Are stem cells from blood as effective as from bone or fat?

Is PRP a stem cell therapy?

**Even among physicians and researchers,
a most common naming convention
has relegated the term stem cell therapy
to bone marrow and adipose methods.**

**Mesenchymal stem cells are
most commonly cited
as the target cell for
regenerative injections.**

**CD34+ cells in blood are erroneously thought of
as just hematopoietic (blood) stem cells,
and overlooked or ignored as the key measure
of valuable stem cell content in blood based PRP.**

The prevailing school of thought contents that mesenchymal stem cells (MSCs) do not express CD34, and this sets MSCs apart from hematopoietic stem cells (HSCs), which express CD34. However, the evidence for MSCs being CD34 is largely based on cultured MSCs, not tissue-resident MSCs, and the existence of CD34 HSCs is in fact well documented... In particular, two studies that analyzed CD34 expression in uncultured human bone marrow nucleated cells both found that MSCs (BMSCs) existed in the CD34+ fraction. Several studies also found that freshly isolated adipose-derived MSCs (ADSCs) expressed CD34. In addition, all of these ADSC studies and several other MSC studies observed disappearance of CD34 expression when the cells were propagated in culture. Thus, available evidence points to CD34 being expressed in tissue-resident MSCs, and its negative finding being a consequence of cell culturing.

--- Lin C-S, Ning H, Lin G, Lue TF. Is CD34 Truly a Negative Marker for Mesenchymal Stem Cells? Cytotherapy. 2012 Nov; 14(10): 10.3109/14653249.2012.729817. doi: 10.3109/14653249.2012.729817

Instead,
further investigation shows that
CD34+ stem cells sourced from peripheral blood
are of great value in regenerative therapies,
in part because of they transform into
mesenchymal stem cells according to need.

CD34 is a transmembrane phosphoglycoprotein, first identified on hematopoietic stem and progenitor cells. Clinically, it is associated with the selection and enrichment of hematopoietic stem cells for bone marrow transplants. Due to these historical and clinical associations, CD34 expression is almost ubiquitously related to hematopoietic cells, and it is a common misconception that CD34-positive ($CD34^1$) cells in nonhematopoietic samples represent hematopoietic contamination. The prevailing school of thought states that multipotent mesenchymal stromal cells (MSC) do not express CD34. However, strong evidence demonstrates CD34 is expressed not only by MSC but by a multitude of other non-hematopoietic cell types including muscle satellite cells, corneal keratocytes, interstitial cells, epithelial progenitors, and vascular endothelial progenitors. In many cases, the $CD34^1$ cells represent a small proportion of the total cell population and also indicate a distinct subset of cells with enhanced progenitor activity.

Despite consistent evidence of expression by many cell types, there is still a misconception that CD34 represents a cell of hematopoietic origin, and experimentally, $CD34^1$ cells are often regarded as hematopoietic contamination and subsequently disregarded. This review presents evidence establishing CD34 as a general marker of progenitor cells.

-- Sidney LE, Branch MJ, Dunphy SE, Dua HS, Hopkinson A. Concise Review: Evidence for CD34 as a Common Marker for Diverse Progenitors. Stem Cells: 2014;32:1380–1389 www.StemCells.com

Until recently, adult stem cells were presumed to be committed to differentiation of specific tissues. Adult hematopoietic stem cells (HSCs) originally believed to be limited to hematopoietic differentiation are capable of dedifferentiation and transdifferentiation to generate cells of all lineages. Mesenchymal stem cells (MSCs) have also been shown to transdifferentiate into various tissues. This capability is referred to as stem cell plasticity.

-- Kevy SV, Jacobson MS, Mandle RJ, Point of Care Concentration of Bone Marrow. Paper No: 1738, 52nd Annual Meeting of the Orthopedic Research Society. https://www.ors.org/Transactions/52/1738.pdf

BACKGROUND: Bone marrow (BM) has been considered as a major source of mesenchymal stem cells (MSCs), but it has many disadvantages in clinical application. However, MSCs from peripheral blood (PB) could be obtained by a less invasive method and be more beneficial for autologous transplantation than BM MSCs, which makes PB a promising source for articular cartilage repair in clinical use.
RESULTS: ...No significant difference was found concerning the morphology, immune phenotype, and anti-apoptotic capacity between PB MSCs and BM MSCs. Significantly, MSCs from both sources compounded with decalcified bone matrix showed the same ability to repair cartilage defects in vivo. For multipluripotency, BM MSCs exhibited a more osteogenic potential and higher proliferation capacity than PB MSCs, whereas PB MSCs possessed a stronger adipogenic and chondrogenic differentiation potential than BM MSCs in vitro.
CONCLUSION: Although there are some differences in the proliferation and differentiation aspects between the 2 sources, PB MSCs share certain similar biological characteristics in vitro and chondrogenesis in vivo as BM MSCs.
CLINICAL RELEVANCE: These results suggest that PB MSCs are a new source of seed cells used in articular cartilage repair.

-- Fu WL1, Zhou CY, Yu JK. A new source of mesenchymal stem cells for articular cartilage repair: MSCs derived from mobilized peripheral blood share similar biological characteristics in vitro and chondrogenesis in vivo as MSCs from bone marrow in a rabbit model. Am J Sports Med. 2014 Mar;42(3):592601. doi: 10.1177/0363546513512778. Epub 2013 Dec 10.

