# Mechanism of Action of Bone Marrow Concentrates

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LAS VEGAS, NV

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# Disclosures

RELEVANT FINANCIAL RELATIONSHIPS IN THE PAST TWELVE MONTHS BY PRESENTER OR SPOUSE/PARTNER.

**SPEAKERS BUREAU: The Orthobiologic Institute (TOBI), Boston Biolife, Global Regenerative Academy**
**STOCK SHAREHOLDER: N/A**
**GRANT/RESEARCH SUPPORT: N/A**
**CONSULTANT: Benchmark Biomedical, MiniTC**
**MEDICAL ADVISORY BOARDS: Altis.AI,, DataBiologics, MONJ, ROAM Robotics**

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# Objectives

- Understand the principles of the use of Bone Marrow Concentrate (BMC) in regenerative musculoskeletal care
- Understand what a Mesenchymal Stromal/Stem Cell is
- Understand the differences between BMC and PRP
- Learn some of the evidence that supports BMC
- Understand the importance of draw technique, equipment and image guidance.

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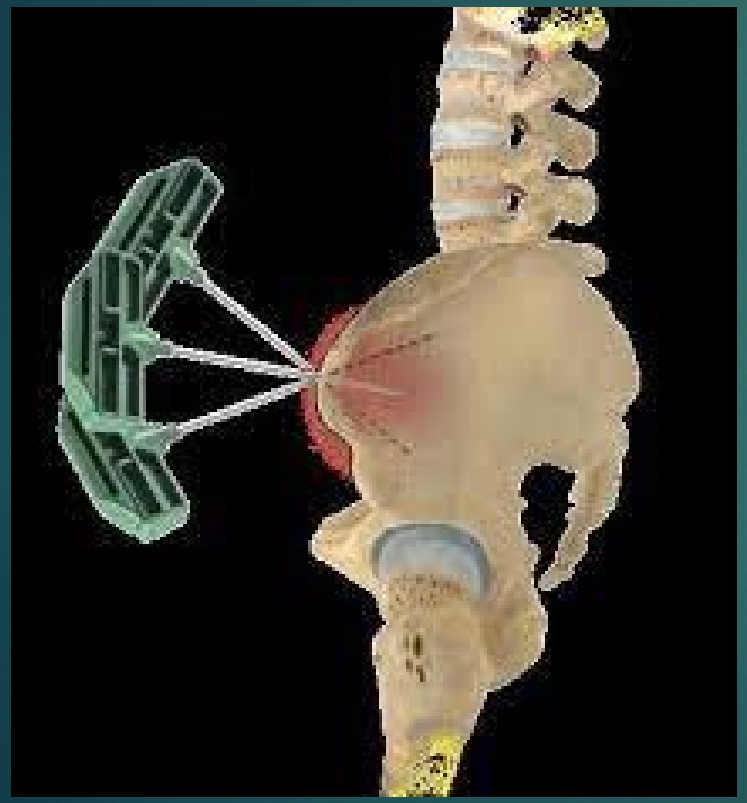
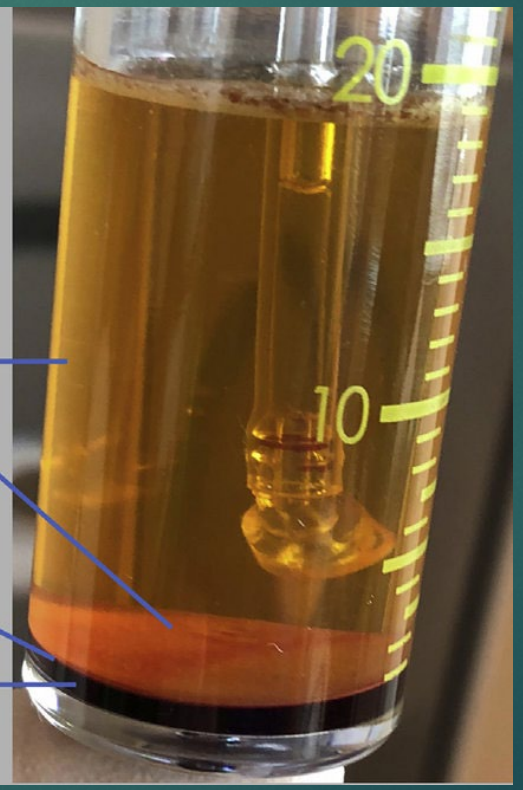
# Bone Marrow Concentrates



**Density separation in g/mL.**

| | |
|---|---|
| Plasma: | 1.025 – 1.029 |
| Platelets: | 1.060 – 1.067 |
| Monocytes: | 1.062 – 1.068 |
| Lymphocytes: | 1.068 – 1.072 |
| HSCs: | 1.069 – 1.071 |
| MSCs: | 1.073 – 1.077 |
| Neutrophils: | 1.080 – 1.090 |
| Erythrocytes: | 1.086 – 1.100 |



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# The Promise

Orthobiologics like BMAC are safe, minimally invasive and may provide a definitive solution for patients in the treatment gap.

Reducing the need and use of corticosteroids, NSAIDS and Opiods

Reducing unnecessary, overly invasive surgery

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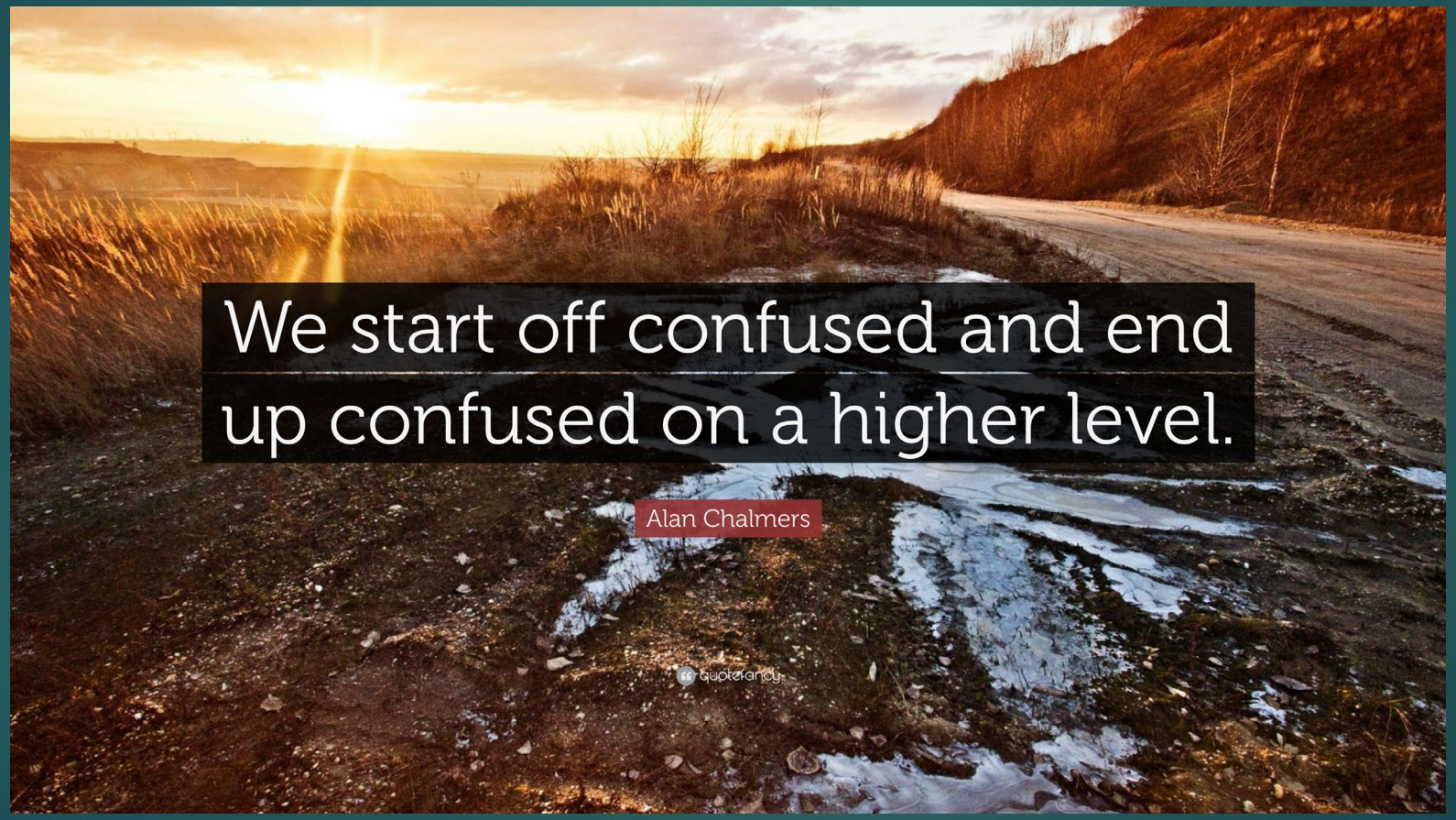
# The Reality

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# Bone Marrow Concentrate Landscape

- Orthopedic surgeons have used Bone Marrow Aspirate for decades
- Hernigou et al, has been injecting BMAC into AVN of the hip and knees as a successful alternative to joint replacement for decades
- Mesenchymal Stem/Stromal Cells (MSC's) are multi-potent and can differentiate into bone, cartilage, fat, etc.
- MSC's, HSC's are found in Bone Marrow Aspirates
- Cortisone has been shown to have adverse side-effects with repeated exposure
- Regulatory controversies

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# Is it Safe?

