (26,28)
VEGF Vascular Endothelial Growth Factor	✓ (9)	✓ (8,9,10)	✓ (22)	✓ (26)
IGF 1&2 Insulin-like Growth Factors	✓ (9)	✓ (8)	✓ (22)	✓ (26)
PD-EGF Platelet Derived Epidermal Growth Factor			✓ (22)	
PDGF-α and β Platelet Derived Growth Factor	✓ (9)	✓ (8,9)	✓ (22)	✓ (26)
FGF-2 Fibroblast Growth Factor 2	✓ (16)		✓ (22)	✓ (26)
IL-6 Interleukin 6	✓ (17)	✓ (8,10)	✓ (22)	✓ (28)
IL-8 Interleukin 8	✓ (18)	✓ (8)	✓ (22)	✓ (26,28)
IL-1β Interleukin 1β	✓ (19)	✓ (8,10)	✓ (22)	
IL-10 Interleukin 10	✓ (20)	✓ (8,10)	✓ (22)	✓ (28)
IL-12p70 Interleukin 12p70			✓ (22)	
TNF-α Tumor Necrosis Factor Alpha	✓ (21)	✓ (8,10)	✓ (22)	✓ (26,28)
NGF Nerve Growth Factor	✓ (9)	✓ (8,9)	✓ (23)	
HGF Hepatocyte Growth Factor	✓ (9)	✓ (8,9)	✓ (24)	✓ (26,28)
EGF Epidermal Growth Factor	✓ (9)	✓ (8,9)	✓ (25)	✓ (26,28)

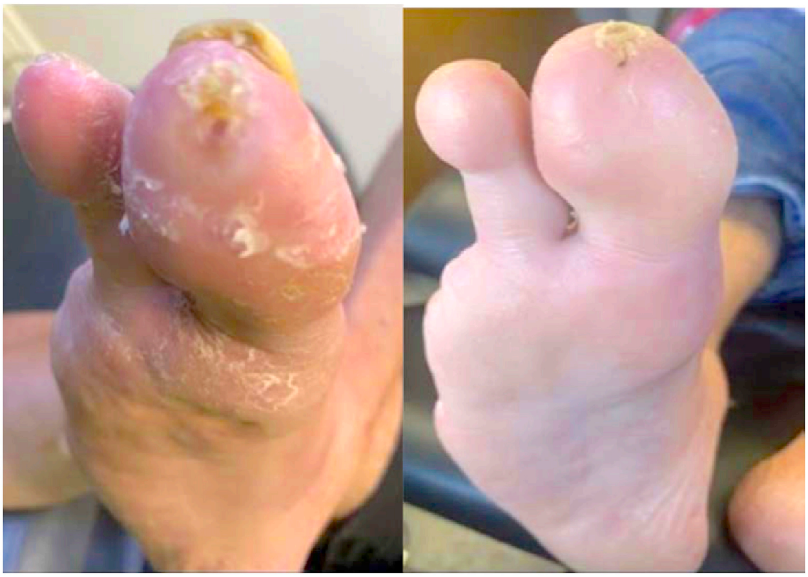
GCSF Granulocyte-Colony Stimulating Factor				✓ (26)
M-CSF Macrophage Colony Stimulating Factor				✓ (26)
FGF4,7,19,21 Fibroblast Growth Factors 4,7,19,21				✓ (26)
SCF Stem Cell Factor				✓ (26)
Fibrinogen			✓ (26)	
PF5 Platelet Factor 5			✓ (26)	
P-selectin			✓ (26)	
α-granules, dense granules, and lysosomes			✓ (13)	
			Over 300 proteins identified including 125 proteins related to wound healing; 4 for collagen biosynthesis;2 proteins for glycosaminoglycan biosynthesis process; 13 proteins for glycosaminoglycan binding. (27)	Over 300 cytokines reported in main categories of host defense, proliferation/ differentiation, cell adhesion/cell-cell interactions, angiogenesis (28)

(D) High Dose Light Activated PRP is a Better Therapeutic Modality Within the Field of Stem Cell Medicine

As shown in the Table above, PRP contains the identical known growth factors and cytokines found within adipose tissue and its derivative SVF soluble fraction. Moreover, PRP contains what we feel are the explosive and highly active α-granules (13) which some practitioners may refer to as “VSELS-Very Small Embryonic Like Stem Cells”.

