

# Mesenchymal Stem Cell Exosomes

The New Frontier of Regenerative Medicine

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# MSC Exosomes

## Arnold Caplan:

- 2006 - “The trophic effects of MSCs may have profound clinical use.”<sup>1</sup>
- 2017 - “I now urge that we change the name of MSCs to Medicinal Signaling Cells ...”
- “It is, indeed, the patient’s own site-specific and tissue specific resident stem cells that construct the new tissue as stimulated by the bioactive factors secreted by the exogenously supplied MSCs”<sup>2</sup>

Revisionist Viewpoint!

- 1917 - Julius Wagner-Jauregg
  - Patients with dementia paralytica after febrile illness frequently become lucid, sane. He began treating dementia paralytica patients with malaria for 8-12 febrile paroxysms then rescued with Quinine Sulfate and they became lucid.
- 1927 - Nobel Prize in Medicine awarded to Julius Wagner-Jauregg for the healing properties of malarial fever
- 1928 - Alexander Fleming discovers Penicillin
- 1938 - Dr. Howard Florey reads Fleming's paper. He is joined by Dr. Ernst Chain and Dr. Norman Heatley
- 1942 - Mass production of Penicillin
  - WW I death rate from pneumonia: 18%
  - WW II death rate from pneumonia: <1%

# Tomorrow's Regenerative Medicine Textbooks

- Bone Marrow Harvest MSCs

- Adipose Harvest MSCs



Malaria

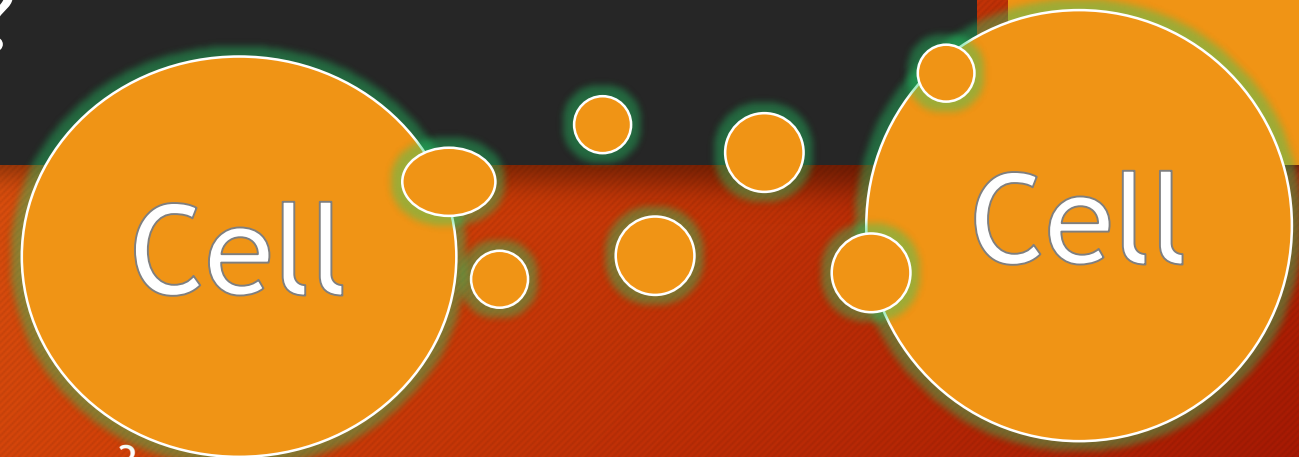
- Exosomes



Penicillin

# What are MSC Exosomes?

- Vesicles from 40-100 nm
- Formed by a two-step budding process <sup>3</sup>
  - Inward budding of membranous vesicles in a multivesicular-body
  - MVBs fuse with the plasma membrane to release their cargo
  - **Transmembrane proteins are conserved!**
- Composed of lipids, proteins, mRNA, and miRNAs

