Introduction to Stem Cell Therapy

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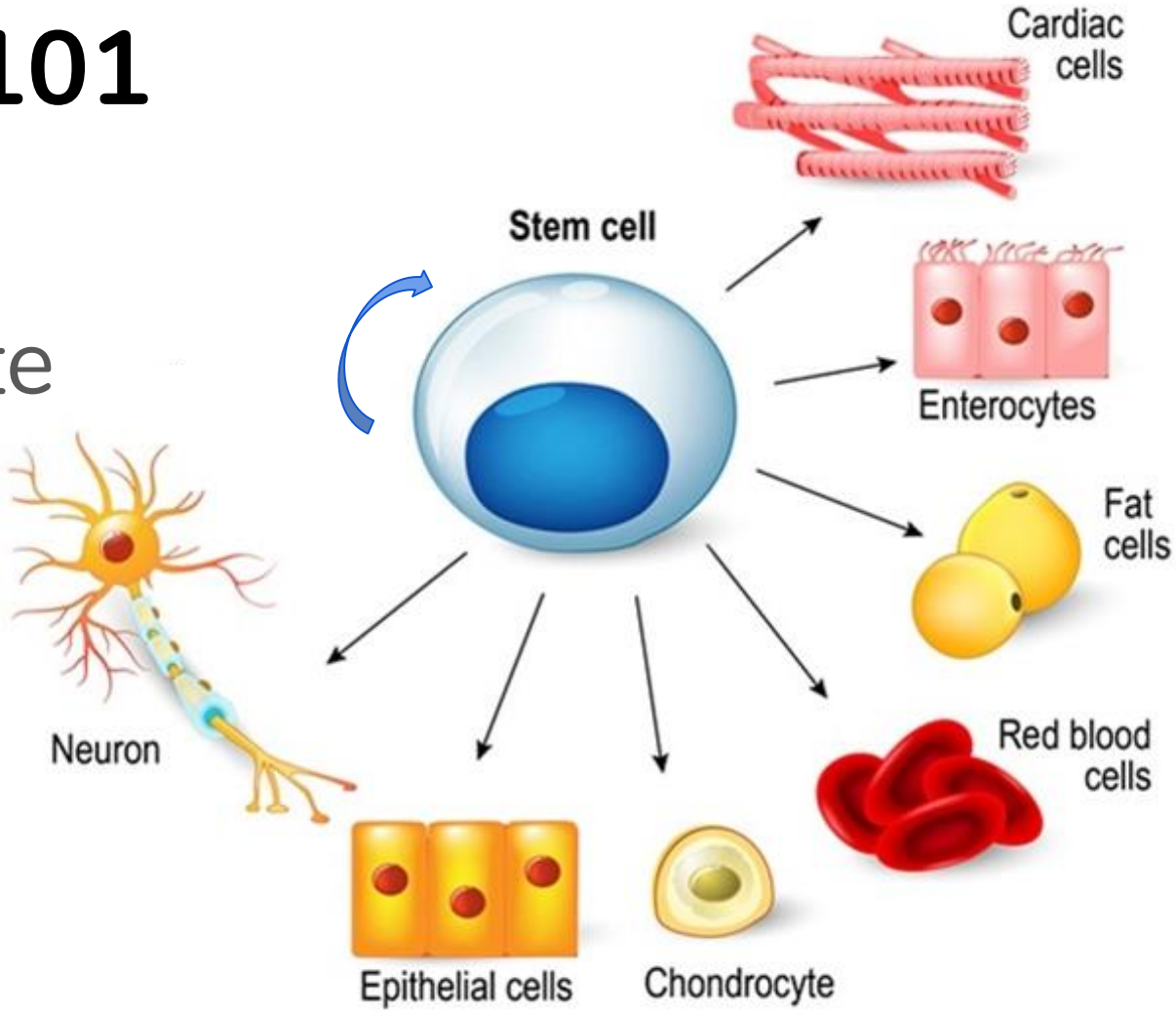
Founder/CEO