Mesenchymal stem cells (MSCs) from adult exhibit self-renewal and multi-lineage differentiation capacities, making the MSCs promising candidates for cell therapy and tissue engineering. Although bone marrow (BM) is the most universal source of MSCs, other tissues may also contain MSCs. Peripheral blood (PB), in particular, arises as the most attractive source of MSCs due to easy accessibility and noninvasive procedure. However, it is not certain that PBMSCs have the equal biological characteristics to those of BMMSCs. The purpose of this study was to compare the biological characteristics between BMMSCs and PBMSCs.

Although the BMMSCs showed stronger osteogenic and adipogenic differentiation, PB-MSCs displayed a more chondrogenic capacity. Further, BMMSCs have greater proliferation ability. Apoptosis resistance and cellular senescence were similar in MSCs derived from both sources. The results of our study demonstrate that PBMSCs have similar biological characteristics to those of BMMSCs despite certain minor differences, suggesting PB as a possible alternative source for MSCs.

-- Fu WL, Zhang JY, Fu X, Duan XN, Leung KK, Jia ZQ, Wang WP, Zhou CY, Yu JK. Comparative study of the biological characteristics of mesenchymal stem cells from bone marrow and peripheral blood of rats. Tissue Eng Part A. 2012 Sep;18(17-18): 1793-803. doi: 10.1089/ten.TEA.2011.0530. Epub 2012 Jul 30.

Blue slides that follow are from a presentation by researchers at Harvard Medical School, and the Harvard affiliated blood lab.

Defining the Concentration and Composition of Platelet-Rich Plasma (PRP) and Bone Marrow Concentrate (BMAC) for use in Regenerative Medicine

Sherwin V. Kevy, M.D.
Immune Disease Institute, Boston, MA
Children's Hospital, Boston, MA
Harvard Medical School, Boston, MA

May S. Jacobson, Ph.D.
Department of Orthopedic Surgery
Children's Hospital, Boston, MA
Harvard Medical School, Boston, MA

Robert J. Mandle, Ph.D.
BioScience Research Associates
Cambridge, MA

3/9/11

STEM CELL PLASTICITY

- Adult hematopoietic stem cells (HSCs) have been shown to transdifferentiate into mature nonhematopoietic cells.

- Plasticity has only been demonstrated in response to inflammation and tissue injury.

- Recent studies indicate that primitive adult hematopoietic stem cells can differentiate to osteoblasts through a mesenchymal intermediate.

- HSCs are a heterogeneous mixture of cells that range from the most primitive to lineage-committed cells.

A Comparative Analysis of the Cellular Composition by Means of Flow Cytometry

	Harvest PRP				Cascade PRP			
Donor ID	Vol. mL	WBC x10^6/mL	CD34+ x10^3/mL	Total CD34+	Vol. mL	WBC x10^6/mL	CD34+ x10^3/mL	Total CD34+
59	7.1	23.6	21.1	149,810	4.5	0.9	0.6	2,700
61	6.7	22.9	64.1	429,470	3.8	0.5	0.4	1,520
62	7.0	19.2	18.8	131,600	3.7	0.6	0.3	1,110

Analysis of the Cellular Composition of the Harvest APC-60 PRP by Flow Cytometry

n=11

WBC X 10^6/mL	CD 34+ X 10^3/mL	Total CD 34+
22.2 ± 3.60	17.47 ± 9.32	171,571 ± 88,064

- CD 34+ - Hematopoietic Stem Cells
- Results were obtained from actual patient samples
- CD 34+ is used as a stem cell marker

CD34+ cells "are drivers of tissue regeneration".

CD34+ is a marker on the Stem cell/Progenitor cells found in the mononuclear cell population. The don't transdifferentiate. Bone Marrow contains a greater population of stem cells. However Neither BMAC or PRP are considered stem cell transfusions.

Does Adipose contain more stem/progenitor cells than marrow. No. Adipose contains more MSCs than Marrow. MSCs are signaling cells. Marrow provides greater CD34+ cells which are drivers of tissue regeneration Yashura et.al. 2010, Herman et. al. Hermann et. al. Marrow contains more cell types.

-- May S. Jacobson, Ph.D.
Department of Orthopedic Surgery
Children's Hospital, Boston, MA
Harvard Medical School, Boston, MA
personal communication 4/27/15

The prevailing school of thought contents that mesenchymal stem cells (MSCs) do not express CD34, and this sets MSCs apart from hematopoietic stem cells (HSCs), which express CD34. However, the evidence for MSCs being CD34 is largely based on cultured MSCs, not tissue-resident MSCs, and the existence of CD34 HSCs is in fact well documented. Furthermore, the Stro1 antibody, which has been extensively used for the identification/isolation of MSCs, was generated by using CD34+ bone marrow cells as immunogen. Thus, neither MSCs being CD34 nor HSCs being CD34+ is entirely correct. In particular, two studies that analyzed CD34 expression in uncultured human bone marrow nucleated cells both found that MSCs (BMSCs) existed in the CD34+ fraction. Several studies also found that freshly isolated adipose-derived MSCs (ADSCs) expressed CD34. In addition, all of these ADSC studies and several other MSC studies observed disappearance of CD34 expression when the cells were propagated in culture. Thus, available evidence points to CD34 being expressed in tissue-resident MSCs, and its negative finding being a consequence of cell culturing.

-- Lin C-S, Ning H, Lin G, Lue TF, Is CD34 Truly a Negative Marker for Mesenchymal Stem Cells? Cytotherapy. 2012 Nov; 14(10): 10.3109/14653249.2012.729817.