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Int Orthop. 2016 Aug;40(8):1755-1765. doi: 10.1007/s00264-016-3162-y. Epub 2016 Mar 30.

# A multi-center analysis of adverse events among two thousand, three hundred and seventy two adult patients undergoing adult autologous stem cell therapy for orthopaedic conditions

Christopher J Centeno [1], Hasan Al-Sayegh [2], Michael D Freeman [3] [4], Jay Smith [5], William D Murrell [6], Rostyslav Bubnov [7]

- 3012 MSC based procedures
- 325 Adverse events
  - Pain (3.9%), and pain due to progressive joint disease (3.8%)
- 2.2 years Bone Marrow and/or Adipose
- 3.9% pain from procedure
- 3.8% pain from DJD
- 7 neoplasms total= LOWER incidence than the general population with BMC being the lowest incidence
- Bone Marrow Concentrate (BMC) safer than Adipose or BMC+Adipose or cultured cells (illegal in the US)

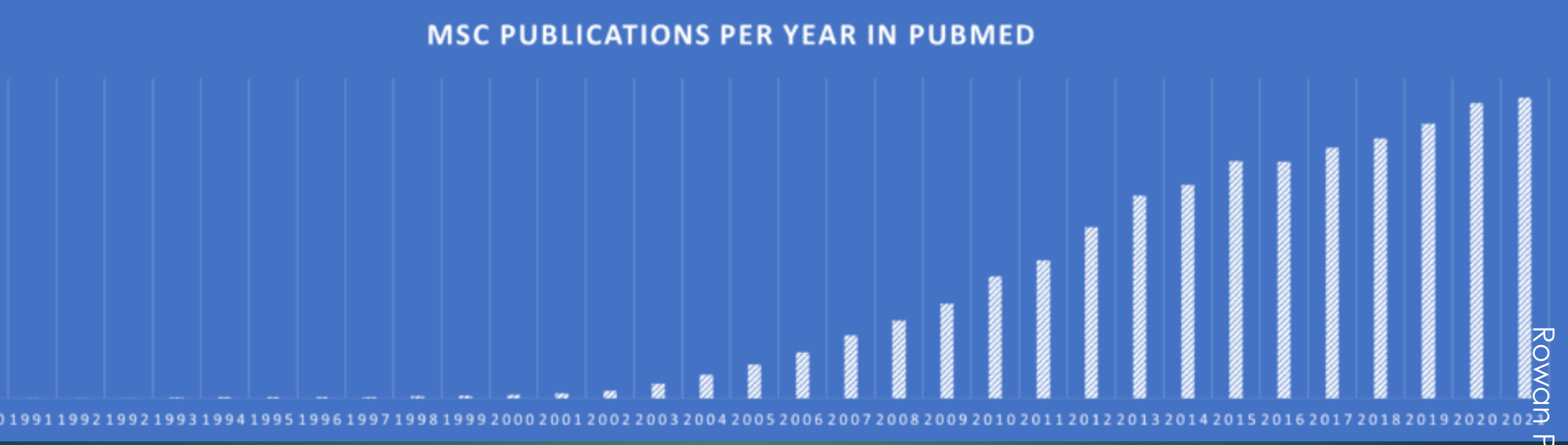
J Bone Joint Surg Am. 2013 Dec 18;95(24):2215-21. doi: 10.2106/JBJS.M.00261.

# Cancer risk is not increased in patients treated for orthopaedic diseases with autologous bone marrow cell concentrate

Philippe Hernigou [1], Yasuhiro Homma [2], Charles-Henri Flouzat-Lachaniette [1], Alexandre Poignard [1], Nathalie Chevallier [1], Helene Rouard [1]

- 12.5 year follow-up data
- 1837 patient s/p BMAC
- No increased cancer/tumor risk at the site of placement or other distant site

# Are there data for Bone Marrow Concentrates?

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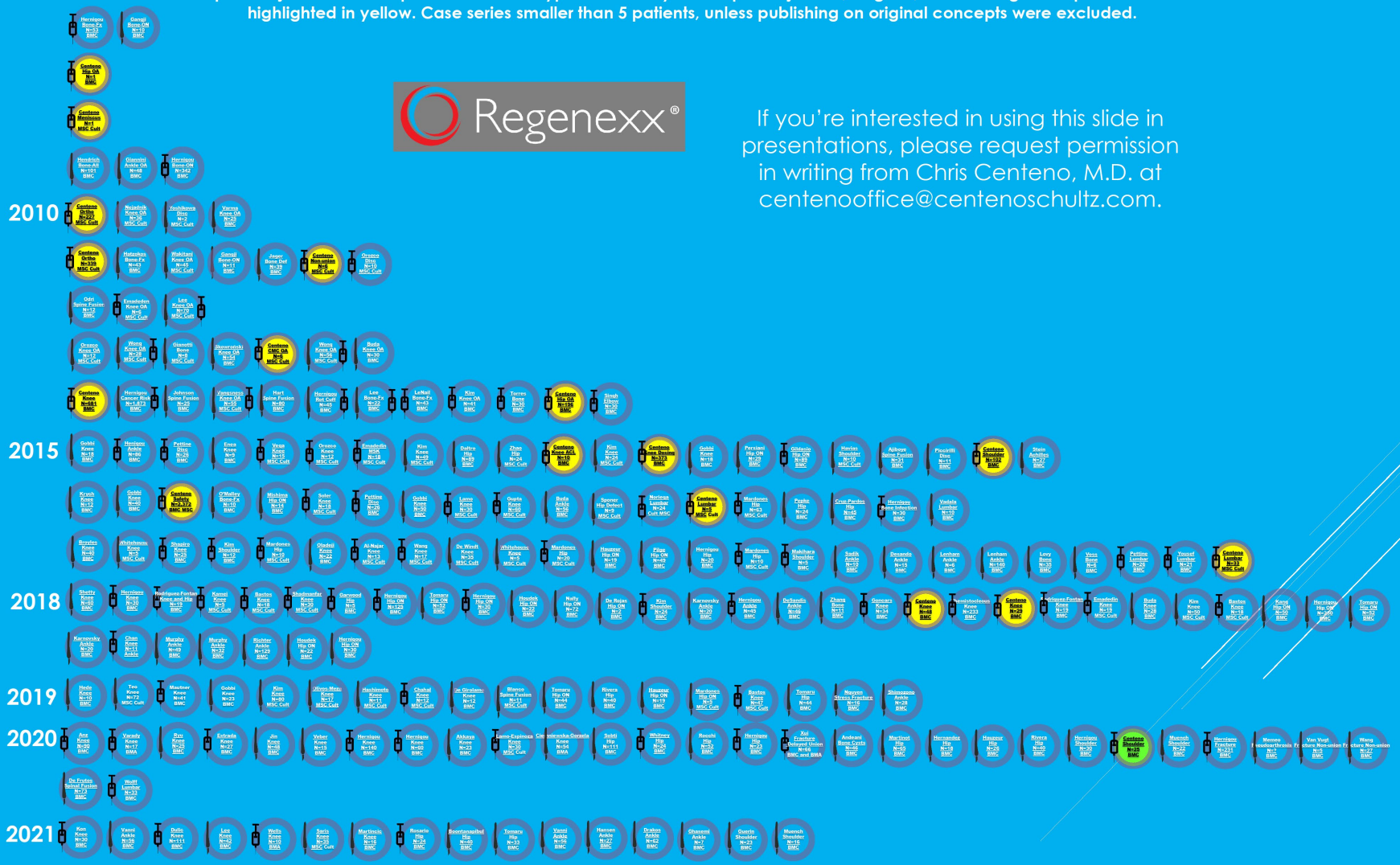
# MSC Publications- The hype AND the data are building

https://regenexx.com/blog/mesenchymal-stem-cell-research-continues-to-increase/

# 2021 Bone Marrow Stem Cell Infographic

Some physicians and scientists argue that we don't have much research supporting the use of bone marrow stem cells to treat orthopedic conditions. Here I list all significant publications from the late 90s through April 2021. The list was complied by running various searches on body parts and bone marrow cell types on PubMed. Each circle represents a research study and is hyperlinked to the abstract in the U.S. National Library of Medicine or the full text article. The main author is listed, the area of treatment, the number of patients involved or treated with bone marrow cells, and the type of bone marrow stem cells used (either Autologous Bone Marrow Concentrate (BMC), Bone Marrow Aspirate (BMA) or Cultured Mesenchymal Stem Cells (MSC Cult) [autologous or allogeneic]. The scalpel or injection icons represent the three types of delivery techniques: injection, surgical, or both. Regenexx publications are highlighted in yellow. Case series smaller than 5 patients, unless publishing on original concepts were excluded.