Stem Cells 101

- Self-renew
- Differentiate



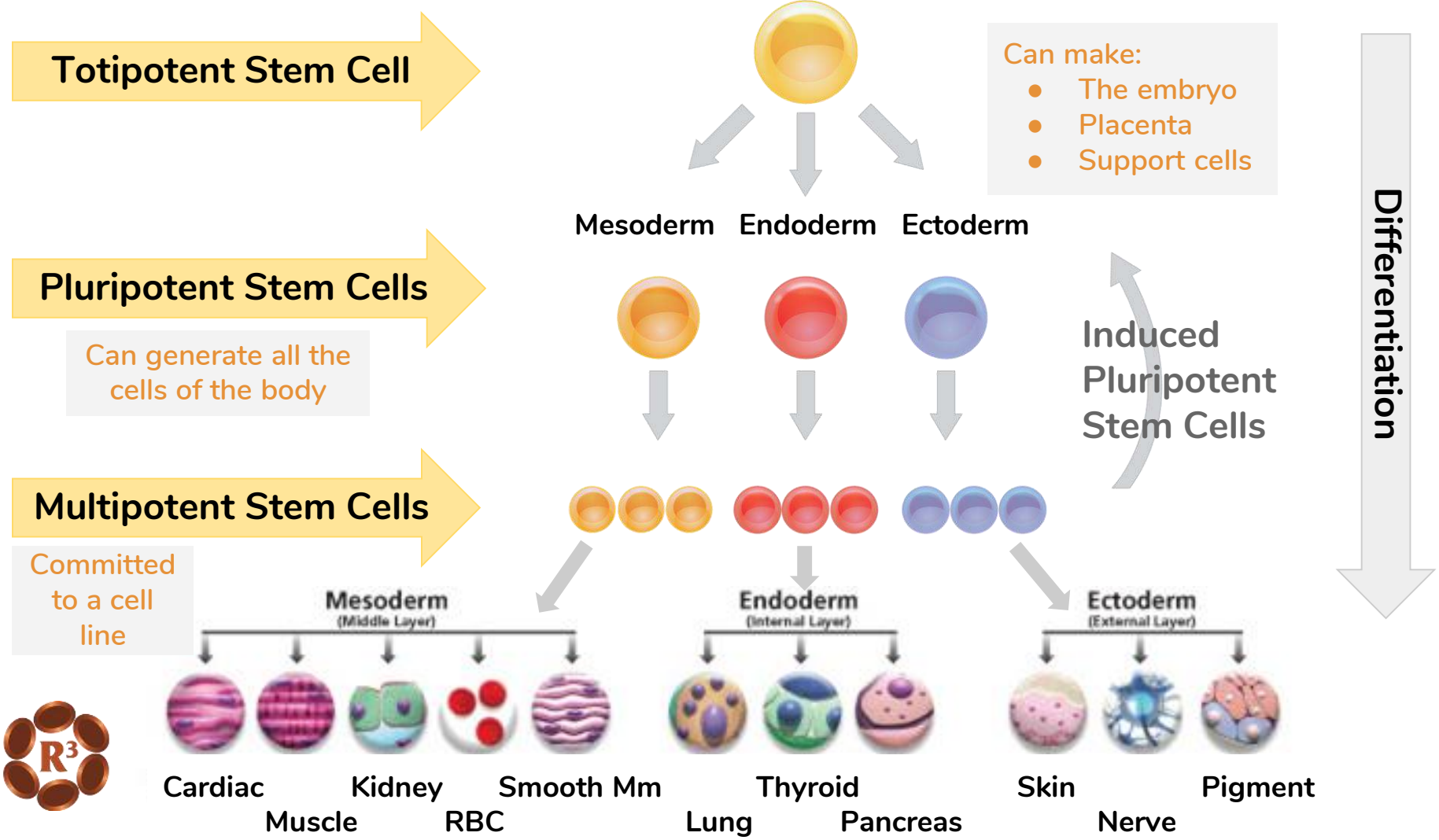
Classification

Source of Origin

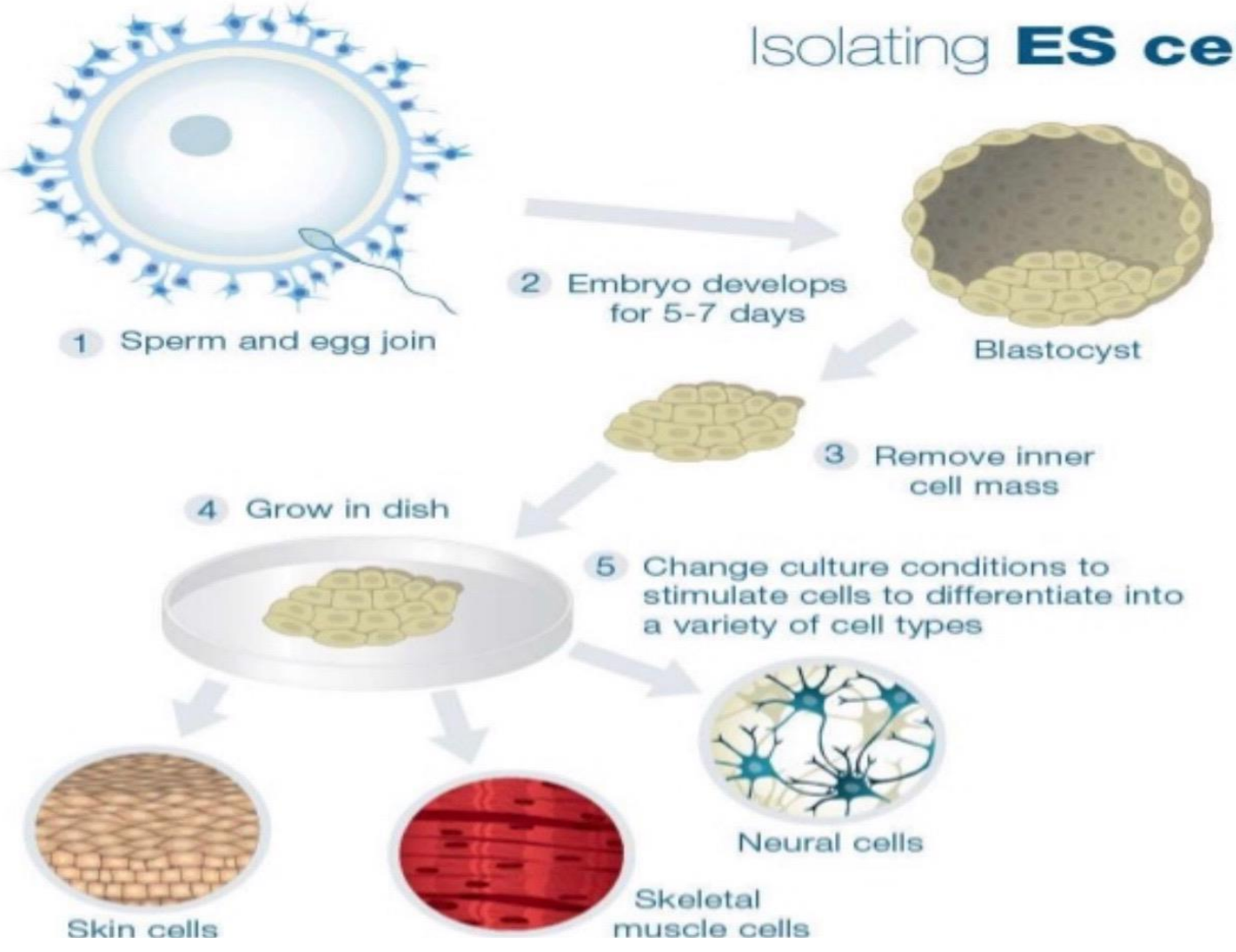
- Embryonic
- Adult

Action Potential

- Totipotent
- Pluripotent
- Multipotent
- Unipotent


Totipotent Stem Cell
Can make:
The embryo
Placenta
Support cells
Mesoderm
Endoderm
Ectoderm
Pluripotent Stem Cells
Can generate all the cells of the body
Induced Pluripotent Stem Cells
Differentiation
Multipotent Stem Cells
Committed to a cell line
Mesoderm
(Middle Layer)
Endoderm
(Internal Layer)
Ectoderm
(External Layer)
Cardiac Muscle
Kidney
RBC
Smooth Mm
Thyroid
Lung
Pancreas
Skin
Nerve
Pigment

Isolating **ES cells**



Adult Stem Cells

Bone Marrow

Adipose Tissue

Post Partum Birth Tissues

Mother/Donor

Donor's provide full consent and undergo comprehensive panel testing to determine donor eligibility.

Birth/Collection

Donated tissue is collected by a licensed professional at time of birth and shipped overnight to our laboratory.

Processing

Upon receipt of tissue Invitrx lab technicians utilize Invitrx proprietary methods to process tissue.

Cryopreservation

During processing a cryopreservative is introduced to the tissue. This allows the tissue to be stored at -80°C.