Recently, researchers have observed in animal studies that hematopoietic stem cells appear to be able to form other kinds of cells, such as muscle, blood vessels, and bone. If this can be applied to human cells, it may eventually be possible to use hematopoietic stem cells to replace a wider array of cells and tissues than once thought.

Plasticity of Hematopoietic Stem Cells

A few recent reports indicate that scientists have been able to induce bone marrow or HSCs to differentiate into other types of tissue, such as brain, muscle, and liver cells. These concepts and the experimental evidence supporting this concept are discussed in Chapter 4. The Adult Stem Cell.

https://stemcells.nih.gov/info/2001report/chapter5.htm

Recently, some studies have documented that peripheral blood-derived mesenchymal stem cells (PBMSCs) own biological characteristics which are similar to that of BMMSCs and UCMSCs, and also possess much more effective cost, less trauma and no anesthesia.

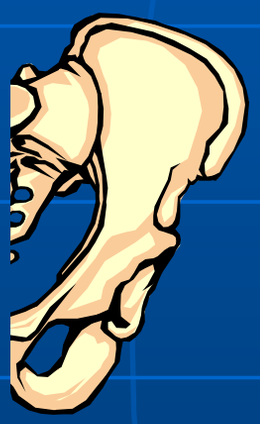
-- Fu Q, Liu Y, Liu X, Zhang Q, Chen L, Peng J, Ao J, Li Y, Wang S, Song G, Yu L, Liu J, Zhang T. Engrafted peripheral blood derived mesenchymal stem cells promote locomotive recovery in adult rats after spinal cord injury. Am J Transl Res. 2017; 9(9): 3950–3966.

Even in bone marrow,
mesenchymal stem cells are
far more rare than
CD34+ hematopoietic stem cells

CELLULAR COMPOSITION OF BONE MARROW

There are two principal multipotent stem cells in the marrow:

BM-MNC's



Multipotent Stem Cells

Stromal Stem Cells

Hematopoietic Stem Cells

- **ONE STROMAL CELL IN EVERY 250,000 CELLS IN THE MARROW AT AGE 35, BUT THIS RATIO DECREASES WITH AGE.**

- **ONE HEMATOPOIETIC STEM CELL FOR EVERY 10-15,000 CELLS IN THE MARROW AND DOES NOT DECREASE WITH AGE**

Concentration of Mesenchymal Stem Cells in Bone Marrow

Age	Cell Concentration
Teenager	1/100,000
35 yrs	1/250,000
50 yrs	1/400,000
80 yrs	1/1,200,000

Caplan-Bioassays 1987;6:171

Because of this,
CD34+ hematopoietic stem cells
are a key measure of effectiveness
of bone marrow and BMAC.

ANALYSIS OF CELLULAR COMPOSITION

MEAN VALUES: ± SD N = 25

Normal Donors

NCC x10^6/ml BMA	NCC x10^6/ml BMAC	MNC x10^6/ml BMA	MNC x10^6/ml BMAC	CD34+ x10^3/ml BMA	CD34+ x10^3/ml BMAC	% Yield TNC	% Yield MNC	% Yield CD34+
23.1 ± 5	89.1 ± 8	4.51 ± 0.9	18.80 ± 3.41	183 ± 60	800 ± 180	61.8 ±10.4	69.0 ± 19.4	75.3 ± 13.7

NCC = nucleated cell count
BMA = bone marrow aspirate
MNC = mononuclear cell
BMAC = bone marrow concentrate

TOTAL CELLS DELIVERED

Total NC x10^6	Total MNC x10^6	Total CD34+ x 10^6
1782	376	16

Vol. BMA = 120 ml
Vol. BMC = 20 ml

Comparative Analysis of Bone Marrow Preparations

System	NC X10⁶/ml BMA	NC X10⁶/ mL BMAC	Plts X10³/ μL BMA	Plts X10³/μL BMAC	MNC X10⁶/ ml BMA	MNC X10³/ m L BMAC	CD34+ X10³/ mL BMA	CD34+ X10³/ mL BMAC	% Yield NC	% Yield MNC	% Yield CD34+
Harvest BMA-57mL	46	182	137	755 *(96.6%)	10.3	53.5	500	2118	69.4	91.0	74.3
Arteriocyte BMA-57mL BMAC-10mL	46	44	137	521 *(66.7%)	10.3	18.0	500	1141	17.0	30.6	40.0

NC = nucleated cells
Plts = platelets
BMA = bone marrow aspirate
BMAC = bone marrow concentrate
MNC = mononuclear cell
* Per cent yield

Total Cells Delivered

System	NC x10⁶	MNCX10⁶	CD34+x0⁶
Harvest	1820	535	21.2
Arteriocyte	440	180	11.4

Total Cells Delivered Normal Patients

HARVEST N = 25			MAGELLAN N = 3		
Total TNC $X10^6$	Total MNC $X10^6$	Total CD34+ $X10^6$	Total TNC $X10^6$	Total MNC $X10^6$	Total CD34+ $X10^6$
891 (yield 61.8%)	**188 (yield 69.0%)**	**8.0 (yield 76.3%)**	**337 (yield 37.8%)**	**53 (yield 30%)**	**2.2 (yield 58.5%)**

TNC = Total nucleated cells
MNC = Total mononucleated cells

Vol. BMA – Magellan 60 ml = 40 ml BM + 20 ml WB
Vol. BMA - Harvest 60 ml

Bone marrow concentrate has more stem cells, but not that much more than PRP.