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# What is in Bone Marrow?

- Cellular and non-cellular components
- Marrow stroma- heterogenous population of cells
  - Fibroblasts, adipocytes, osteoblasts, osteoclasts, macrophages, endothelial cells
  - MSCs and HSCs
  - Platelets and erythrocytes
  - Mononuclear cells
    - Endothelial progenitors

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# ISCT Classification for MSCs

**Table 1:** Negative and Positive MSC Markers as Proposed by the International Society for Cell Therapy (ISCT)

| Negative HSC Marker | Used to Exclude | Peer-reviewed References |
|---|---|---|
| CD34[20,21] | Primitive hematopoietic cells and endothelial cells[22] | 23, 24 |
| CD45[20,21] | Leukocytes[25] | 26, 27 |
| CD11b and CD14[20,21] | Monocytes and macrophages[28,29] | 27, 30 |
| CD79 alpha and CD19 alpha31 | B cells[32] | 33, 34 |
| HLA Class II31 | Antigen presenting cells and lymphocytes[1,35*] | 36, 37 |
| **Positive MSC Markers** | **Biological Role** | **Peer-reviewed References** |
| CD73/5′-Nucleotidase[21] | Catalyzes production of extracellular adenosine from AMP[38] | 39, 40 |
| CD90/Thy1[20] | Wound repair, cell-cell and cell-matrix interactions[41]; see Figure 2 | 27, 42 |
| CD105/Endoglin[21] | Vascular homeostasis; modulates TGF-beta functions via interaction with TGF-beta RI and TGF-beta RII[43] | 24, 36, 44 |

*HLA Class II may be expressed on stimulated MSCs.

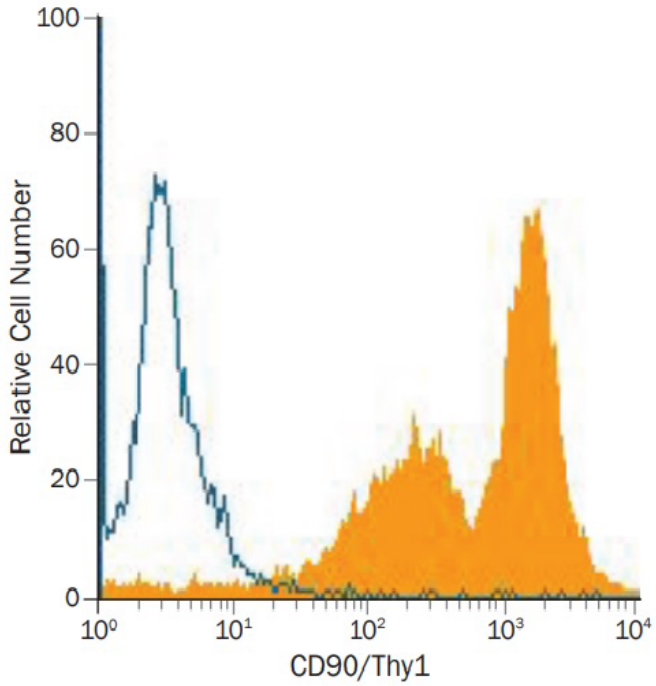


**Figure 2. Detection of CD90/Thy1 in Human Mesenchymal Stem Cells by Flow Cytometry.** Human mesenchymal stem cells were stained with Sheep Anti-Human/Porcine/Canine CD90/Thy1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2067, filled histogram) or isotype control antibody (Catalog # 5-001-A, open histogram),

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Post 2006 Markers for MSC's: Stro-1, Cd271/NGF R, CD200, GangliosideGD2, Frizzled-9, TNAP

# ISCT MSC recommendation

- Cells must adhere to plastic in culture conditions
- Must express CD markers 73,90,105
- Cannot express CD34, 45, 14, 11b, 19
- Cannot express HLA-DR
- Should be able to differentiate into osteoblasts, chondroblasts, adipocytes in vitro

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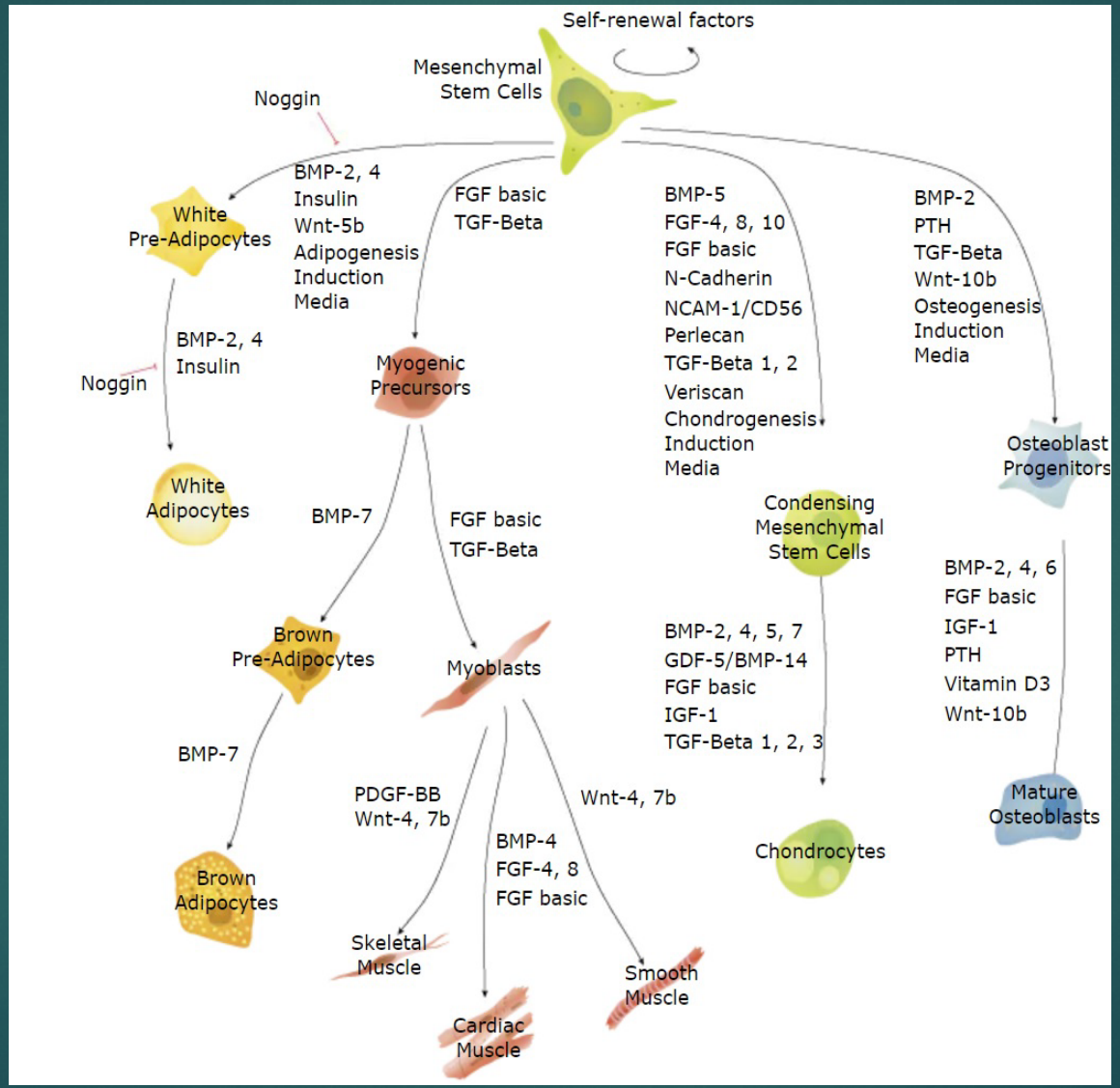
# Immunomodulation by Therapeutic Mesenchymal Stromal Cells is Triggered Through Phagocytosis of MSC by Monocytic Cells

Samantha F.H. De White et al 2018 Stem Cells

- "Primed Monocytes"
  - ↓Decreasing M1 markers , ↑ M2 markers
  - Via  MSC debris uptake in the blood stream
  - Decreased
    - Classical (Non-primed monocytes)
    - TNF-A
  - Increased
    - Intermediate Monocytes
    - PD-L1, CD90 expression
    - IL-6, IL-8, IL-1B expression
    - TGF-B and IL-10

# Traditional thinking on mechanism




https://www.rndsystems.com/pathways/mesenchymal-stem-cell-differentiation-pathways-lineage-specific-markers