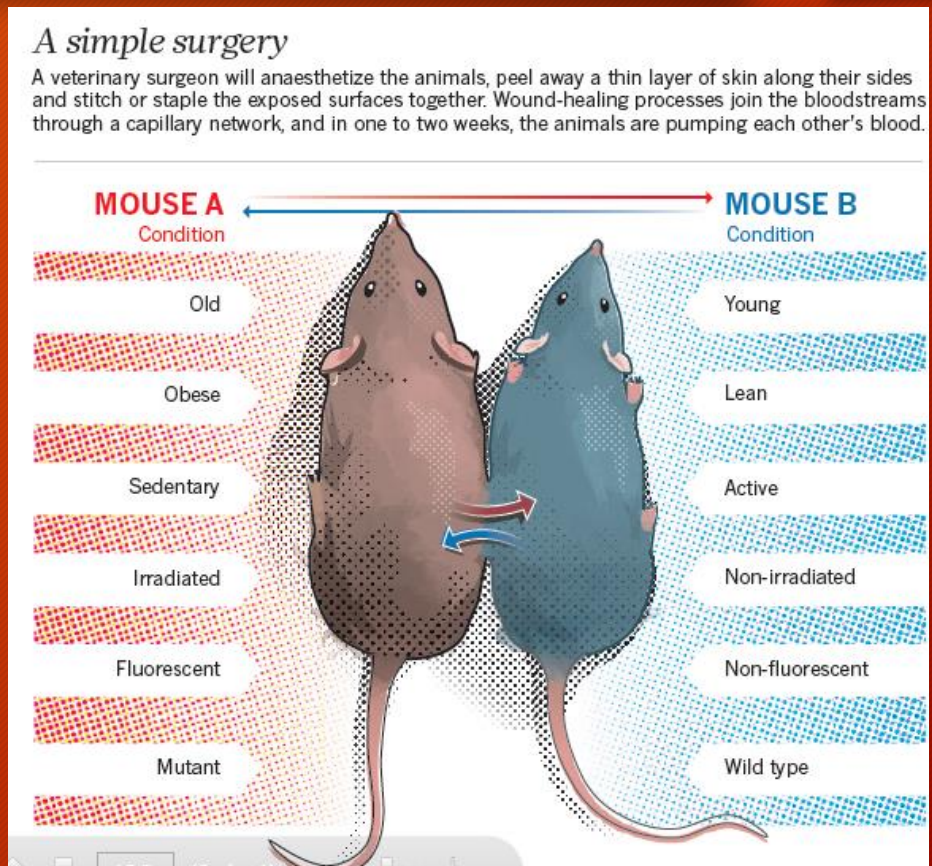


# I said transmembrane proteins are conserved from MSCs. Why?

- MSCs are recruited at the site of injury by receptor-mediated interaction
- MSC-derived vesicles bear the same membrane receptors of MSCs and it is likely for this reason that they accumulate at the site of injury by the same mechanism <sup>4,5</sup>
- MSC exosomes via IV infusion were taken up by M2 Macrophages in spinal cord of injured rats <sup>8</sup>

# What can Science teach us regarding Aging?

By splicing the blood circulation of 2 animals together we have shown that young blood rejuvenates old tissues, bones, muscles, brain, and nerve tissue.



# What can Science teach us regarding aging? (Continued)

A Hong Kong billionaire backed a scientist from Stanford (Wyss-Coray) to start Alkahest, Inc, in Menlo Park, California on 2014.

To Treat Alzheimers they are sharing plasma from adults < 30 and transfusing it into Alzheimers patients.



# What can Science teach us regarding aging? (Continued)

A second company, Ambrosia has started a similar trial in California, giving adults age 35+ transfusions from younger adults <25.

This one-time infusion of a 2L bag of plasma (no blood cells) is offered for \$8,000.

# Why does plasma transfer work?

Are we transfusing stem cells?

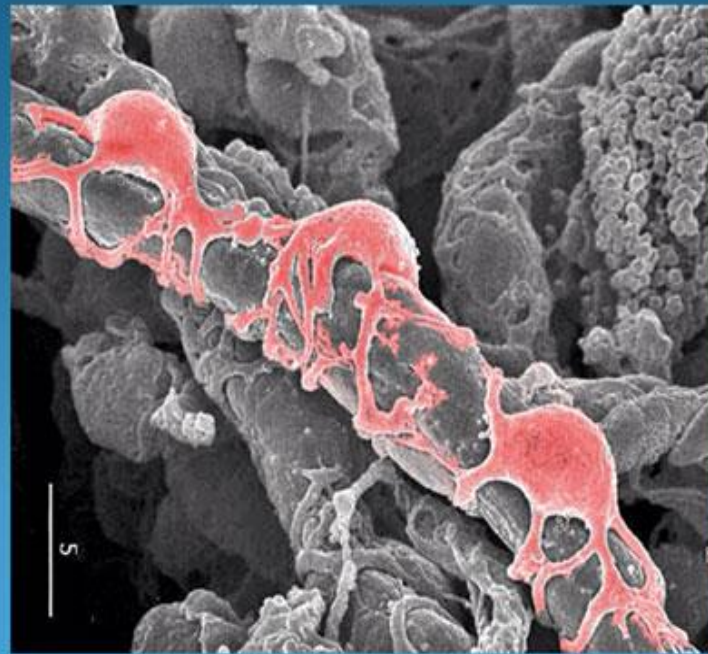
No. Simply, there are not enough stem cells in blood/plasma. In fact, factors present in the plasma seem to upregulate the older stem cells themselves.