The number of CD34+ stem cells in PRP is less than the number in BMAC, but by less than some suspect. In a personal communication on 5/1/15, Robert J. Mandle, Ph.D. of the Harvard School Affiliated BioSciences Research Associates, Inc. identified characteristic CD34+ content of (Harvest) PRP at about *.8%*, and the CD34+ content of BMAC at about *1-2%*.

And,
PRP has other functions
that are advantageous
for tissue regeneration procedures.

PRP activates dormant stem cells when injected in tendon.

PRGF (activated PRP) significantly enhanced cell DNA synthesis, improved viability and promoted proliferation, while facilitating cell migration and the recruitment of TDSCs.

-- Xu K, AlAni MK, Sun Y, Xu W, Pan L, Song Y, Xu Z, Pan X, Yang L, Platelet-rich plasma activates tendon-derived stem cells to promote regeneration of Achilles tendon rupture in rats. J Tissue Eng Regen Med. 2017 Apr;11(4):11731184. doi: 10.1002/term.2020. Epub 2015 Mar 11.

Stem cells in PRP are inherently better guided via the homing mechanism, due to the presence of SDF-1A and other factors in PRP.

How Do Stem Cells Find Their Site

- Migration of stem cells to different organs requires active navigation, a process termed homing
- Homing is a multistep process signaled by stromal derived factor 1 alpha (SDF-1α), stem cell factor (SCF), and activation of lymphocyte function – associated antigen
- Stem cells finalize their homing uniquely, by selective access and anchorage to their specialized niches

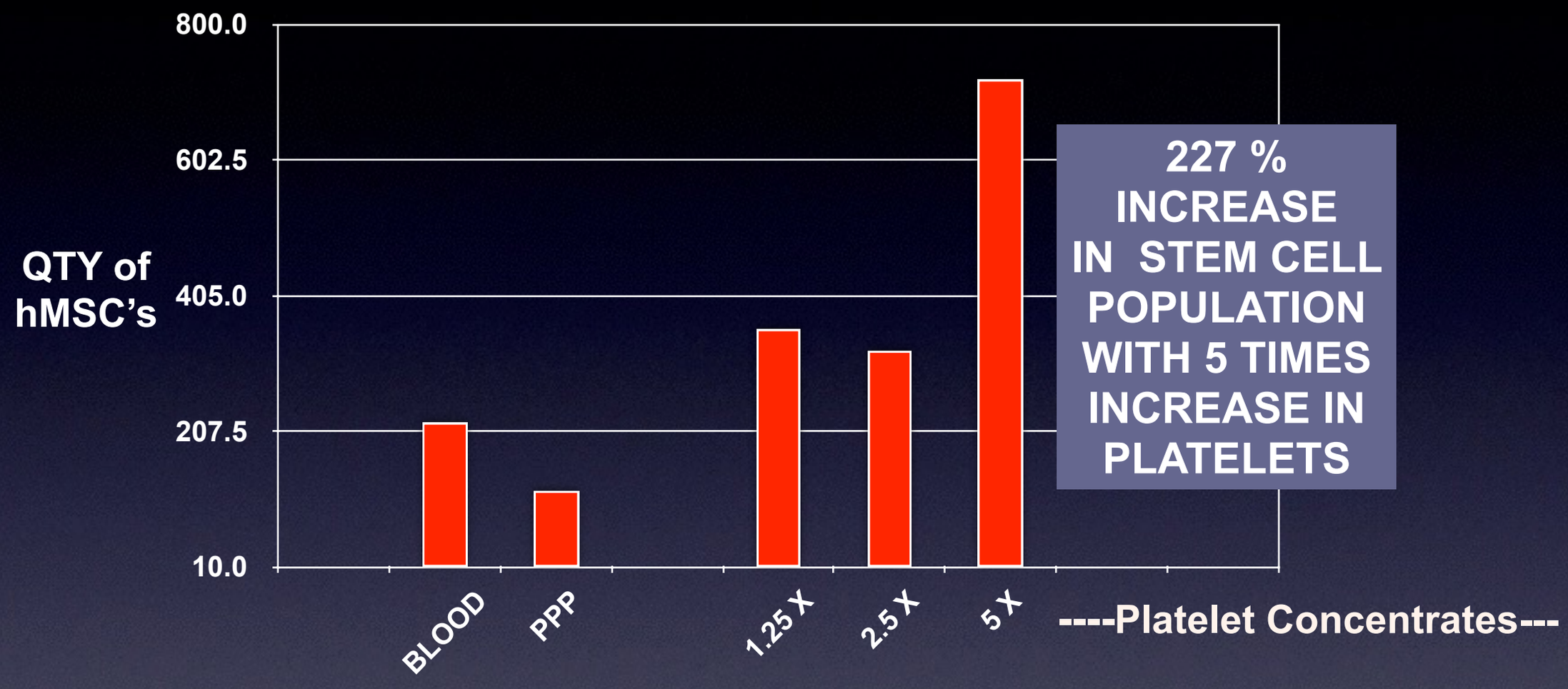
The Importance of Stromal Derive Factor-1 Alpha SDF-1α

- Unlike growth factors and stem cells, it is rarely referred to in articles.
- It is released from platelets and endothelial cells.
- Without its presence stem cells would not know where to go.

PRP injection causes chemotactic attraction of another 227% in stem cells to the injection site.