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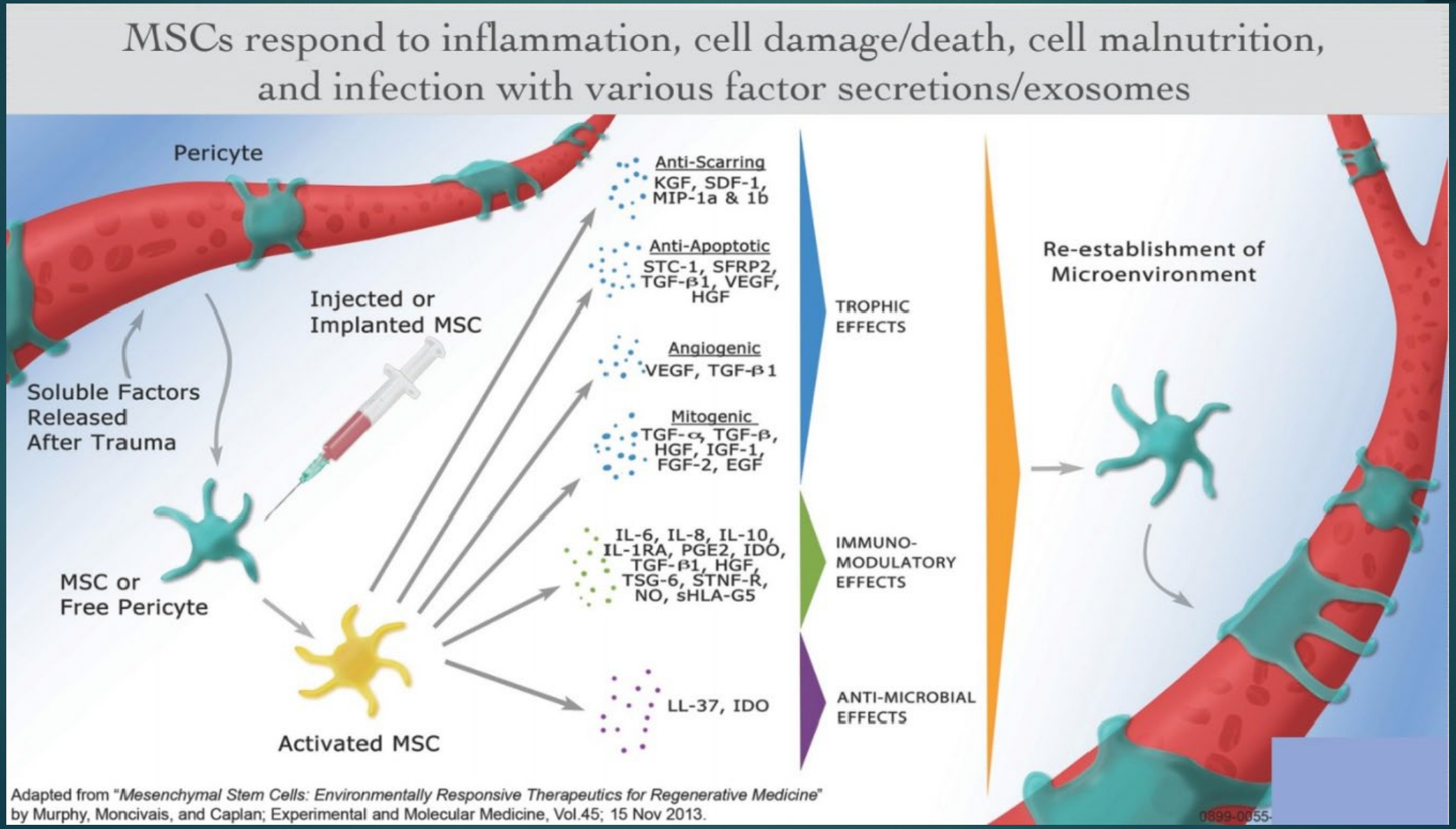
# Updated thinking on mechanism

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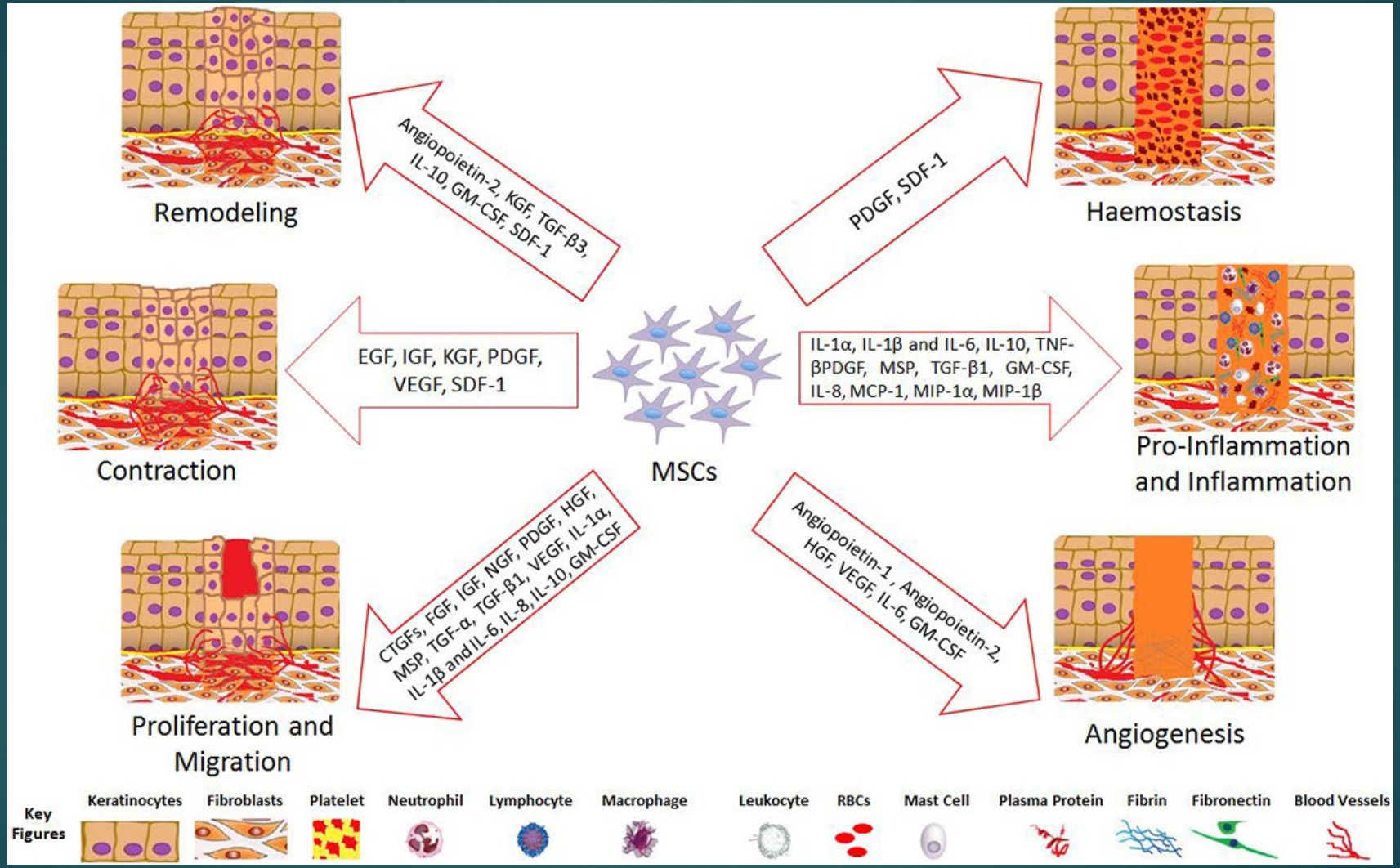
# Paracrine Effect




https://www.intechopen.com/source/html/50942/media/fig2.png

# BMC is NOT PRP with a few Stem Cells

## Cassano KSSTA 2016

- N=29, mean age 48 years, (23-68)
- Bone Marrow
  - CFU, Multi-lineage Differentiation
  - Flow Cytometry:
    - CD271, CD73, CD146, CD34, CD45
- Whole Blood, PRP, BMC in common have
  - Cytology:
    - Leukocytes (Lymphocytes, Neutrophils, Monocytes, Eosinophils, Basophils)
    - Platelets

  Cytokines:

  Anabolic: PDGF TGF- B 1,2,3, FGF, IL-1ra

  Catabolic: IL-1B, IL-6, Il-8, TNF-a, IFNy

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# BMC is NOT PRP with a few Stem Cells

## Cassano KSSTA 2016

| | PRP | BMC | P value |
|---|---|---|---|
| WBC | 3.1 +/- 1.2 | 36.7 +/- 16.9 | <0.0001 |
| Neutrophils | 0.9 +/- 0.4 | 17.5 +/- 9.8 | 0.0002 |
| Monocytes | 0.3 +/- 0.2 | 3.3 +/- 2.3 | 0.0005 |
| Platelets | 202.3 +/- 86.1 | 151.6 +/- 11.2 | 0.46 |

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# BMAC equipment and draw technique matters more than other Orthobiologics

DataBiologics

Second Annual

**Regenerative Orthopedic Patient Outcomes Report**

2022

## Bone Marrow Aspiration Concentrate

### The Research

In clinical studies, bone marrow aspiration concentrate (BMAC) injections have been evaluated as an alternative to knee, hip or spine surgery.[27] Bone marrow cells and platelets along with cytokines and growth factors in the bone marrow, may help in tissue healing.[14] In addition, BMAC has been shown to decrease inflammation, assist with wound healing and repair non healing bone or cartilage injuries.[11,12,14] Although BMAC has shown promising results, much like PRP, more research will be necessary to fully understand the benefits of this emerging treatment.