# What might these factors be?

# EXOSOMES

Pericytes: cells on capillaries and micro vessels.

ALL MSCs are PERICYTES!



modified by  
**BRUNO PAULT** from <http://www.geocities.co.jp/HeartLand-Suzuran/9389/kekka>

Mesenchymal Stem Cells

[Photograph] Retrieved from <http://stemcellorthopedic.com/mesenchymal-stem-cells/>

# Translational Studies Employing MSC-derived exosomes

- **TARGET TISSUES**

- Heart
- Brain
- Liver
- Lung
- Intestine
- Skin/Wound
- Limb Ischemia
- Skeletal Muscles
- Spinal Cord
- Immune System

These studies demonstrate that MSC-derived exosomes recapitulate the broad therapeutic effects previously attributed to MSCs via horizontal transfer of mRNAs, miRNAs, and proteins<sup>6</sup>

# Mechanisms of MSC Trophic Activity Within the Joint Space

- Trophic factors influence anabolic tendencies of:
  - chondrocytes
  - chondrocyte progenitor cells (CPCs)
  - cartilage-derived stem/progenitor cells (CSPCs)
  - synovium-resident multipotent progenitor cells
  - osteoblasts/osteoclasts/resident MSCs within the subchondral bone
  - chondrogenic cells within the infrapatellar fat pad

# MSC Trophic Activities relevant to MSK Therapy<sup>7</sup>

- Angiogenesis
- Neurogenesis
- Osteogenesis
- Musculogenesis

“Mesenchymal stem cells derived exosomes and microparticles protect cartilage and bone from degradation in osteoarthritis”

-Cosenza, et al.

Microparticles and exosomes exerted similar chondroprotective and anti-inflammatory function *in vitro* and protected mice from developing osteoarthritis *in vivo*.

# Key Advantages of Exosomes:

1. Can travel via systemic therapy without risk of clumping
2. Can travel via local therapy
3. Cross the “Blood-Brain Barrier”
4. Deliver miRNA and mRNA
5. Can home
6. Not perceived as foreign
7. No first-pass lung removal as in MSCs
8. Can not transdifferentiate into other cells or into malignant cells
9. Easy to administer, store, freeze (Out of the box stem cell therapy!)
10. Easily controlled dosage
11. Potency related to age of parent MSC



# Key Therapeutic Effects of MSC Exosomes

1. Influence Growth of Target Cells
2. Influence Phenotype
3. Contribute to Cell Fate Decision
4. Promote Regeneration
5. Immunomodulation
6. Anti-Inflammatory
7. Anti-Fibrotic

# Some Key Immune and Growth Factors Present in MSC Exosomes

- BMP-5 Stimulates bone growth
- GDF-15 Growth Differentiation Factor-15
- OPG Stimulates bone growth/blocks osteoclast precursor formation
- G-CSF Granulocyte Colony Stimulating Factor
- SCF Stem Cell Factor shown to be responsible for stem cell and melanocyte growth
- TGF- $\beta$ 3 The most important anti-inflammatory protein. Converts inflammatory T-Cells to anti-inflammatory regulatory T cells.
- VEGF Vascular Endothelial Growth Factor
- ICAM-1 Binds inflammatory ligands on white cells

# Some Key Immune and Growth Factors Present in MSC Exosomes

- IL-1 $\alpha$  Binds and sequesters the inflammatory cytokine IL-1
- IL-6 Responsible for macrophage activation
- IL-10 An anti-inflammatory cytokine responsible for immunomodulation and regulatory T cell conversion
- MCP-1 Monocyte Chemoattractant Protein-1 is a chemokine that recruits mononuclear cells to the treatment area
- MIP-1 Also known as CCL-4, this chemokine recruits mononuclear cells to the treatment area
- PDGF- $\beta\beta$  Platelet Derived Growth Factor Beta

# Some Key Immune and Growth Factors Present in MSC Exosomes

- TIMP-1
  - TIMP-2
  - TNF-RI
- Tissue Inhibitor of Matrix Metalloproteinases, blocks collagen and extracellular matrix degradation. Important for cartilage repair
- Binds and inactivates the inflammatory TNF- $\alpha$

# Contraindications to Stem Cells/Exosomes

- Cancer
- Myeloproliferative Disease
  - Bone Marrow Dysplasia
  - Sickle Cell
- Primary Pulmonary Hypertension
- Acute Bacterial Infection
- Recent Dental Work
- Macular Degeneration with neovascularization
- Any abnormal neovascularization
- Immuno-compromised