High Dose Light Activated PRP therapy is already showing promise in recent studies. For example, a light activated PRP fraction from 120cc of blood cured a 6 month old diabetic wound in the foot within 2 weeks of IV injection, while also resolving inflammation and peripheral neuropathy (Phillip B. Yoo, DC, PhD, personal communication, June 19, 2023):

As Illustrated in the Before and After Photos Below



Before: non-healing wound, edema Peripheral neuropathy

After: complete wound healing after Hi-dose PRP & Vsel Light activation

Photo courtesy of Phillip B. Yoo, Stem Cell Clinician in California

Most importantly, and of no small consideration, is that High Dose PRP therapy avoids surgical liposuction, which continues to make stem cell therapy both expensive and somewhat unavailable.

High Dose PRP Therapy could be accomplished by many practitioners in an office setting because, from the patient standpoint, only a blood draw with eventual intravenous re-injection of concentrated PRP would be needed.

Two purposes of this paper are to demonstrate the surprising identity of PRP growth factors and cytokines to SVF; and to define a protocol for **High Dose Light Activated PRP Therapy**

2. Methodology and Protocol For High Dose Light Activated PRP Therapy

Some of the important considerations include:

— Blood volume of 60-120 ml: High dose PRP is considered to be a blood draw from the patient of at least 60 ml, adjusted as required to yield 1.5-3 billion platelets. Most “regular” PRP therapies are currently using no more than 30 ml blood.

—Large bore needle required: Using too small a needle can unintentionally activate the platelets during the blood draw. Choosing the best gauge will undoubtedly depend upon the condition and age of the patient. Many protocols call for a 22 gauge needle for the blood draw. 21 gauge is reported to be the most widely used needle for routine blood draws.

— Testing circulating platelet concentration will help to determine the correct amount of blood to draw, since this will differ widely due to age and condition of each patient.

— Temperature: Most practitioners proceed expeditiously through the collection and concentration phase so as to avoid long delays in the handling of the product. Staying at 21°C-24°C is best. Heat can activate platelets prior to use; cold can inhibit platelet activation with light.

— Choice of anticoagulant: Only sodium citrate is appropriate, EDTA to be avoided

— Light activation is mandatory, and time will depend on the light box used. 4 wavelengths are required; 630 nm; 663 nm; 805 nm; 855 nm for usually about 5-20 min. As time varies by intensity, a practitioner can follow manufacturer guidelines.

Protocol:

1. Clinical judgment as to appropriateness of procedure: A complete medical history is needed as well as medications. Contraindications to PRP therapy are:

Critical thrombocytopenia
Hypofibrinogenaemia
Haemodynamic instability
Sepsis
Acute and chronic infections
Chronic liver disease
Anti-coagulation therapy

And not recommended for patients with these conditions:

Hepatitis C
Human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)
Any type of blood cancer
Cardiovascular disease that requires taking a blood thinner

2. Determine platelet count (via finger prick sampling)

3. Calculate amount of blood draw needed to achieve at least 1.5 x 10^9 platelets

4. Draw appropriate amount of blood using a 100 ml or 120 ml collection bag and no smaller than 22 gauge needle. A 60 ml syringe with butterfly needle can be used if only drawing 60 ml. Gravity feed into the bag at floor level may work best. We will assume the 60 ml minimum amount collected into a 60 ml syringe preloaded with 8 cc sodium citrate as an anticoagulant.