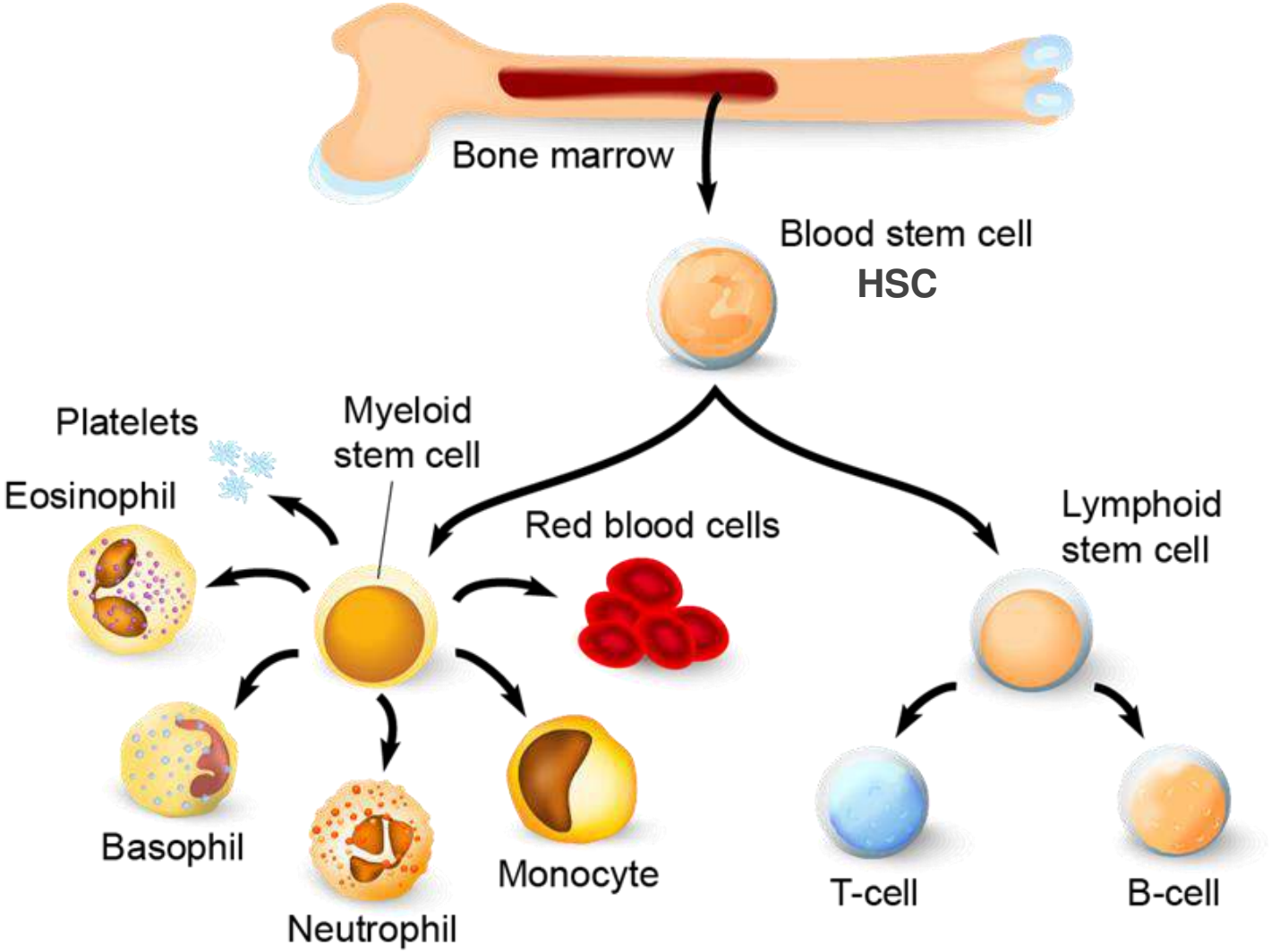
Quarantine

Newly processed allografts are quarantined for a period of two weeks pending in which the tissue is tested for infectious disease and microbial contamination.

Release

Quality Assurance identifies allograft to have passed all required testing including infectious disease and microbial testing.

Bone Marrow Stem Cells (HSC)



Bone Marrow Aspiration & Concentration- BMAC

FDA Approved

PROS:

- Autologous

CONS:

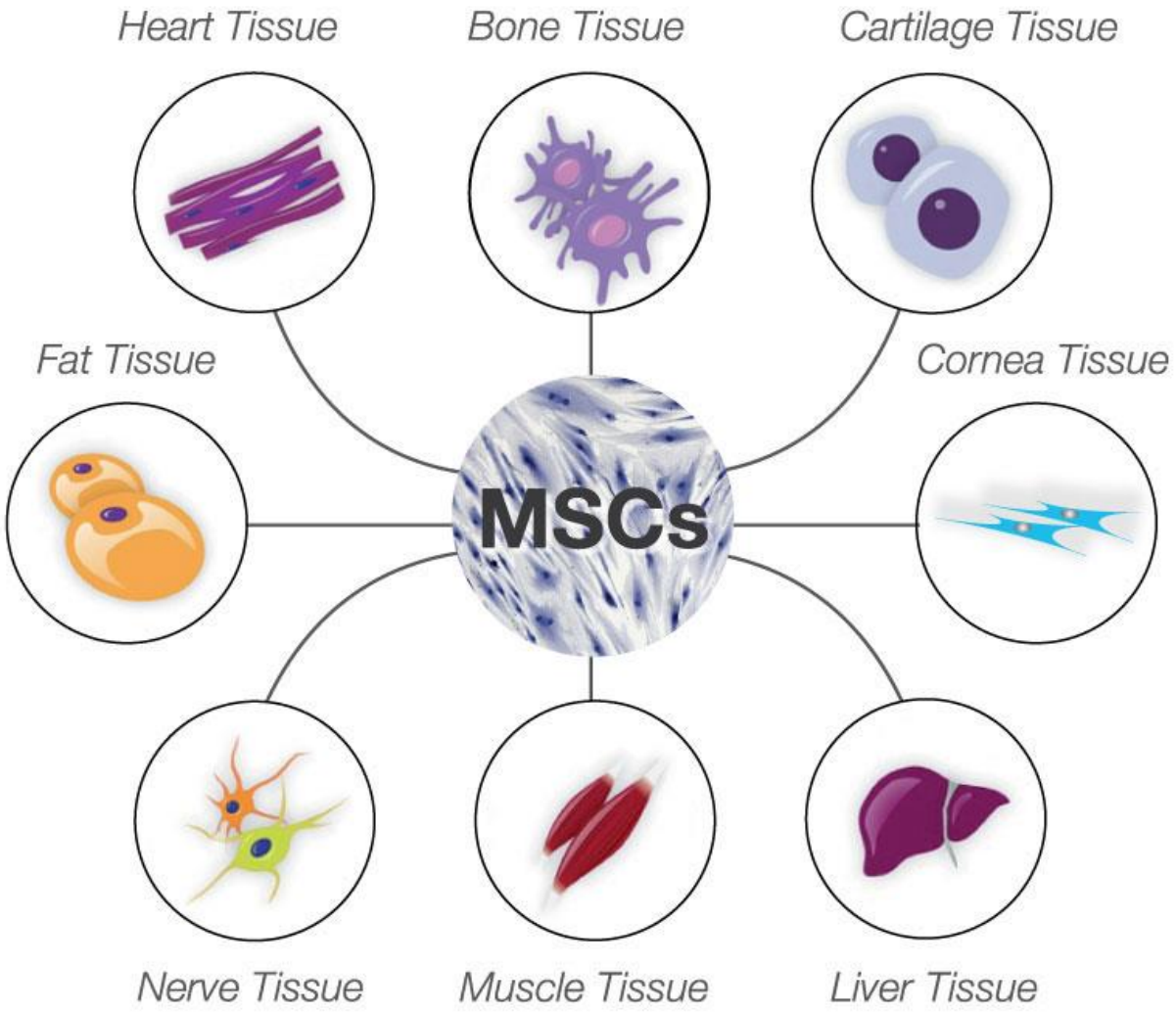
- painful
- time consuming
- risk of infection
- mainly HSCs

Stem Cell Fitness *MSCs*

Mesenchymal stem cells (MSCs) are ***ADULT STEM CELLS*** *traditionally found in the bone marrow. However, MSCs can also be isolated from other tissues including* ***discarded cord blood and umbilical cord tissue from donors following normal C-section births****. Multipotent stem cells, MSCs* ***differentiate to form adipocytes, cartilage, bone, tendons, muscle, and skin.***

Chart adapted from: Caplan, AI. 2007. Adult Mesenchymal Stem Cells for Tissue Engineering Versus Regenerative Medicine: Journal of Cellular Physiology.

Adult Multipotent Stem Cells (MSCs)