Stem Cell Recruitment: blood, PPP, PRP

Dose-Dependent Mitogenic Effects of Platelet Releasate on hMSC's



Conclusions:

Platelet-Leukocyte Concentrate and VEGF stimulate chemotactic migration of hMSC's in a dose-dependent manner.

Platelet-Leukocyte Concentrate stimulates proliferation of hMSC's in a dose-dependent manner

Mitogenic Stimulation of Human Mesenchymal Stem Cells by Platelet Releasate Suggests a Mechanism for Enhancement of Bone Repair by Platelet Concentrate, Poster AAOS Meeting 2002

Haynesworth, SE; Kadiyala, S; Liang, L; Bruder, SP; DePuy AcroMed, DePuy Orthopedics, and Case Western Reserve University.

Chemotactic and Mitogenic Stimulation of Human Mesenchymal Stem Cells by Platelet Rich Plasma Suggests a Mechanism for Enhancement of Bone Repair

Stephen E. Haynesworth PhD,[1,2] Sudha Kadiyala PhD,[1] Li-Nuo Liang PhD,[1,2], Tina Thomas, BS,[1,2], Scott P. Bruder MD, PhD[1,3],
Skeletal Research center[1] and Department of Biology[2], Case Western Reserve University, Cleveland, OH, and
DePuy AcroMed[1], Raynham, MA

INTRODUCTION

Platelets are known to perform multiple functions during injury and tissue repair. While their role in hemostasis is well understood, their mechanism of action in promoting wound healing requires further characterization. As a repository of multiple growth factors such as PDGF, EGF, VEGF, and TGF-ß, degranulation of platelets at wound sites serves to initiate or enhance the healing cascade. Armed with this knowledge, clinicians have used platelet concentrates in conjunction with bone graft materials to enhance osseous repair[1,2]. In addition, experimental evidence has shown that when PDGF or platelet concentrates are combined with demineralized bone or certain other materials, augmentation of bone formation ensues[3].

The purpose of this study was to begin elucidating the cellular mechanisms that underlie these observations. Since mesenchymal stem cells (MSCs) are known to be an essential component of the tissue repair process, we sought to characterize elements of their response to platelet concentrates in the controlled in vitro environment.

Donor Preps (n)	Donor Age (years)	Hematocrit (%)	Initial Platelet Count ($x10^3/\mu l$)	Platelet Yield (%)
21	30 ± 6.2	38.6 ± 2.9	223.3 ± 45.80	70.6 ±11.0

Isolation of Concentrated Platelets using the SYMPHONY Platelet Concentration System. Values equal averages ± S.D.

PLATELET CONCENTRATION

Platelet rich plasma (PRP) was isolated from approximately 55 ml of fresh human blood (IRB-approved protocol) using the Symphony™ Platelet Concentration System (DePuy AcroMed, Raynham, MA), designed to be used at the point-of-care for obtaining a platelet concentrate from a small amount of blood. Samples of the starting material and platelet concentrates were analyzed to determine the absolute concentrations and yields of platelets. PRP, platelet poor plasma (PPP) and whole blood were clotted with thrombin (1000 U/ml in 10% $CaCl_2$) by adding 1 part thrombin stock solution to nine parts PRP, PPP or blood to yield a final thrombin concentration of 100 U/ml. The soluble platelet releasates from the clotted preparations were isolated by centrifugation and cleared by ultrafiltration.

PRP and PPP releasates were diluted in serum-free DMEM to generate appropriate final dilutions of platelet releasate. Similar to previously published results[4], we obtained very high efficiency of platelet concentration. Also, the efficiency of the platelet concentration was reproducible across the various samples as can be seen by the low standard deviation. The specific levels of various growth factors were not measured in this study. However, it has been previously reported that in platelet concentrates processed using the current system, the concentration of growth factors increase linearly with the platelet concentration[4,5].

Presented at the 48th Annual Meeting of the Orthopaedic Research Society, Dallas, TX, 2002 and 28th Annual Meeting of the Society for Biomaterials, Tampa, FL, 2002

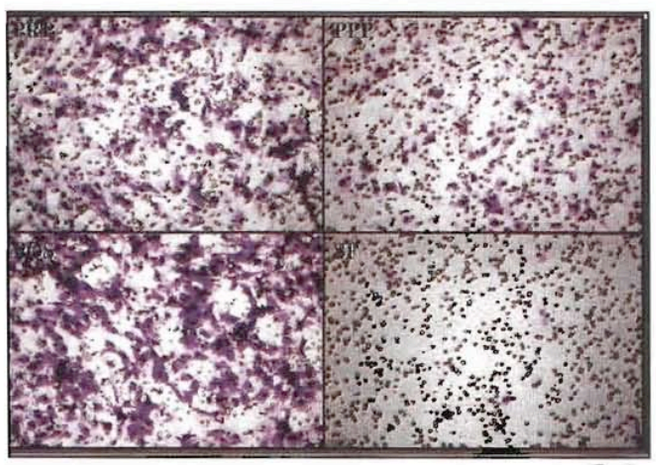
CELL PREPARATION

In order to evaluate the mitogenic activity of PRP, human MSCs (hMSCs) were isolated and culture-expanded from bone marrow (IRB-approved protocol) using published techniques[6]. The growth media (GM) for the selection and culture-expansion of hMSCs consisted of DMEM supplemented with 10% fetal bovine serum (FBS). The specific lot of FBS was chosen for it's ability to optimize MSC selection and growth[7].