6. Use a PRP collection kit to separate out and concentrate the PRP. There are many kits available with new developments occurring all the time. As of this writing, the Pure PRP SupraPhysiologic appears to be very popular because of its 60 ml tubes for centrifugation. Systems which have too much whirring and spinning may cause too much jostling of the biological products causing activation and loss of of the α-granules (VSELs). The "Accelerate" kit is another option. Inject the anti-coagulated blood into the 60 ml PRP kit.

7. Centrifugation. You need 600G x 7 minutes for the first spin (RPM will vary with centrifuge) to separate Red Blood Cells, and draw off the platelet/plasma suspension. A second spin at 2000G x 5 minutes will yield the "buffy coat" containing the platelet rich plasma, yielding about 7 ml of concentrated PRP.

8. Light Activation of PRP. Although the idea of light activation of PRP in the clinic appears relatively new, those that are doing light activation are most likely using the Adilight-2 by Adistem (which has been used many years for stem cell activation in the SVF protocol). Other practitioners are also experimenting with just using a light box such as "LightStim" being sold for pain. Some specify 4 wavelengths 630 nm; 663 nm; 805 nm; 855 nm for usually about 5 -10 minutes; others specify 600-1200 nm Near Infra Red. So, as light activation has just recently been determined to be required, we do not yet have a consensus as to which specific wavelengths and how long. Will various syringe plastics also cause varying results requiring adjustment in activation times?

9. Inject the activated high dose PRP preparation IV using butterfly needles (20-22 gauge). A syringe driver (battery operated syringe pump) could also be considered for infusion.

3. Discussion And Conclusion

Previously, in their Primer for Physicians, Laurence V. Hicks, Sr. and Geoffrey N. Hicks presented an excellent comparison of SVF and PRP both in terms of processing as well as in current therapeutic uses (29). And as shown by the Table above, PRP contains essentially the same growth factors and cytokines found in adipose tissue, as well as in the soluble SVF fraction. Consequently it would seem likely that similar if not superior therapeutic results could be achieved compared to the SVF cellular fraction, particularly in cases of systemic disease and autoimmune diseases. In the now widely accepted SVF therapy, only stem cells are injected. We rely on the light activated stem cells to go on to secrete growth factors and cytokines *in vivo*

to yield therapeutic benefits. *In a sense, we are relying on the injected stem cells to secrete the very molecules we already discarded in the cell-free SVF fraction.*

The direct IV injection of identical growth factors and cytokines found within PRP, albeit now in a concentrated high dose form, would be expected to yield superior therapeutic effects without the need for liposuction and at a very much reduced cost. This reduced cost alone would allow patients to not only pay for the procedure if not covered by insurance, but also encourage repeat treatments as might be necessary.

Some already believe that **High Dose Light Activated PRP Therapy** is the stem cell treatment of choice now. This article puts the field in perspective:

> "Traditionally speaking, PRP therapy has been used in Orthopedics for intra-articular injections. It has been known to treat more chronic conditions, such as arthritis, as well as acute sports injuries including a torn meniscus, tendonopathies, muscle injuries, and more. However, it is now being explored through other routes of administration and producing incredible results. **PRP therapy is now being given intravenously to treat more systemic health conditions. Patients suffering from neurological diseases including Parkinson's, Multiple Sclerosis, and Cerebral Palsy, are seeing positive results with autologous platelets given IV**. **Other conditions such as Lupus, Rheumatoid Arthritis, and Chronic Fatigue have also shown remarkable improvement** (14).

We believe that **High Dose Light Activated PRP** will have the same or better curative effects on our most debilitating diseases and conditions as has been shown for SVF therapy.

This paper is important for establishing that PRP has at least the identical profile of important growth factors and cytokines as shown for SVF; and for the proposition that **High Dose Light Activated PRP** should be the 1st choice of stem cell practitioners in the consideration of available therapeutic modalities.

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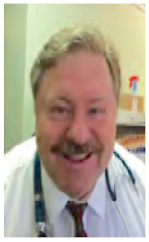

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