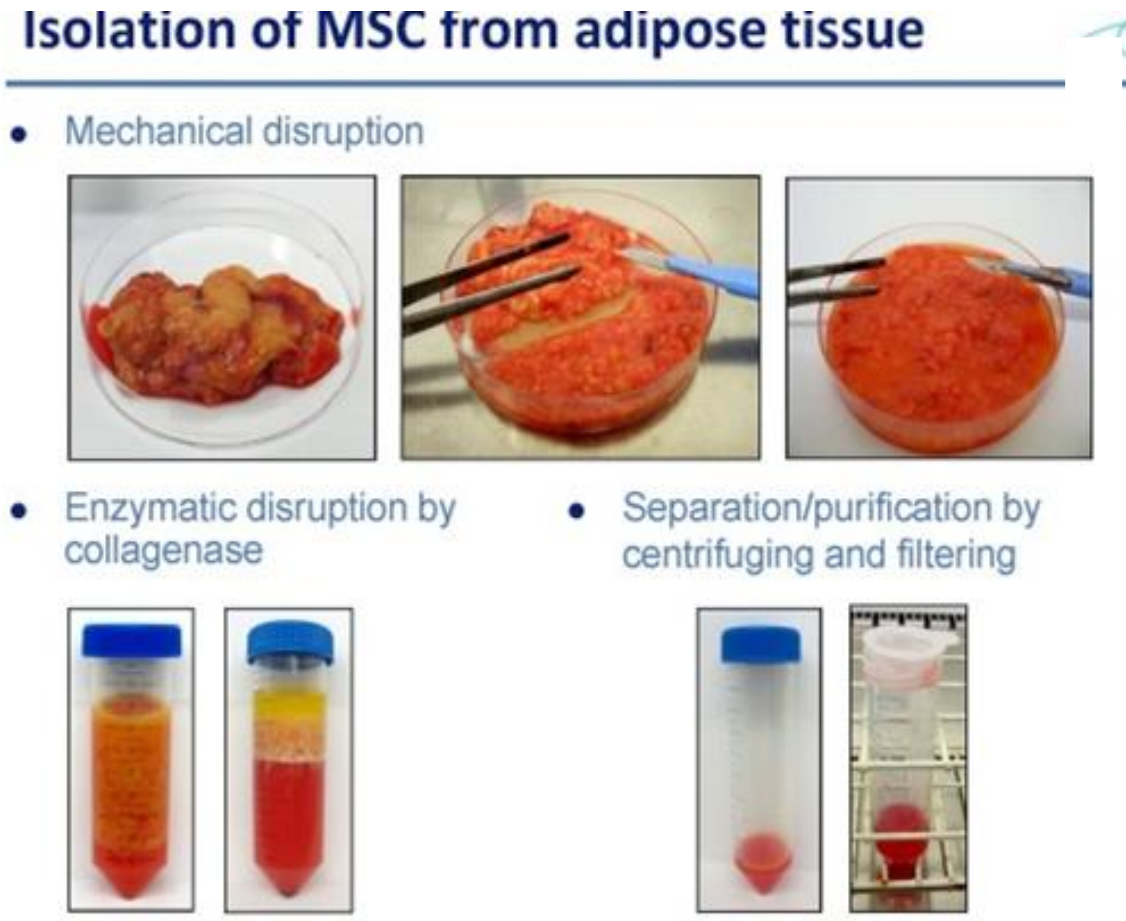


Adipose Stromal Vascular Fraction

Good source of **older** adult MSCs.

PROS:
- Autologous

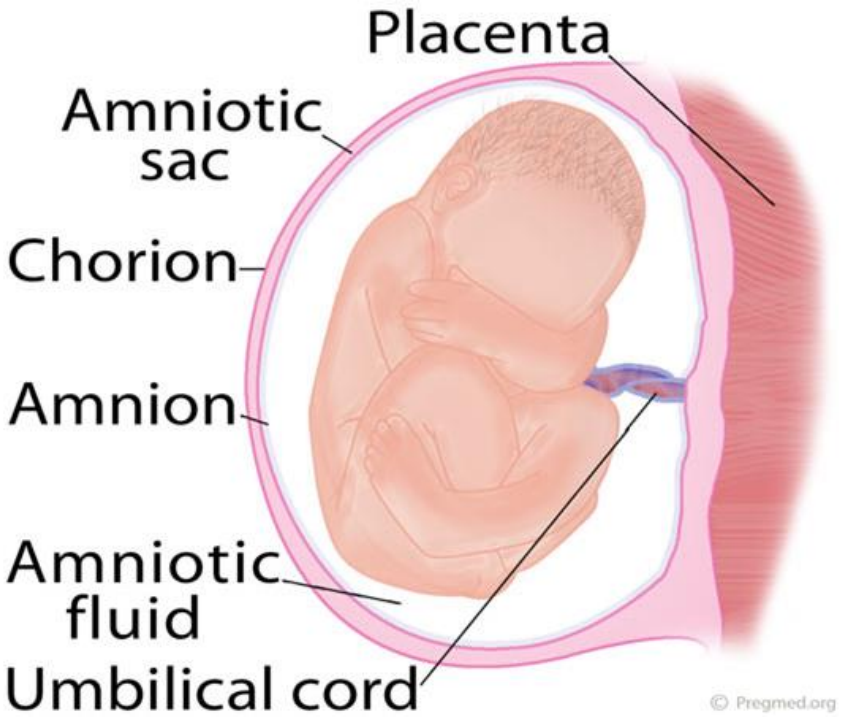
CONS:
- painful
- time consuming
- risk of infection
- potential tumor promoter

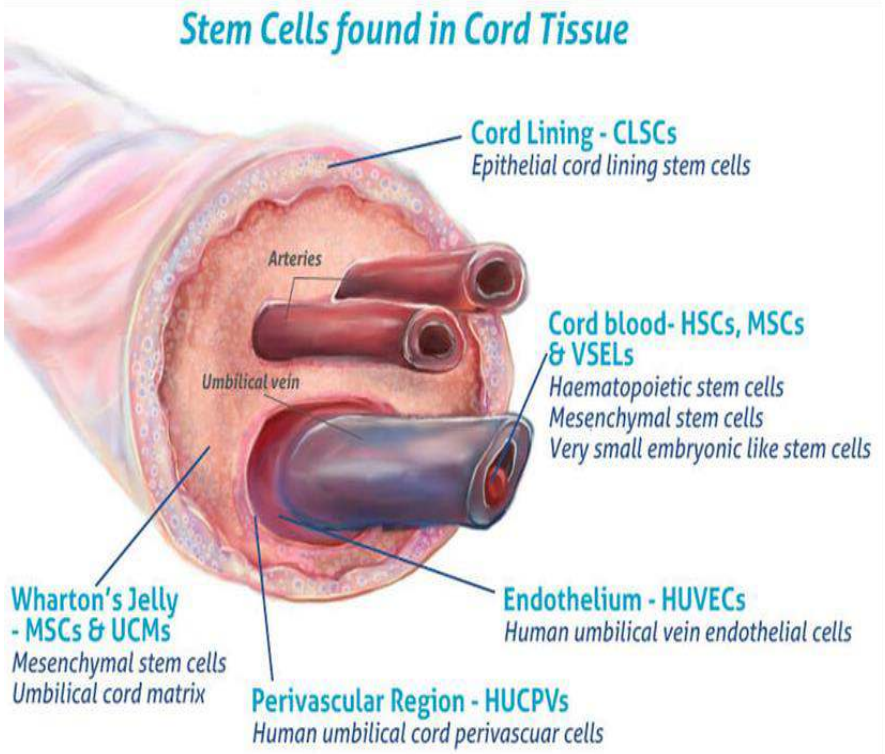


Older Adult Mesenchymal Stem Cells

- More likely to have accumulated cellular damage
- Less vigorous response
- Require typing and cross matching
- 1:2,000,000 (scarce)

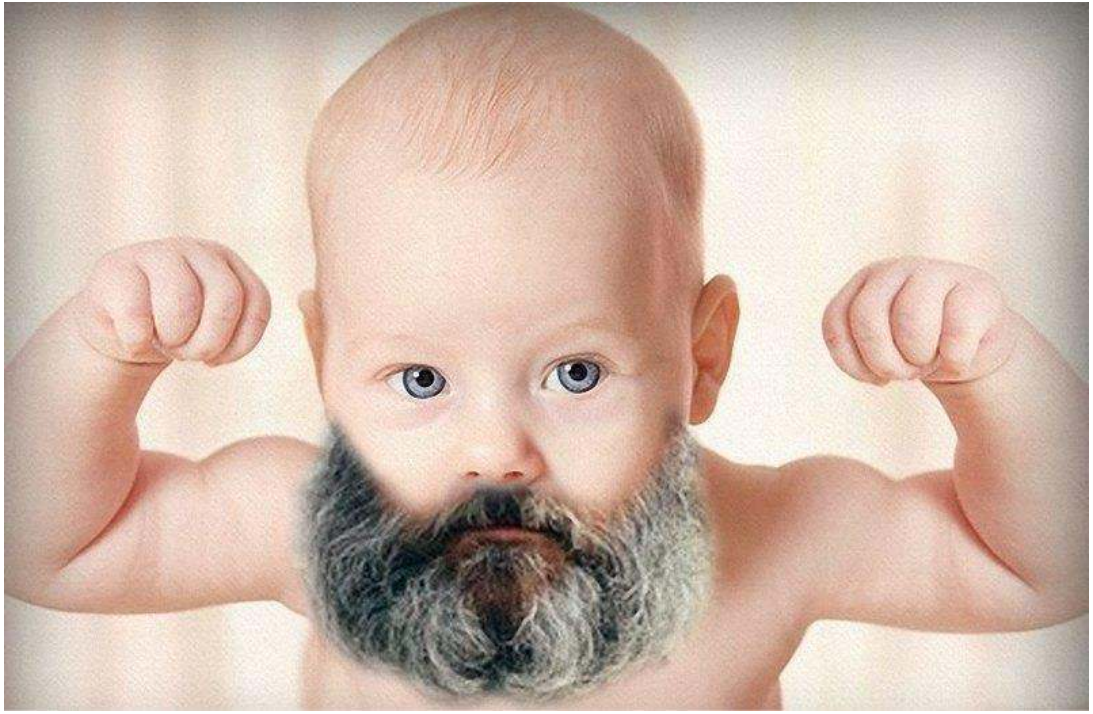
Post Natal Adult Mesenchymal Stem Cells





Post Natal Mesenchymal Stem Cells

- Robust
- Less likely to have genetic errors
- Not the same as mature adult
- Immune Priviledged
- 1:10,000 (Plentiful)