Strong Evidence

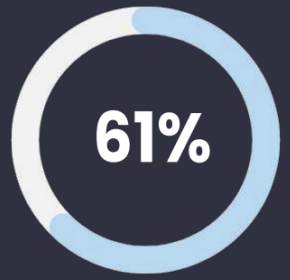
Supports the use of bone marrow injection for **knee osteoarthritis** and **low back pain**.[11,28]

Emerging Evidence

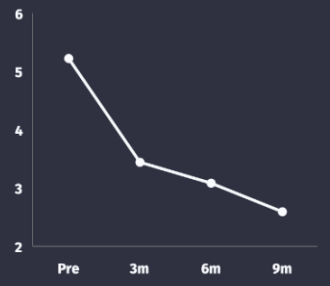
For **degenerative meniscal tear**, or **hip capsular injury, intervertebral disc repair** and **patellar tendinopathy**[29,30,31]

### BMAC processing methods

### Our Outcomes Data




**of patients experienced a meaningful reduction in pain at 12-months following treatment**




**Average patient reported pain (0-10) following BMAC treatment across all conditions**

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# To centrifuge or not to centrifuge?

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# Numbers of MSC's in Bone Marrow Aspirate

| | Bone Marrow Aspirate |
|---|---|
| TNC's or WBC | 10-25 Million TNC/cc |
| MSCs | 0.025--.01% of TNC's |
| HSC's, platelets, plasma proteins | YES |

Using The Midpoint of the Range

17,500,000/cc (TNC) x 0.00625% = 1094 MSC/cc of BMA
ONLY IF DRAW TECHNIQUE IS METICULOUS

Concentrating systems for BMA can further concentrate the MSC/cc from 3-6 times over BMA

=> 4.5x concentration x 1094= 4923 MSC/CC of BMC

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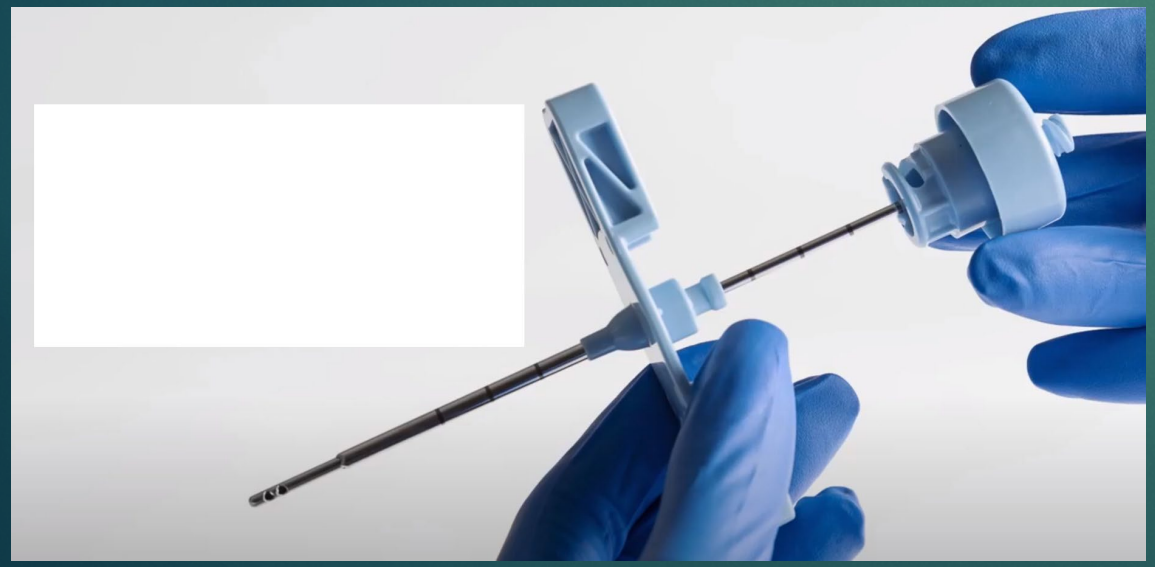
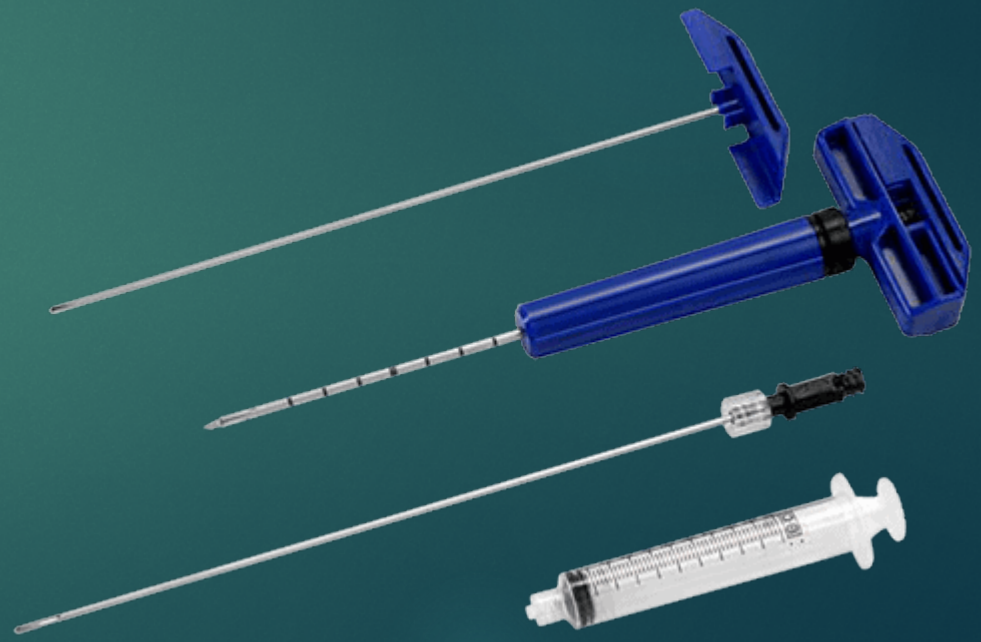
# BMAC 2.0
# Regenerative Medicine Specific Needles
# &
## BMC (Centrifugation) vs Bone Marrow Aspirate





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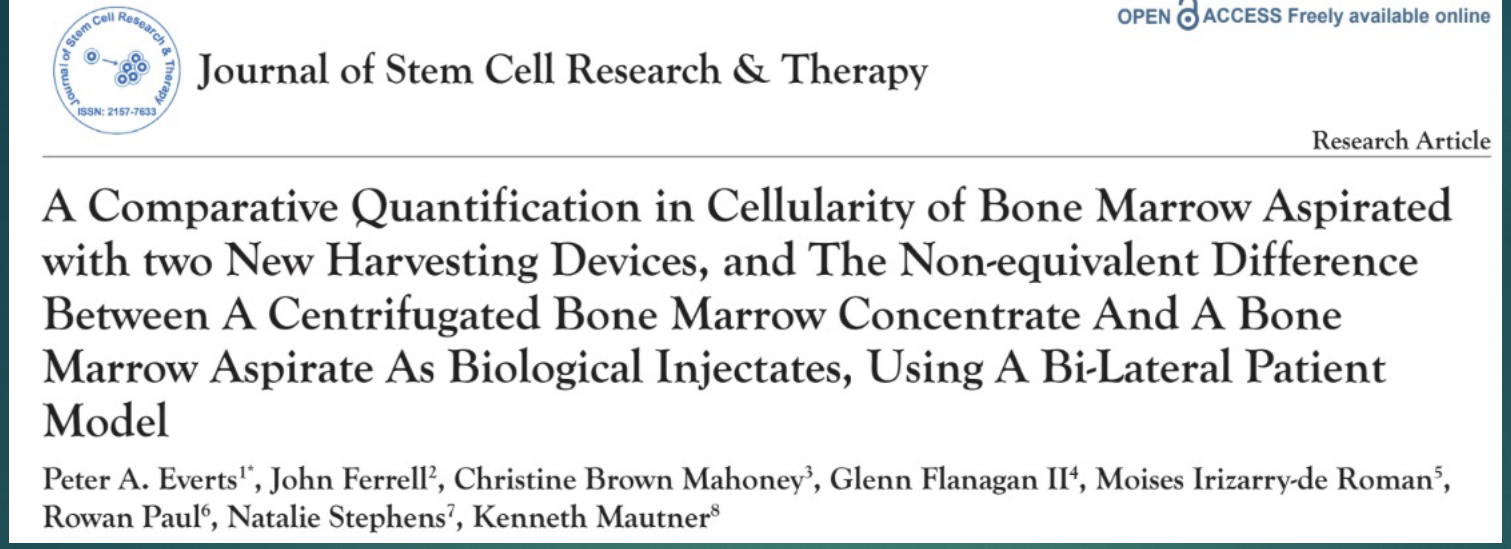

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Journal of Stem Cell Research & Therapy