# Preoperative Labs

- CBC
- CMP
- UA
- CA-125 for females
- PSA for males over 40
- CEA for males and females over 40
- PT/INR
- EKG

# Important Immunological Effects of MSC Exosomes

- Downregulate TH1 and TH17 cells while upregulating T<sub>Reg</sub> cells
- Upregulate M2 macrophages at expense of M1 Macrophages

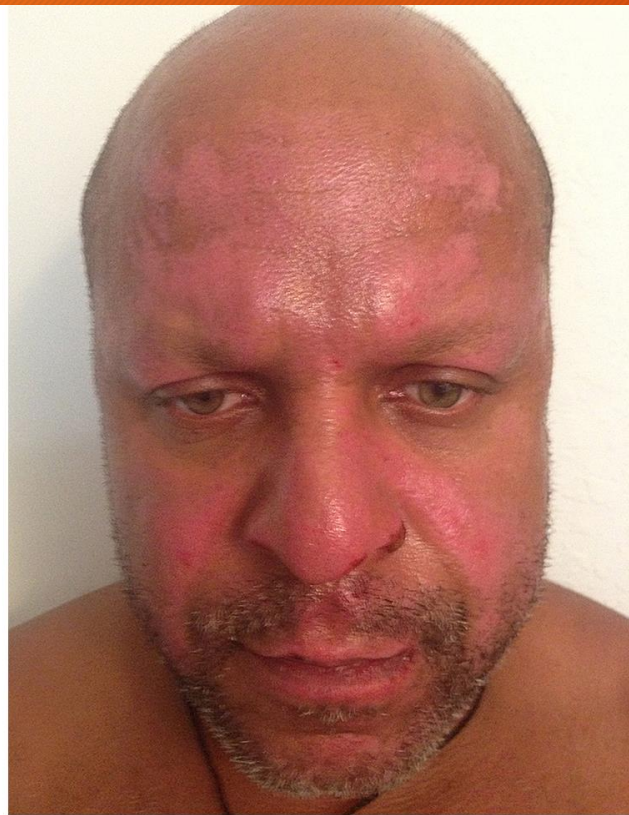
# MSC Exosomes for Hair Growth

- Hair regeneration is controlled by dermal papilla (DP) cells which are located at the base of hair follicles
- 3 Phases:
  - Anagen (Growth)
  - Catagen (Apoptosis)
  - Telogen (Inactive)
- Androgenic alopecia - Intact population of hair follicle stem cells deficient in DP signaling
- MSC extracellular vesicles promote telogen to anagen conversion comparable to Minoxidil<sup>11</sup>
- SCF present in exosomes is required for hair follicle creation from stem cell progenitors





Day 0



Day 7



Day 60

**TOPICAL EXOSOMES**

# Exosomes secreted by human adipose mesenchymal stem cells promote scarless cutaneous repair by regulating extracellular matrix remodelling

Lu Wang, Li Hu, Xin Zhou, Zehuan Xiong, Chenguang Zhang, Hassan M. A. Shehada, Bo Hu, Jinlin Song ✉ & Lili Chen ✉

- 100 Million people annually suffer pain and discomfort caused by scarring.
- Scar is a complex process involving inflammatory, proliferative , and remodeling phases
- Fetuses retain the ability to heal regeneratively without scars
- Scarless wound tissue is marked by fine reticular collagen, less cross-linking, less inflammation, and fewer fibroblasts.
- The remodeling of extracellular matrix is one of the most important factors for scar formation.

# Adipose MSC Exosomes

- Increase ratio of Collagen III to Collagen I
- Prevent fibroblast differentiation into myofibroblasts
- Inhibit granulation tissue formation
- Increase ratio of TGF- $\beta$ 3 to TGF- $\beta$ 1
- After IV administration accumulate in wound area
- Increase ratio of MMP3 to TIMP1

# MSK Protocols

- Joints
  - PRP + Exosomes Day 0
  - PRP + Exosomes Day 14
- Muscles
  - Direction Injection Exosomes
- Tendons/Ligaments
  - PRP + Exosomes (+ Tenotomy for tendons)
- Bones
  - BMAC + Exosomes +/- DBM vs. other scaffold
- Nerves
  - Exosomes +/- PRP
- Discs
  - Exosomes + PRP Day 0
  - Exosomes + PRP Day 14

# Aesthetic Protocols

- Hair  
PRP + Exosomes
- Ulcers/Wounds/Scars/Venous Insufficiency Ulcers  
Debride, Exosomes along periphery of wound and into wound base +/- Tisseel covering
- Erectile Dysfunction  
Exosomes +/- PRP
- Urinary Incontinence  
PRP + Exosomes
- Arterial Insufficiency  
Exosomes

# Thank You!

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