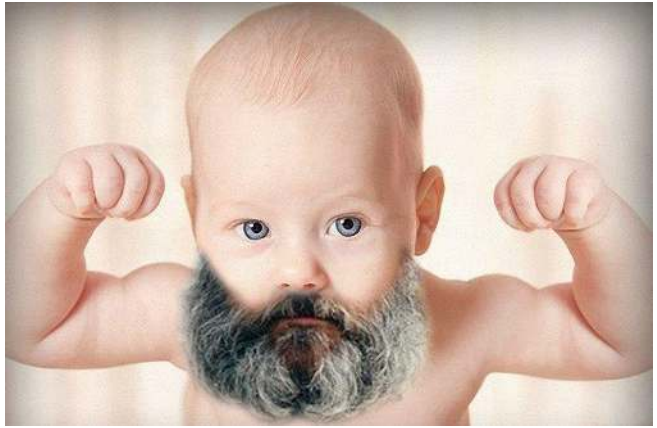


Fan M. The effect of age on the efficacy of human mesenchymal stem cell transplantation after a myocardial infarctation. *Rejuv Research*, 2010.

Post Natal Mesenchymal Stem Cells

- **MSC from young individuals can undergo neuroectodermal differentiation in vitro, but MSC from elderly patients cannot**

  Heremann A. Age dependent neuroectodermal differentiation capacity of human mesenchymal stromal cells: limitations for autologous cell replacement strategies. *Cytotherapy*, 2010.



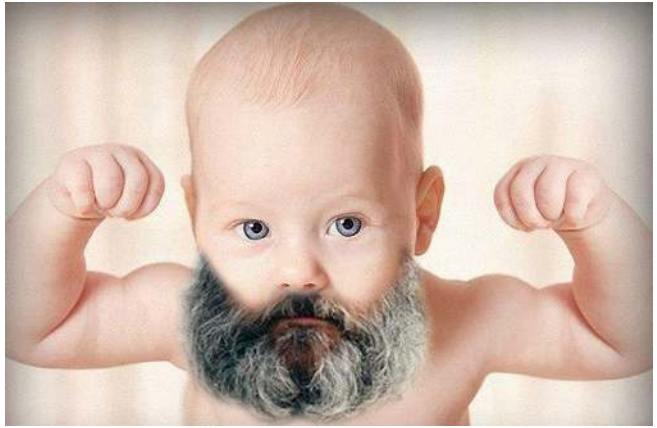
- **Adult MSC are exposed to a lifetime of factors, eg NSAIDS -- may inhibit MSC chondrogenic differentiation and disrupt endochondral bone formation.**

  Pountos I. NSAIDS inhibit in vitro MSC chondrogenesis: implications for mechanisms of bone formation inhibition in man. *J of Cell and Mol Medicine* 2011.

Post Natal Mesenchymal Stem Cells

- **Lifestyle affects the quality of MSCs- animal study where mice fed a high fat diet had a lower endothelial differentiation potential in vitro vs control group.**

  Lamontgne V. High-fat diets-induced metabolic alterations alter the differentiation potential of adipose tissue-derived stem cells. *Open J of Endocr & Metabol* Dis, 2013.



- **Osteogenic potential impaired in obese patients, and diminished capacity of adult stem cells to form a vascular network in patients with metabolic diseases such as DM**

  Roldan M. Obesity short-circuits stemness gene network in human adipose multipotent stem cells. *The FASEB Journal*. 2011.

  Rennert RC. Diabetes impairs that angiogenic potential of adipose-derived stem cells by selectively depleting cellular subpopulations. *Stem Cell Research & Therapy*. 2014.

Measure	Bone Marrow	Adipose Tissue	Umbilical Cord Blood
Proliferative capacity	Growth arrest at pass 11-12	Growth arrest at pass 11-12	Growth arrest at pass 14-16
Growth Rate/ Doubling Time	Moderate	Lowest	Highest
Colony Forming Ability	Moderate	Lowest	Highest
Senescence (Passage 6)	11%	13%	0%
Anti-Inflammation	Some	Some	Greatest

Hye JJ. Comparative analysis of human MSC from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. I*nt J Mol Sci* 2013

Measure	Bone Marrow	Umbilical Cord Blood
Collagen Formation*	1x	3x
Mineralization in bone culture**	Moderate	Highest
Size of chondrogenic pellets	Small	Large

*Fong CY. Human umbilical cord Wharton's jelly stem cells undergo enhanced chondrogenic differentiation when grown in nanofibrous scaffolds and in sequential two-stage culture medium environment. Stem Cell Rev & Report, 2012.

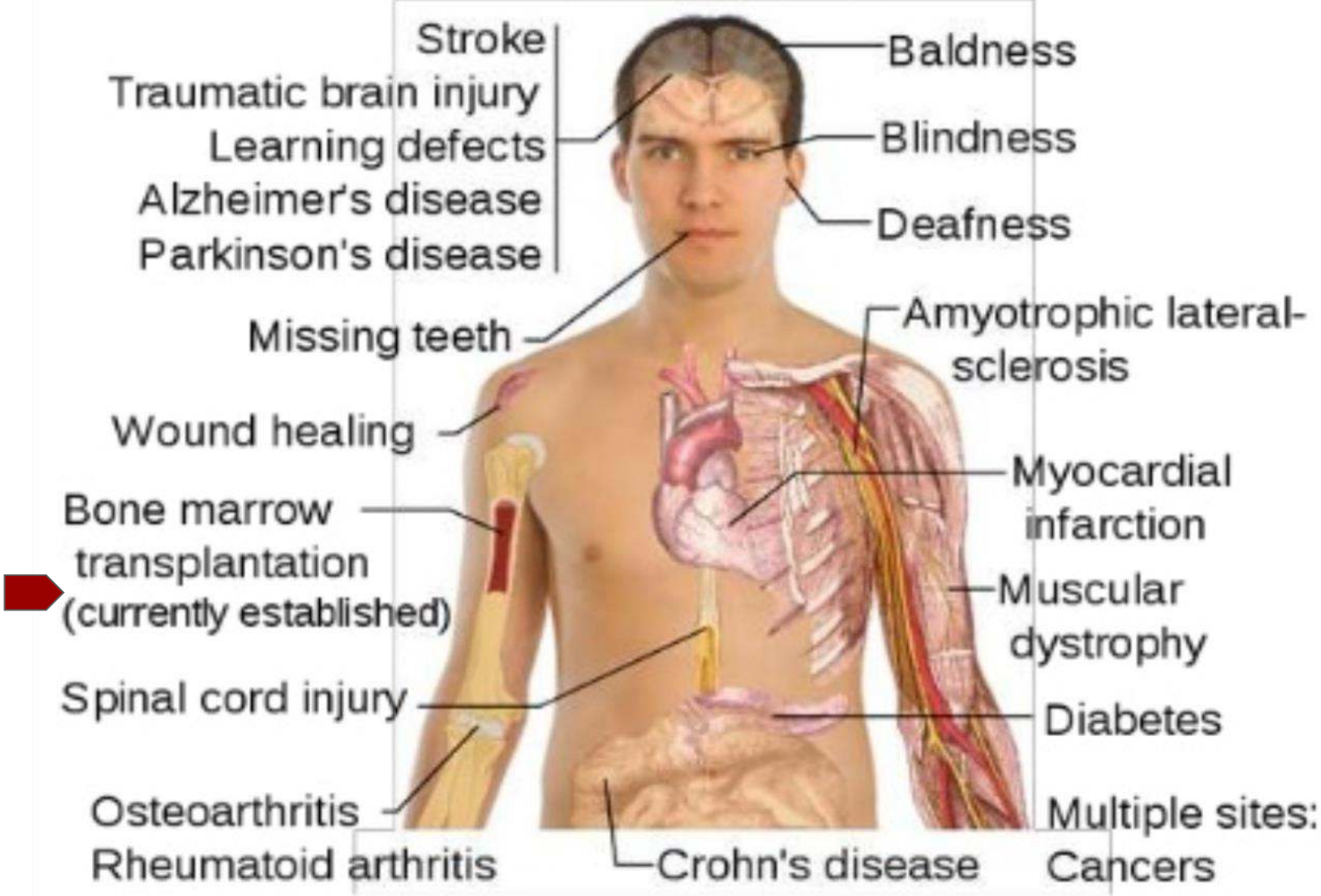
**Baksh D. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow stem cells, 2007.