Research Article

A Comparative Quantification in Cellularity of Bone Marrow Aspirated with two New Harvesting Devices, and The Non-equivalent Difference Between A Centrifugated Bone Marrow Concentrate And A Bone Marrow Aspirate As Biological Injectates, Using A Bi-Lateral Patient Model

Peter A. Everts[1*], John Ferrell[2], Christine Brown Mahoney[3], Glenn Flanagan II[4], Moises Irizarry-de Roman[5], Rowan Paul[6], Natalie Stephens[7], Kenneth Mautner[8]

- AS-BMA >MC-BMA
  - AS-BMA had higher CFU/f and TNC concentrations
  - AS-BMA 1,060/ml, 33.5 × $10^6$/ml vs MC-BMA 610/ml and 28.6 × $10^6$/ml
  - Comparable Platelet and RBC concentration
- After centrifugation
  - AS-BMC>MC-BMA with regard to
    - CFU/f, CD34+ cells, TNCs, platelets, and RBCs
    - Significantly higher cell yields, fewer RBC's, lower hematocrit

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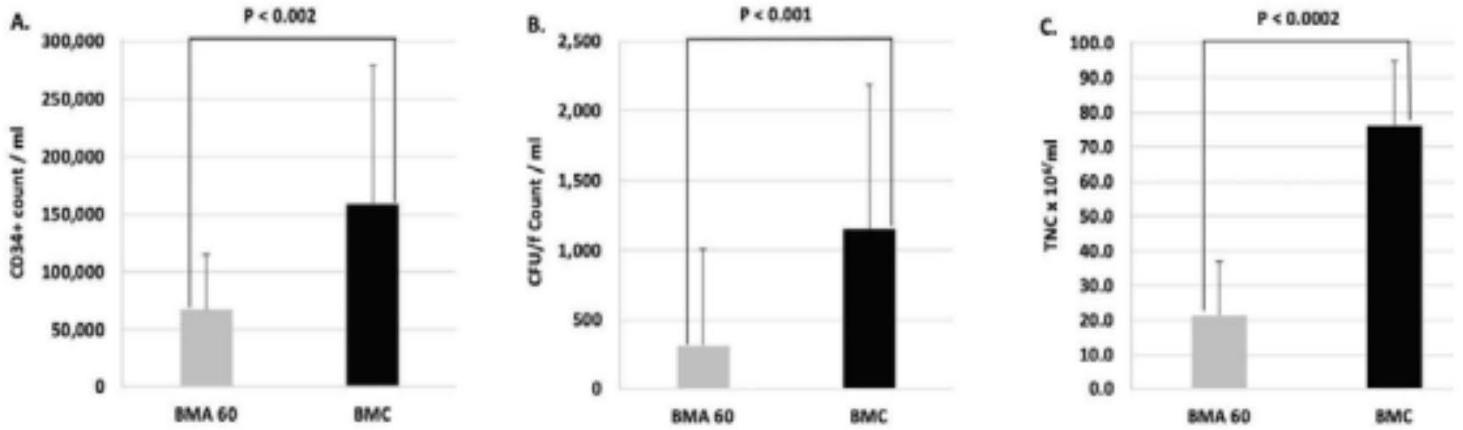



**Figure 5:** Change in CD34 + cells, CFU/f, and TNC concentrations after centrifugation of bone marrow aspirate collected with the AS-BMAD to produce a bone marrow concentrate. Abbreviation: BMA: Bone marrow aspirate; BMC: bone marrow concentrate; CFU/f: colony forming units for fibroblasts; TNC: total nucleated cells.

CFU/f increased from 316-1157 (3.66x)

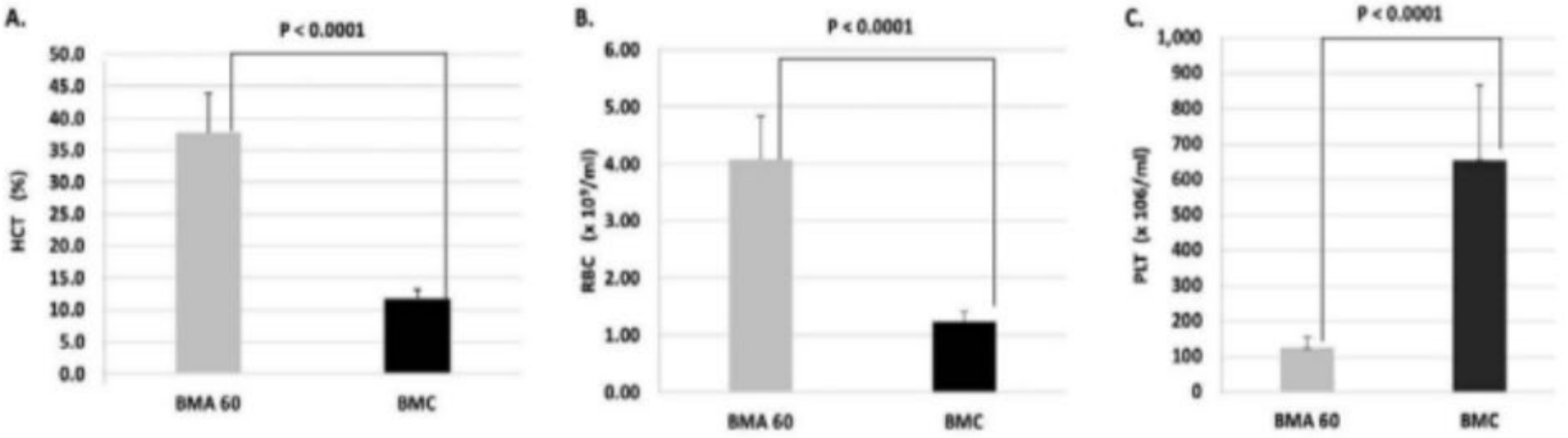



**Figure 4:** Change in hematocrit, RBC, and platelet concentrations after centrifugation of bone marrow aspirate collected with the AS-BMAD to produce a bone marrow concentrate. Abbreviation: BMA: Bone marrow aspirate; BMC: bone marrow concentrate; HCT: hematocrit; RBC: red blood cell; PLT: platelet

By centrifuging, you get about 5x over baseline PRP!

# Benefits of centrifugation

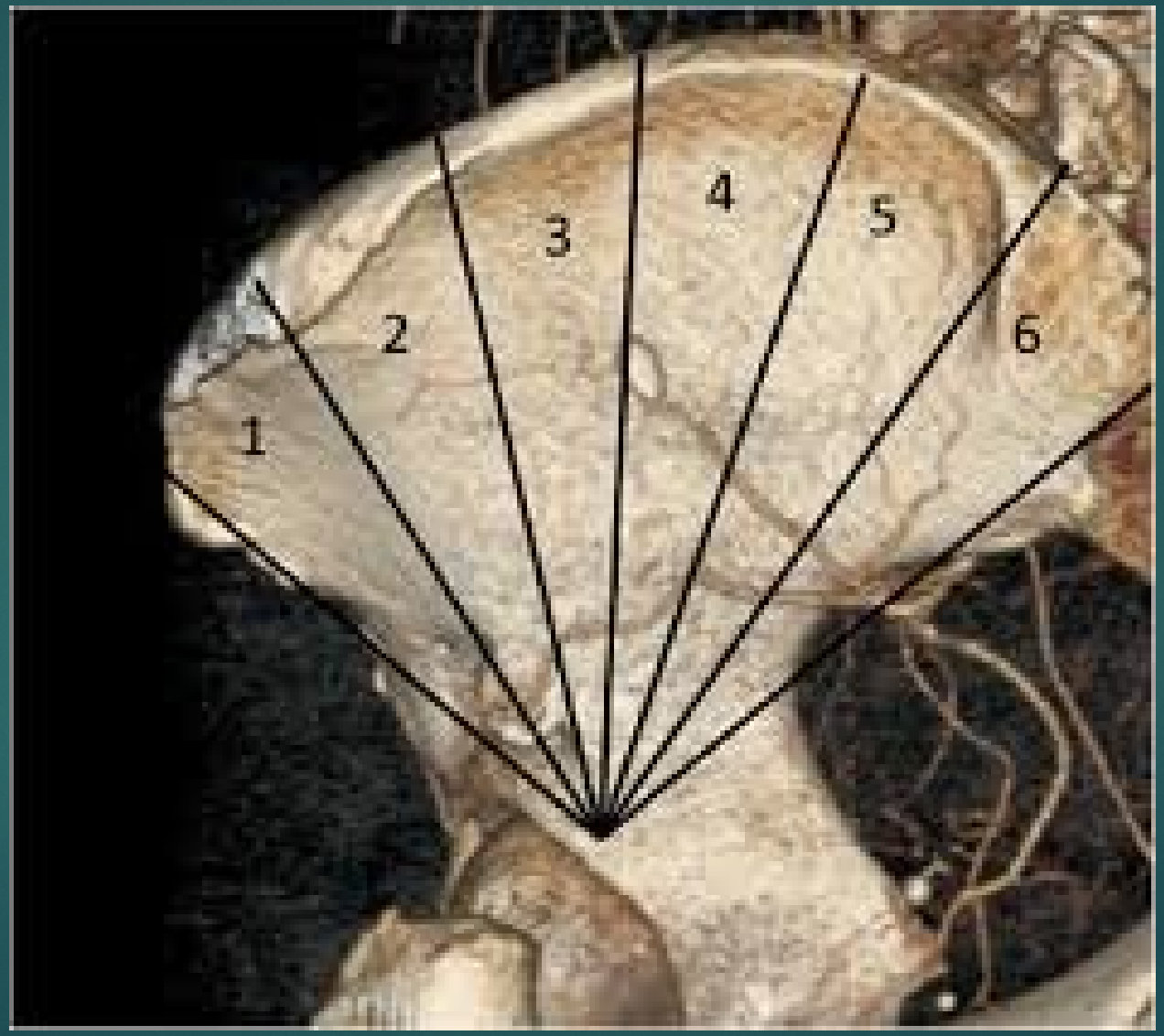
- At least 3-6 x MSC's/Cfu-f
- Potentially 5-6x over baseline PRP
- Decreased RBCs
  - RBC's trigger chondrocyte apoptosis, long term inhibition of proteoglycan synthesis and damage to cartilage
  - Free hemoglobin stimulates expression of ADAMTS-5 and 9 by synovial cells possibly causing cartilage damage.
  - RBC's cause flares in injected areas.
- Concentration into a smaller volumes for smaller target structures