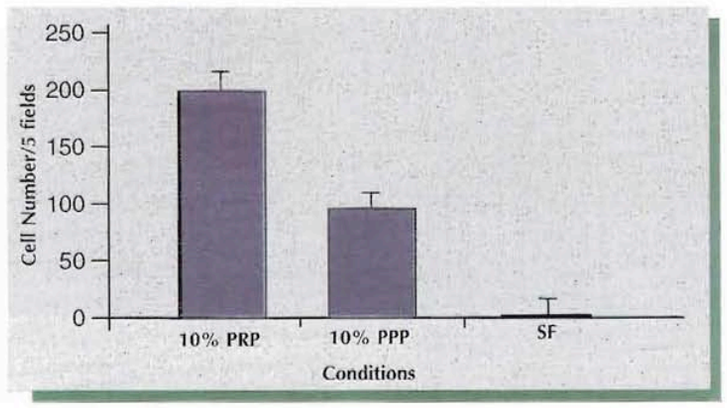
Control media consisted of serum free medium (SF), or DMEM supplemented to 10% (v/v) with the following preparations: PPP releasate alone, or serum from clotted peripheral blood (PB). Test media consisted of DMEM supplemented with undiluted PRP releasate or PRP releasate diluted with PPP, such that the final concentration of PRP releasate ranged from 0.625- to 10-fold of that in media supplemented with peripheral blood. To achieve the 5-fold concentration and the 10-fold platelet concentration, the PRP releasate was added to the media at 10% and 20% (v/v), respectively. To achieve the lower platelet concentrations, PRP diluted with an appropriate amount of PPP was added to the media at 10% (v/v).

CHEMOTACTIC MIGRATION

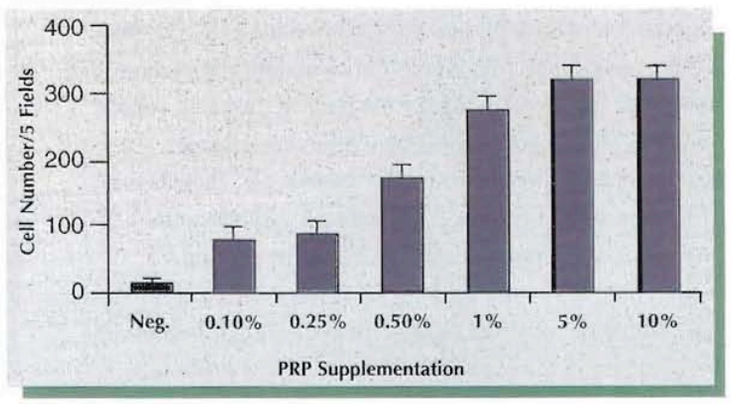
The ability of concentrated platelet releasate to stimulate the chemotactic migration of hMSCs was measured using a Neuroprobe AC48 Boyden Chamber with 5 µm pore size polycarbonate filters. 7,500 hMSCs in 50 µl serum-free medium were added to the upper chambers of each well. Lower chambers contained test media. Cells were allowed to migrate for 4 hours at 37° C, at which time non-migratory cells were scraped from the filter. Migratory cells on the underside were stained with crystal violet dye and counted. PRP releasate and VEGF each stimulate chemotactic migration of hMSCs in a dose-dependent manner. Since VEGF is a component of PRP releasate, it is at least partially responsible for the chemotactic activity of PRP releasate on hMSCs.



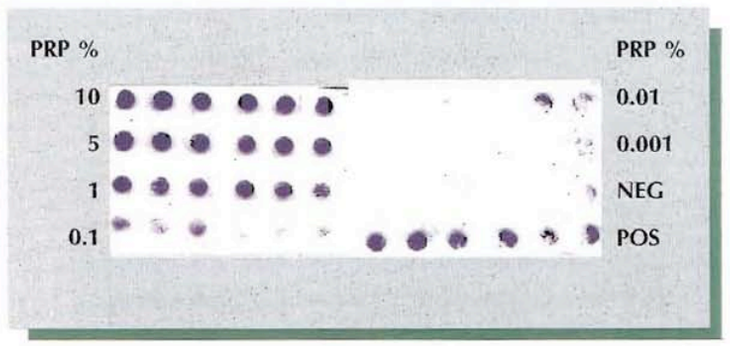
Photomicrographs of hMSCs after chemotaxis due to PRP releasates and proper controls(original magnification 200x).



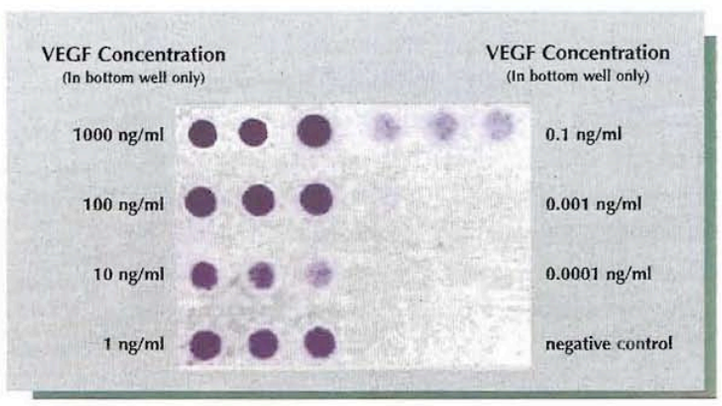
Chemotactic Migration of hMSCs in Response to Various Media Additives