Measure	Adipose Tissue	Umbilical Cord Blood	Wharton' Jelly
Effect of Tumor Growth*	Promoted	Inhibited	
Apoptosis*	Mild	Stronger	
Teratoma/Tumors			Not Induced
Abrogate Solid tumors			Yes

*Akimoto K. Umbilical cord blood derived MSCs inhibit, but adipose tissue-derived MSCs promote glioblastoma multiforme proliferation. *Stem Cells & Development*, 2012.

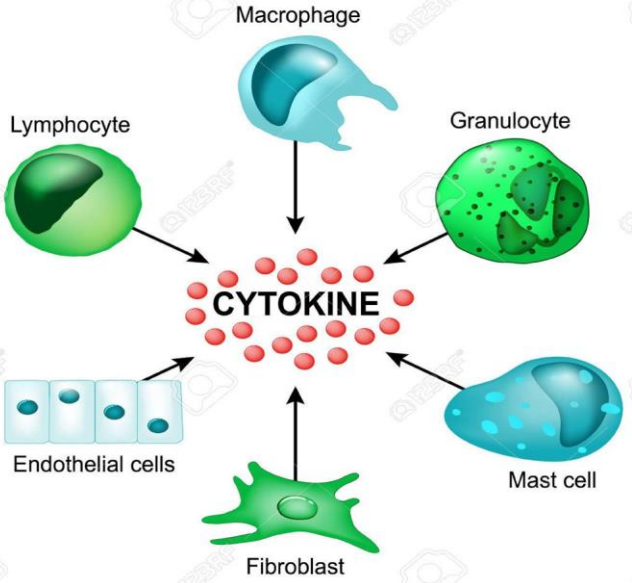
Gauthaman K. Human umbilical cord Wharton's jelly stem cell (hWJSC) extracts inhibit cancer cell growht in vitro. J Cell Biochem, 2012.

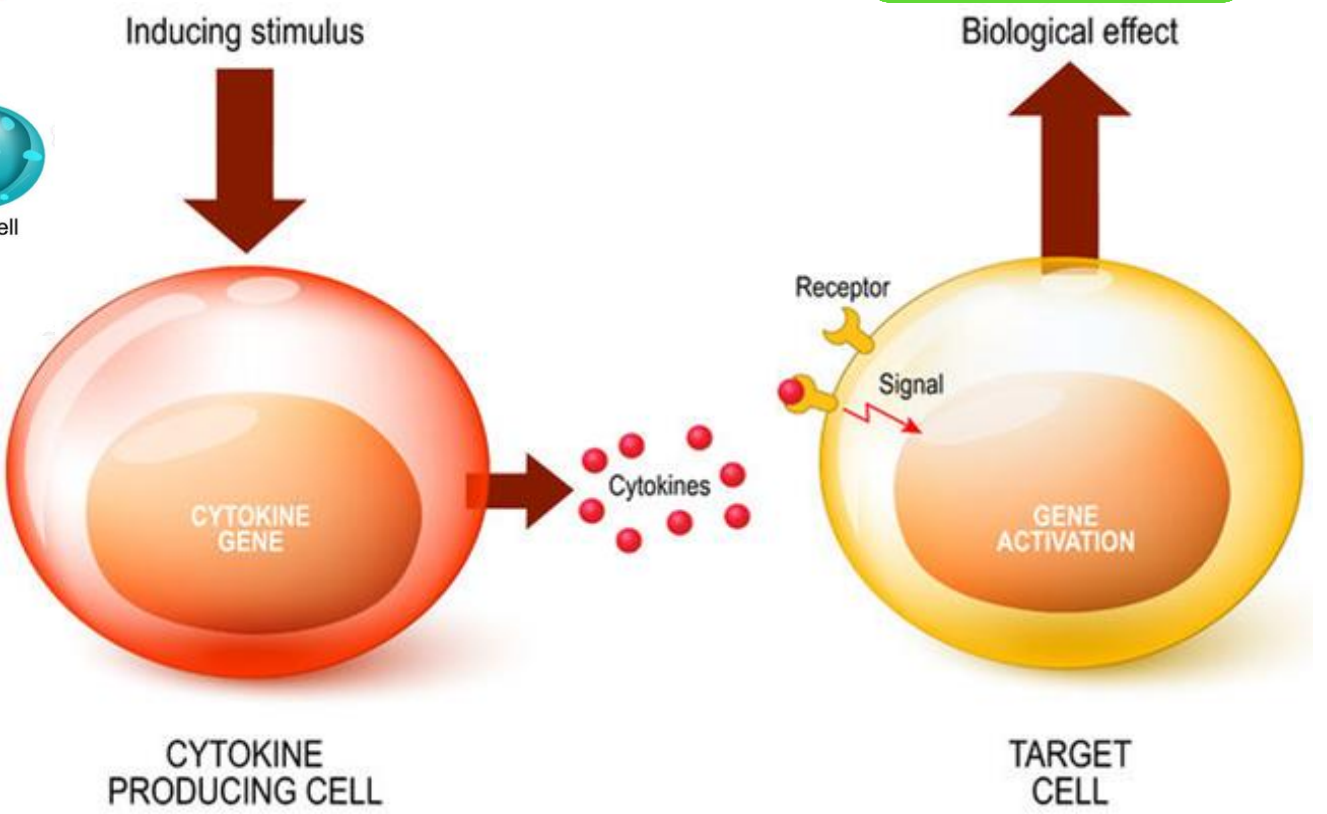
Potential Uses of Stem Cells (MSCs)