# Where is the best place to Aspirate Bone Marrow?

- PSIS Iliac crest- Safest, highest numbers and in vitro potential
- ASIS: acceptable but more risk than PSIS
- PSIS has more than 2x MSC's/TNC's vs Tibia or Calcaneus
- MSC's from PSIS
  - Desirable immunophenotype AND in vitro potential
  - Better differentiation into osteoblasts, chondroblasts

Understanding Bone safety zones during bone marrow aspiration from the iliac crest: the sector rule. Hernigou J, et al. International Orthopaedics. 2014
Bone Marrow Mesenchymal stem cell aspirates from alternative sources: is the knee as good as the iliac crest? Narbone-Carceles J, Injury. 2014

# Hernigou Intl Orthopaedics 2014

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# Does Age Matter? Age and CFU=F

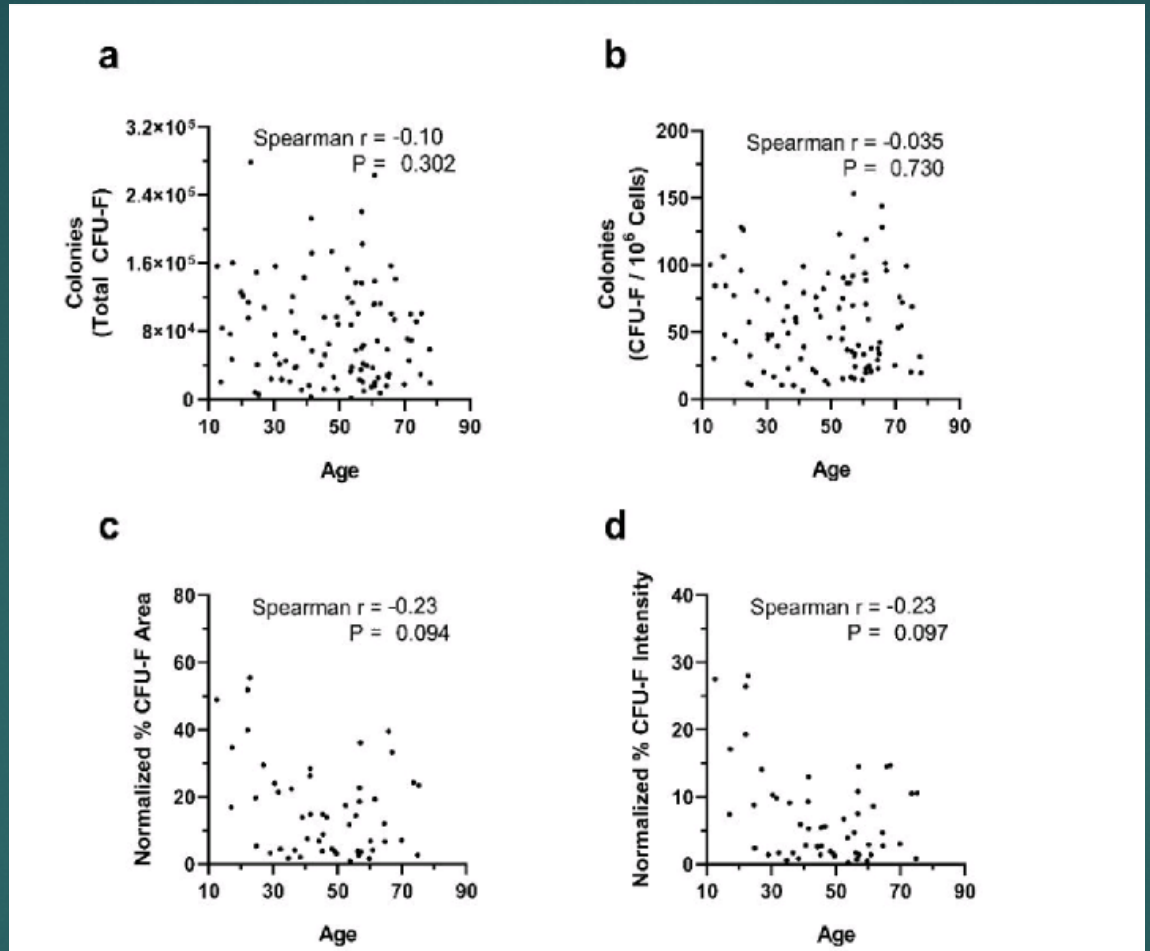



**Fig. 5** Colony formation is not correlated with donor age. Spearman rank order correlations between age and (**a**) total CFU-F, (**b**) CFU-F frequency, (**c**) percent CFU-F area, and (**d**) percent CFU-F intensity in fresh BMAC; (**a-b** n=99, **c-d** n=52)

Centeno, C 9/2019 .Cryopreserved BMC for posthoc CFU analysis

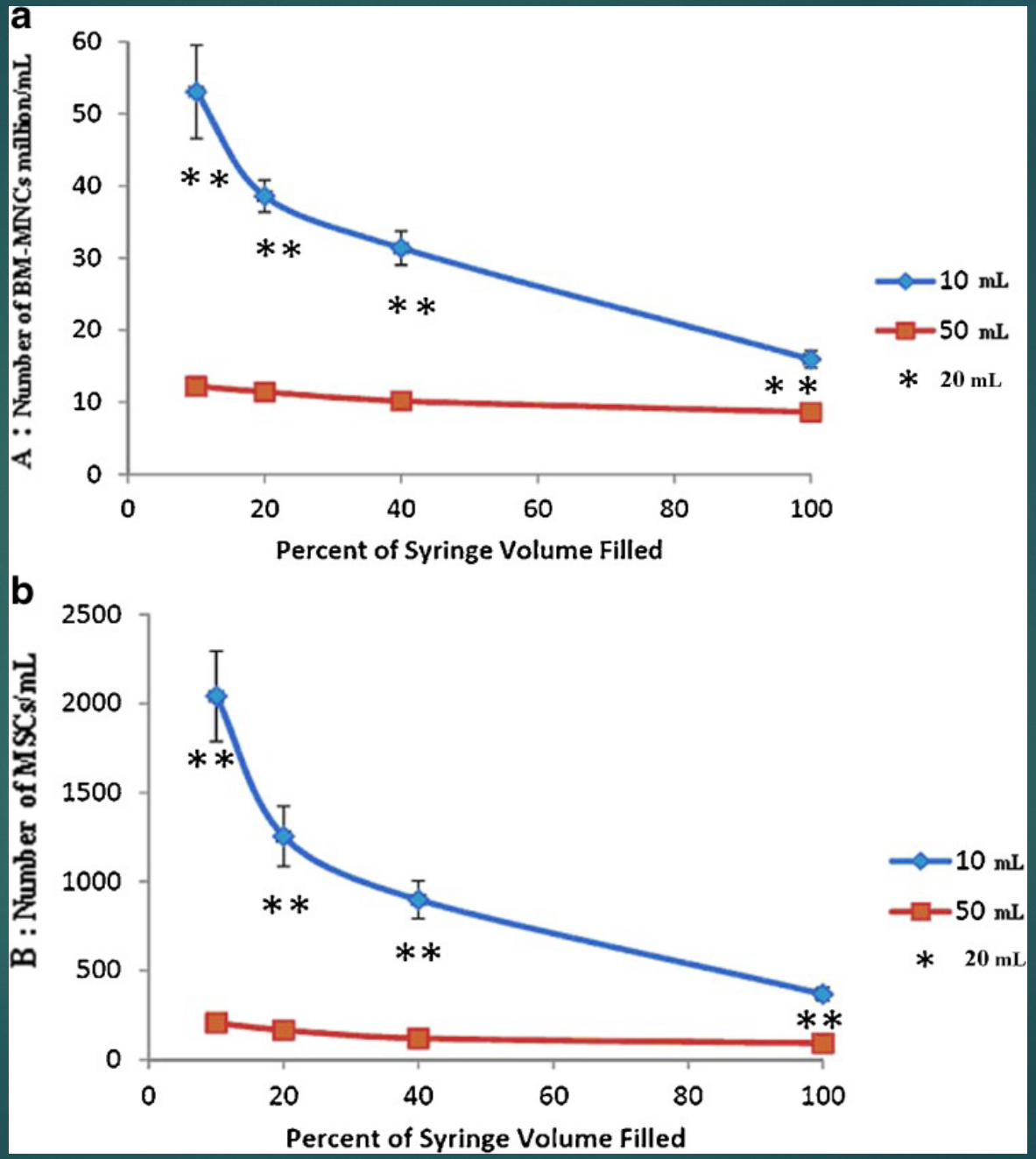
# Bone Marrow Aspiration Technique Matters to maximize dose