Chemotactic Migration of hMSCs in Response to PRP Releasate



PRP-Releasate Stimulates Dose-Dependent Chemotactic Migration of hMSCs



VEGF Stimulates Dose-Dependent Chemotactic Migration of hMSCs

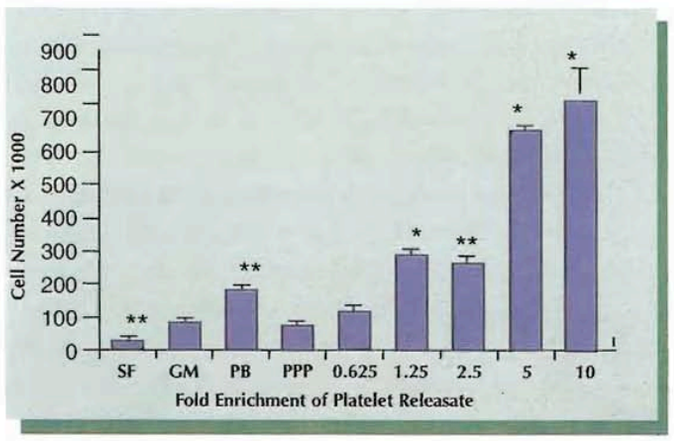
However, extrapolating from the reported levels of VEGF in the PRP[4], it is clear that VEGF by itself is unlikely to account for the majority of the chemotactic effects of PRP. Other known chemotactic molecules, such as TGF-β probably contribute to the chemotactic response of PRP.

MITOGENIC STIMULATION

In order to evaluate the mitogenic activity of PRP, second passage hMSCs were replated at a density of $3x10^3/cm^2$ in serum-free DMEM. Cells were allowed to attach and incubate for 48 hours, at which time culture medium was replaced with the various media. hMSCs were allowed to incubate in test and control media for 7 days with complete media changes taking place on day 4. At the end of the 7 day time course, cells were released with trypsin and counted with a hemocytometer.

PRP releasate stimulates proliferation of hMSCs in a dose-dependent manner. While these experiments demonstrate that serum from a fresh human blood clot, and even PPP, can stimulate hMSC proliferation, approximately 90% of the mitogenic activity in PRP is derived from the platelet releasate.

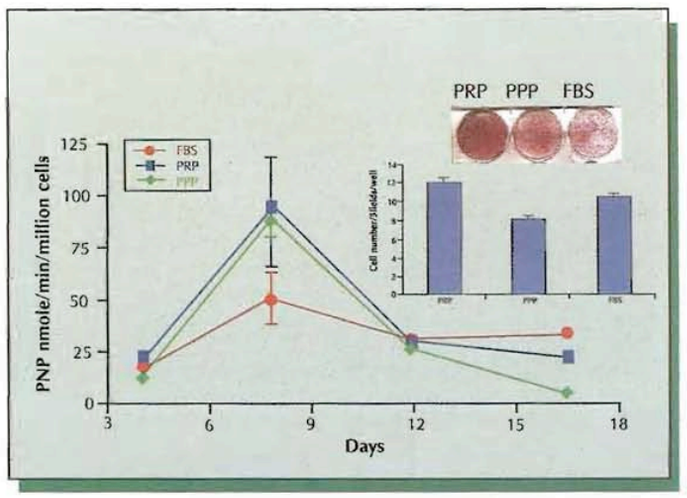
Photomicrographs of hMSCs cultivated in various concentrations of platelet releasate or appropriate controls (original magnification 200x).



*Dose-Dependent Mitogenic Effects of Platelet Releasate on hMSC's. Statistical differences (two-tailed paired t-tests) are shown relative to hMSC Growth Medium (GM) control, *p< 0.05 and **p<0.01*

OSTEOGENIC DIFFERENTIATION

The ability of PRP to support mitotic expansion of hMSCs without loss of their osteogenic potential was demonstrated by first expanding hMSCs in DMEM-LG supplemented to 10% with 5x PRP releasate, PPP releasate, or GM. After 5-7 days of mitotic expansion in the various test media, hMSCs were harvested and reformatted at $3x10^3/cm^2$ and allowed to attach overnight in serum-free DMEM-LG. The next day, culture media were switched to standard GM plus Osteogenic Supplements (OS) consisting of 10^{-7}M dexamethasone, 10m M beta-glycerophosphate and 50 μM ascorbic acid-2-phosphate. On days 4, 8, 12 and 16 cultures were analyzed for alkaline phosphatase expression and calcium deposition into the cell layer.



Osteogenic Differentiation of hMSCs after PRP Releasate-induced Mitotic Expansion

As expected, PRP releasate by itself does not cause osteogenic differentiation of hMSCs. In hMSC samples grown in the presence of PRP releasate without OS, the dominant effect was proliferation with no evidence of differentiation. This trend continued in samples grown with PRP (or PPP) releasates plus OS, as cell proliferation was nearly double that observed in GM plus OS samples. The net effect of this potent mitogenic activity was to keep cells cycling, thus preventing their entry into the osteogenic differentiation pathway.

In samples that were exposed to osteogenic differentiation signals, after rapid expansion in the PRP-supplemented media, the levels of various osteogenic markers were similar or greater than those observed in the controls when normalized to a per cell level. Thus, mitogenic stimulation of hMSCs by PRP releasate occurs without alteration of the cell's phenotype or the loss of its osteogenic development potential. Furthermore, the proliferation rate continued to be higher in the samples initially expanded in PRP, thus leading to an overall increase in osteogenic matrix output in these samples as compared to the controls. This effect was similar to data generated by Slater, et al. using human fetal osteoblastic cells.