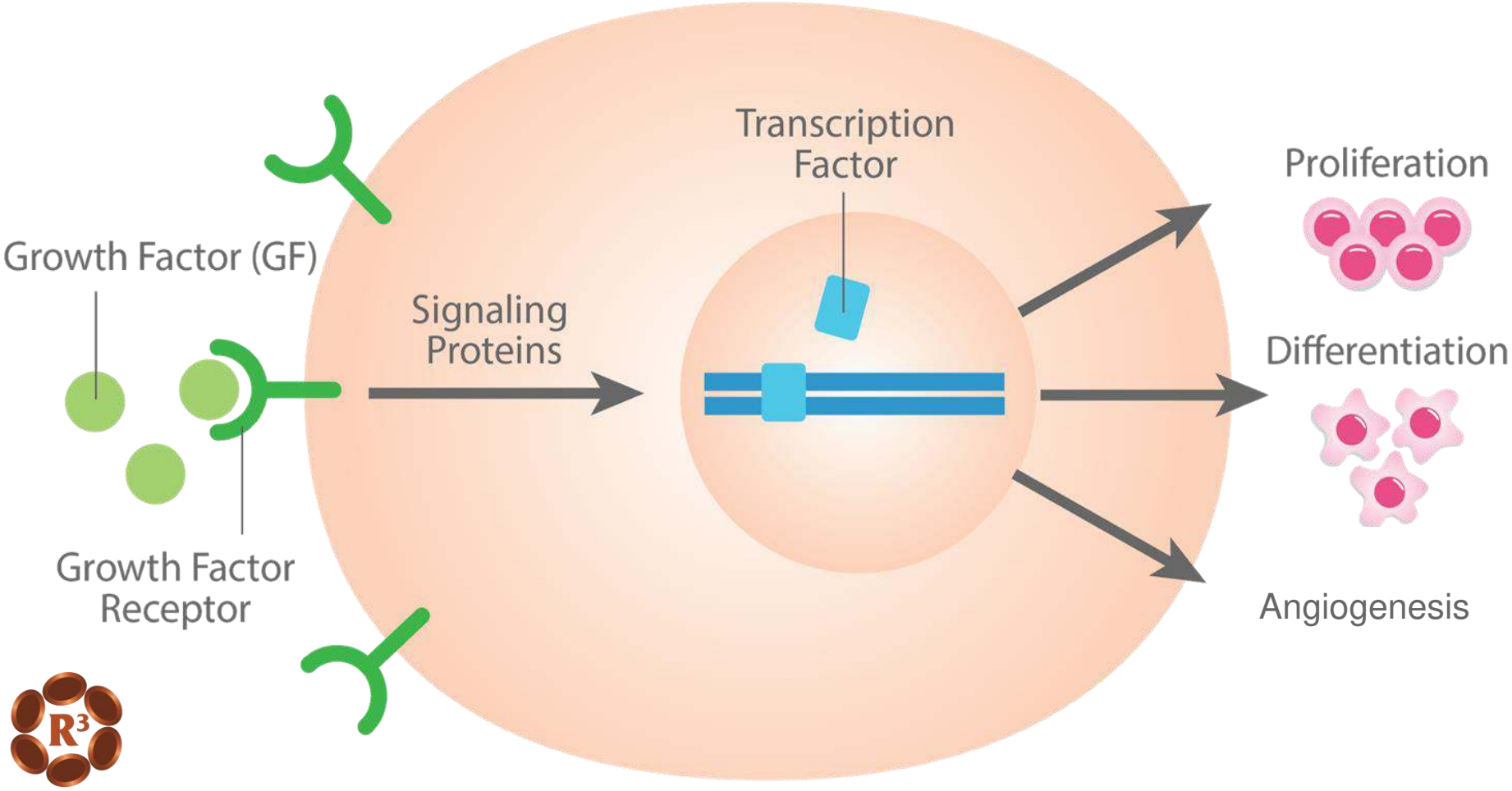


Cytokines

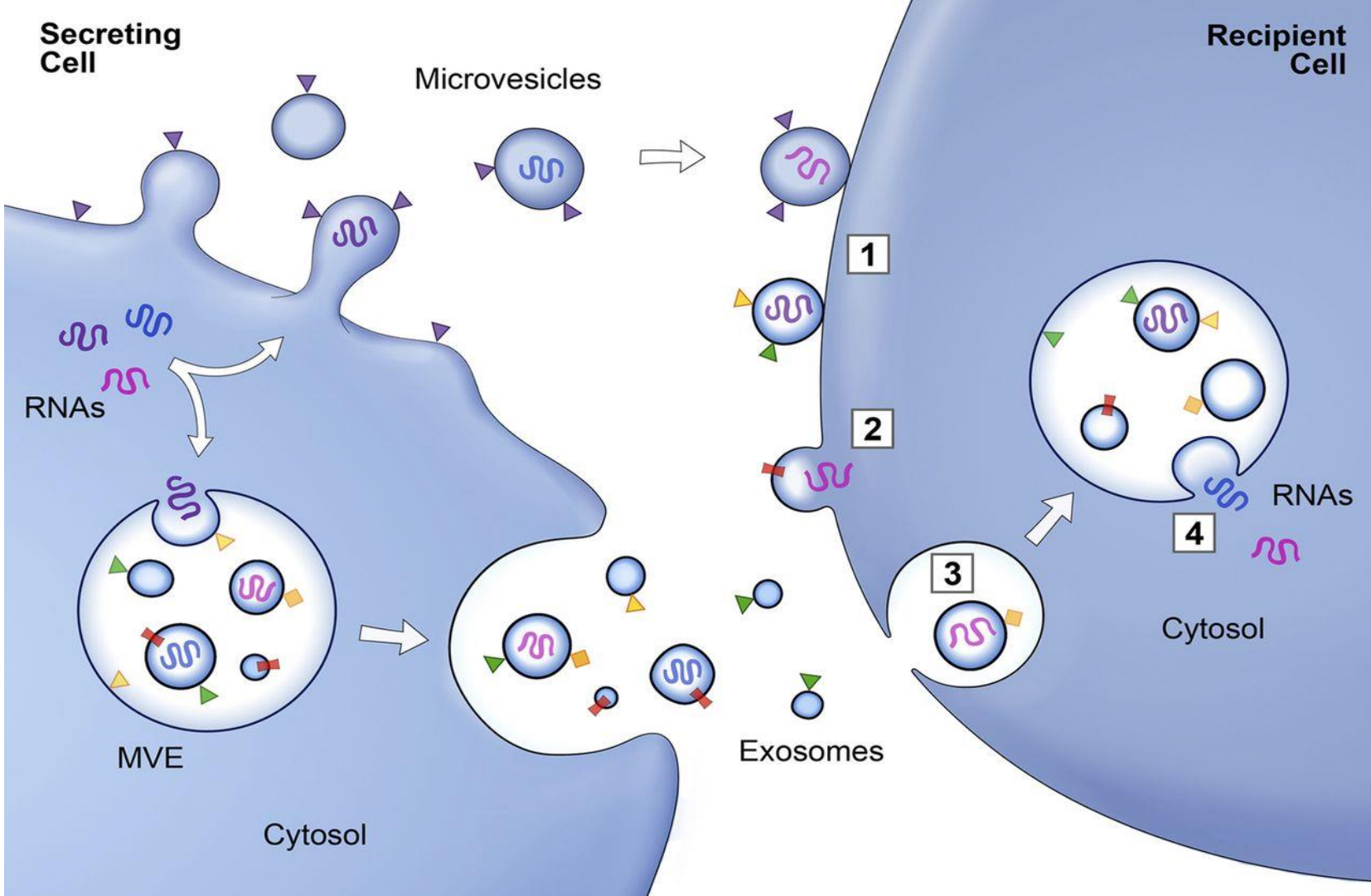
Regulate Cellular Differentiation



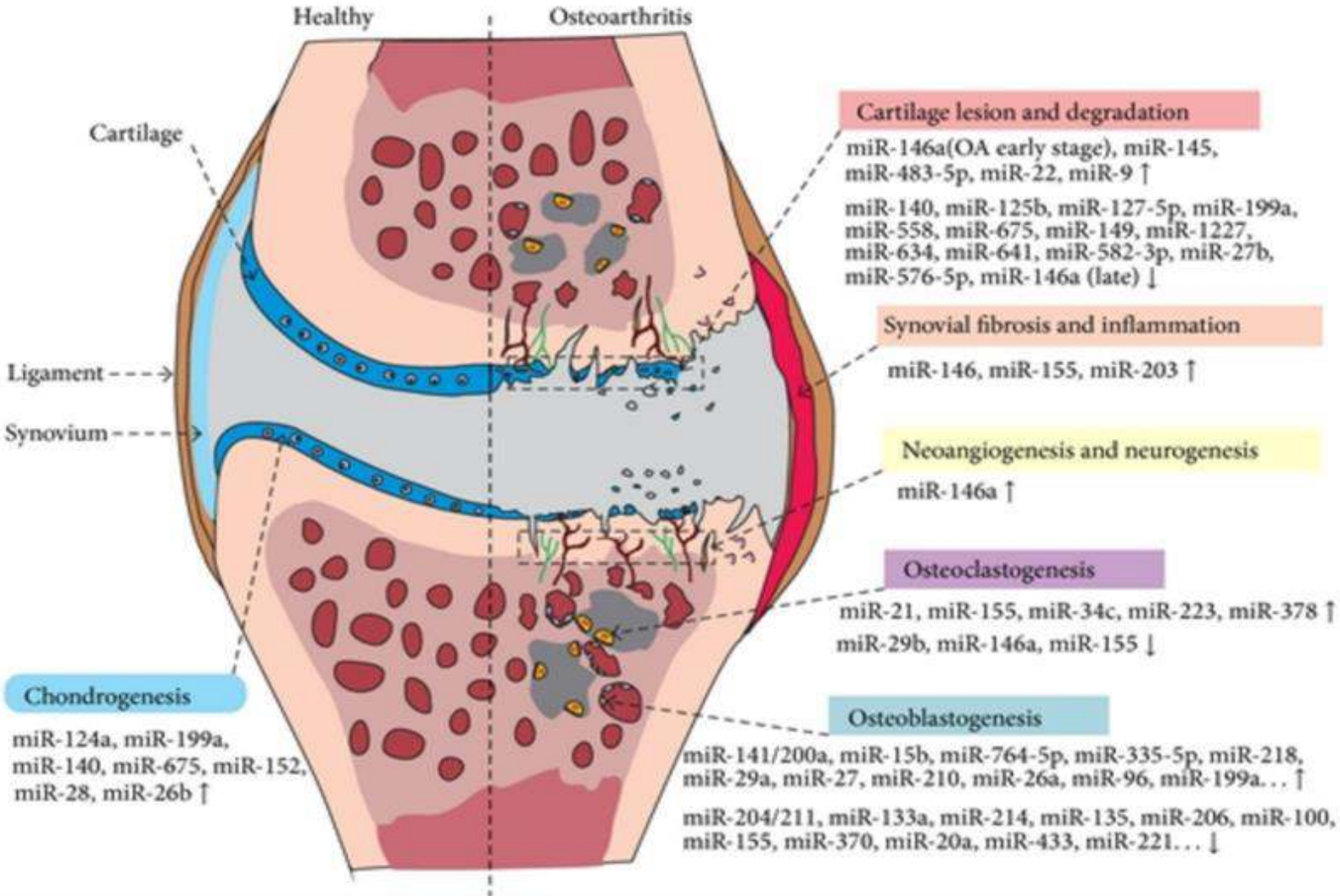



Growth Factor (GF)
Growth Factor Receptor
Signaling Proteins
Transcription Factor
Proliferation
Differentiation
Angiogenesis

Exosomes


Secreting Cell
Microvesicles
Recipient Cell
RNAs
1
2
3
4
RNAs
MVE
Cytosol
Exosomes
Cytosol

Micro RNA


Healthy
Osteoarthritis
Cartilage
Ligament
Synovium
Cartilage lesion and degradation
miR-146a(OA early stage), miR-145, miR-483-5p, miR-22, miR-9 ↑
miR-140, miR-125b, miR-127-5p, miR-199a, miR-558, miR-675, miR-149, miR-1227, miR-634, miR-641, miR-582-3p, miR-27b, miR-576-5p, miR-146a (late) ↓
Synovial fibrosis and inflammation
miR-146, miR-155, miR-203 ↑
Neoangiogenesis and neurogenesis
miR-146a ↑
Osteoclastogenesis
miR-21, miR-155, miR-34c, miR-223, miR-378 ↑
miR-29b, miR-146a, miR-155 ↓
Chondrogenesis
miR-124a, miR-199a, miR-140, miR-675, miR-152, miR-28, miR-26b ↑
Osteoblastogenesis
miR-141/200a, miR-15b, miR-764-5p, miR-335-5p, miR-218, miR-29a, miR-27, miR-210, miR-26a, miR-96, miR-199a... ↑
miR-204/211, miR-133a, miR-214, miR-135, miR-206, miR-100, miR-155, miR-370, miR-20a, miR-433, miR-221... ↓

Mesenchymal Stem Cells

- **Infused MSCs don't persist long** in damaged tissues
- Complement system produces a strong chemoattractant that induces the accumulation and **activation of neutrophils which damage the infused MSCs**
- Mouse model of GvHD- **MSCs underwent perforin dependent apoptosis after encountering cytotoxic immune cells**. Paradoxically, this correlated with the efficacy of therapy

News

Stem Cells Attracted by Tissue Inflammation

na

Pages 6-12 | Published online: 20 Dec 2004

Abstract

A joint effort led by stem cell biologist Evan Y. Snyder, M.D., Ph.D., of The Burnham Institute, and Samia J. Khoury, M.D., of Harvard Medical School and Brigham and Women's Hospital, report data suggesting that stem cells use inflammatory signals to "know" where they must home. ~~Using a simulated stroke model, the~~

Mesenchymal Stem Cells

- Tissue resident MSCs may not be as effective as infused MSCs in restoring immunologic hemostasis
- MSCs release **immunosuppressive** cytokines after exposure to inflammatory cytokines in the microenvironment