- 1-4 ml hard pulls with multiple 10 ml syringes
- Minimizes peripheral blood contamination
- 300% Higher concentrations of MSC with 10ml syringe vs 50 in matched controls ($p<0.01$)

Comparative Study > Int Orthop. 2013 Nov;37(11):2279-87. doi: 10.1007/s00264-013-2017-z. Epub 2013 Jul 24.

**Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells**

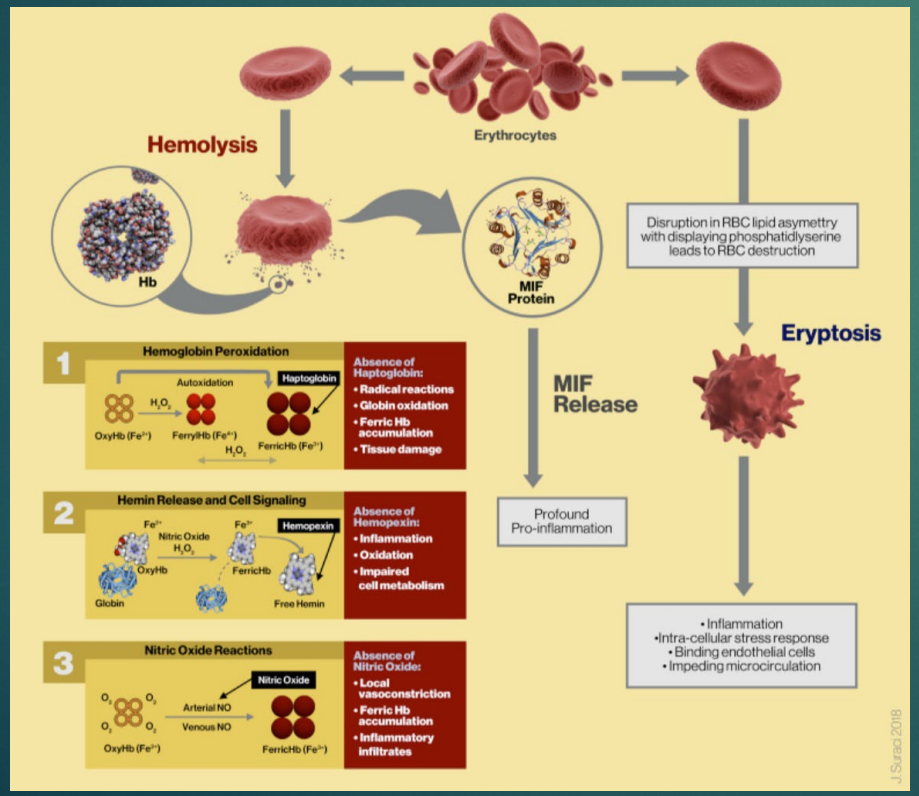
Philippe Hernigou [1], Yasuhiro Homma, Charles Henri Flouzat Lachaniette, Alexandre Poignard, Jerome Allain, Nathalie Chevallier, Helene Rouard




https://pubmed.ncbi.nlm.nih.gov/23881064/#&gid=article-figures&pid=fig-4-uid-3

# Technique matters

- Smooth penetration and fast smooth pulls
- Regenerative medicine specific centrifuges, hand processing and needles

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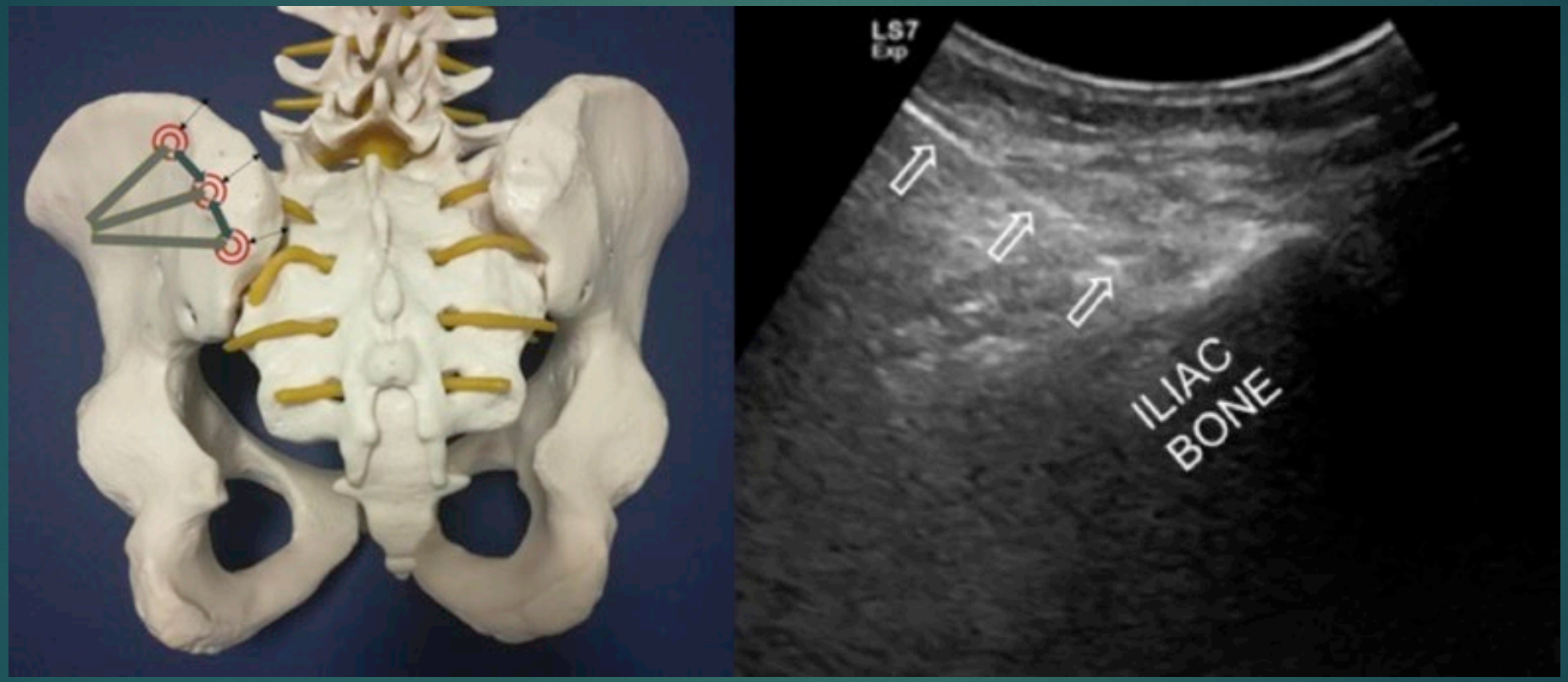
Review > Regen Ther. 2019 May 10;11:56-64. doi: 10.1016/j.reth.2019.03.009. eCollection 2019 Dec.

**Assessing clinical implications and perspectives of the pathophysiological effects of erythrocytes and plasma free hemoglobin in autologous biologics for use in musculoskeletal regenerative medicine therapies. A review**

Peter A Everts [1], Gerard A Malanga [2 3], Rowan V Paul [4 5 6], Joshua B Rothenberg [7 8], Natalie Stephens [9], Kenneth R Mautner [10 11]

# Guidance Matters




https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6349666/

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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6349666/

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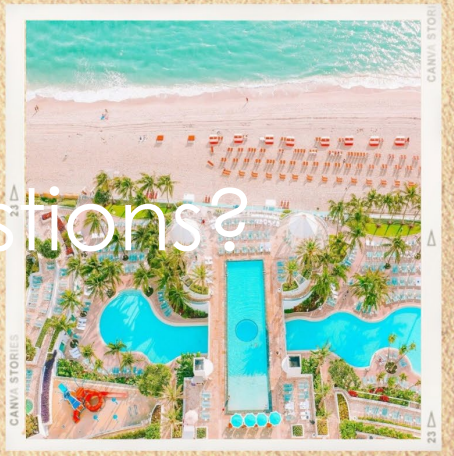

Questions?

# Thank You!

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