CONCLUSIONS & DISCUSSION

- PRP releasate and Vascular Endothelial Cell-Derived Growth Factor (VEGF) each stimulate chemotactic migration of hMSCs in a dose-dependent manner.
- PRP releasate stimulates proliferation of hMSCs in a dose-dependent manner. Approximately 90% of the mitogenic activity in PRP is derived from the platelet releasate.
- Mitogenic stimulation of hMSCs by PRP releasate occurs without alteration of the cell's phenotype or loss of its osteogenic developmental potential.

These observations are consistent with in vivo wound healing models in which degranulated platelets initiate or enhance the healing cascade through the transient chemotactic attraction and mitotic stimulation of reparative cells, which is then followed by morphogenic signals from other sources that induce cell differentiation.
These studies represent the first published data showing a direct effect of PRP releasate on purified human MSCs, which play a pivotal role in the process of musculoskeletal tissue repair. The observation that this easily prepared, autologous source of concentrated growth factors possesses chemotactic and mitogenic activity lends further credence to its therapeutic role in clinical orthopaedics. In view of the data presented, we suggest that local application of PRP causes migration of hMSCs to the wound site, followed by their massive replication to form a repair blastema. As the bioactive factors diffuse away from the fibrin scaffold, now densely populated by hMSCs, the cells cease dividing and are primed to respond to the endogenous inductive cues that stimulate differentiation. The local and transient activity of PRP in this model of tissue repair is responsible for initiating and accelerating the natural healing cascade.

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PRP stem cell therapy
only requires a simple blood draw,
making it safer than
than drilling into bone for marrow,
or performing liposuction for fat.

After bone marrow aspiration procedures, some complications like pain and bleeding at the puncture site may be expected but some serious complications like osteomyelitis and soft tissue infections may also rarely occur.

--- Tural-Kara T, Özdemir H, Fitöz S, Çiftçi E, Yalçınkaya F. Bone marrow aspiration complications: Iliopsoas abscess and sacroiliac osteomyelitis. Turk J Pediatr. 2016;58(5):562-565. doi: 10.24953/turkjped.2016.05.019.

There are a few common sources of stem cells for regenerative medicine. These are blood, fat, and bone marrow. I've seen numerous research articles and web-sites suggesting that a bone marrow aspiration used to obtain stem cells has a high complication rate when compared to liposuction. While we use fat for structural grafts in our Regenexx-AD procedure, we've never considered liposuction safer or more comfortable than a bone marrow aspiration (BMA) procedure... On the complications side, while we've seen very few issues with both procedures, they have different levels of invasiveness. Since liposuction involves placing a larger needle or probe into the fatty area under the skin and moving that probe about to break up the fatty tissue (which can potentially harm arteries, nerves, and other structures), we consider liposuction more invasive than BMA... However, what does the research say about the safety of these two procedures? My analysis today of papers published on both techniques in the National Library of Medicine revealed that the complication rate of a bone marrow aspiration is actually far less than liposuction. Depending on the study, liposuction is approximately 4-100 times more risky in terms of reported complications than a bone marrow aspirate. While serious complications like deaths, skin necrosis, permanent scarring, and blood clots are reported for liposuction, none are reported for bone marrow aspiration.

--- Christopher Centino, MD, writing at: https://regenexx.com/blog/whats-more-risky-bone-marrow-aspiration-or-liposuction/

Complications such as hyperpigmentation of access points, postoperative fluid collection, asymmetry, irregularity, external genital swelling and haematoma were noted. Postoperative fluid collection and haematoma required active intervention. Drainage of fluid collection using a liposuction cannula was effective and prevented recurrence and the need for repeated aspirations.

--- Thomas M, Menon H, D'Silva J. Surgical complications of lipoplasty--management and preventive strategies. J Plast Reconstr Aesthet Surg. 2010 Aug;63(8):1338-43. doi: 10.1016/j.bjps.2009.06.046. Epub 2009 Aug 5.

Although liposuction is minimally invasive and relatively safe, it is a surgical procedure, and it carries the risk of major and minor complications. These complications vary from postoperative nausea to life-threatening events. Common complications include infection, abdominal wall injury, bowel herniation, bleeding, haematoma, seroma, and lymphoedema. Life-threatening complications such as necrotizing fasciitis, deep vein thrombosis, and pulmonary embolism have also been reported.

--- You JS, Chung YE, Baek SE, Chung SP, Kim MJ. Imaging Findings of Liposuction with an Emphasis on Postsurgical Complications. Korean J Radiol. 2015 Nov-Dec;16(6):1197-206. doi: 10.3348/kjr.2015.16.6.1197. Epub 2015 Oct 26.

Obtaining stem cells from blood
is safer and less invasive,
making it less expensive.

The greater safety,
the relative simplicity of the process,
and the lower cost
all make monthly repetition of procedures more possible.

Repetition is a key
to making tissue repair
more thorough, more effective.

Drilling into bone can yield just a bit more stem cells,
but monthly repetition
of our safer, less invasive PRP stem cell therapy process
makes our method effectively grow more new repair tissue.

Conclusions:

Obtaining stem cells from blood is safer, less invasive, more affordable, and effective.

PRP is a stem cell therapy.