- Administration of MSCs early in the disease course or during relapse **(low inflammation), reduces the therapeutic efficacy**

Mesenchymal Stem Cells

- One MSC can inhibit the proliferation of up to 100 T cells
- MSCs suppress proliferation of activated CD4 T and CD8 T cells
- Extensive immunomodulatory potential of MSCs and the ability of **infused MSCs to resolve inflammation and promote tissue repair** are shown in models of disease (GvHD, SLE, MS, renal injury, fibrosis and arthritis)
- **Hypoxia induces MSCs to secrete IL-6, VEGF**, and chemokines in a model of myocardial infarction

Autoimmune Disease- SLE

- Theorized that **autoimmune diseases are stem cell disorders**
- SLE patients found to have functional abnormalities in their BM-MSCs
- SLE patient MSCs vs controls, have defective cytokine secretion and down regulated IL-6, IL-7, and mRNA expression
- 15 patients

Liang et al. *Annals of Rheumatic Diseases*. 2010.

Autoimmune Disease- SLE

- Significant decrease in disease severity at 4 weeks post tx
- Improved serum ANA, anti-dsDNA, serum albumin, and C3
- Remission accompanied by increased T-reg cells, and balanced Th-1 and Th-2 cytokines
- Significantly reduced disease activity, zero 3 year recurrence in all 16 patients

Sun et al. *Arthritis & Rheumatism* Aug 2010. 62:8,

Rheumatoid Arthritis

- 172 patients with active RA
- Treated with DMARDs +/- UCMSC IV
- Significant remission in stem cell group
  - Also better sleep, diet, physical strength
- Joint pain and swelling were alleviated within 12 hours and were maintained throughout the study period

Wang et al, Stem Cells & Development, 22:24, 2013.

Psoriatic Arthritis

- 4 patients underwent autologous BM-MSC with relapse within 21 months
- 9 patients had allogeneic BM-MSC with remission for 4-17 years

Woods et al. *Annals of Rhem Dis*, 2006

Inflammatory Bowel Disease

Autologous BM-MSC:

- 85% 5 year survival, 43% progression free, 30% complete response

Allogeneic BM-MSC

- Higher risk of complication and mortality
- Possible to genetically correct disease with healthy donor
- Increased risk of infectious adoptive autoimmunity transfer of IBD

Duran et al. *Therapeutic Advances in Gastroenterology*, 2016.

Inflammatory Bowel Disease

Allogeneic BM-MSC

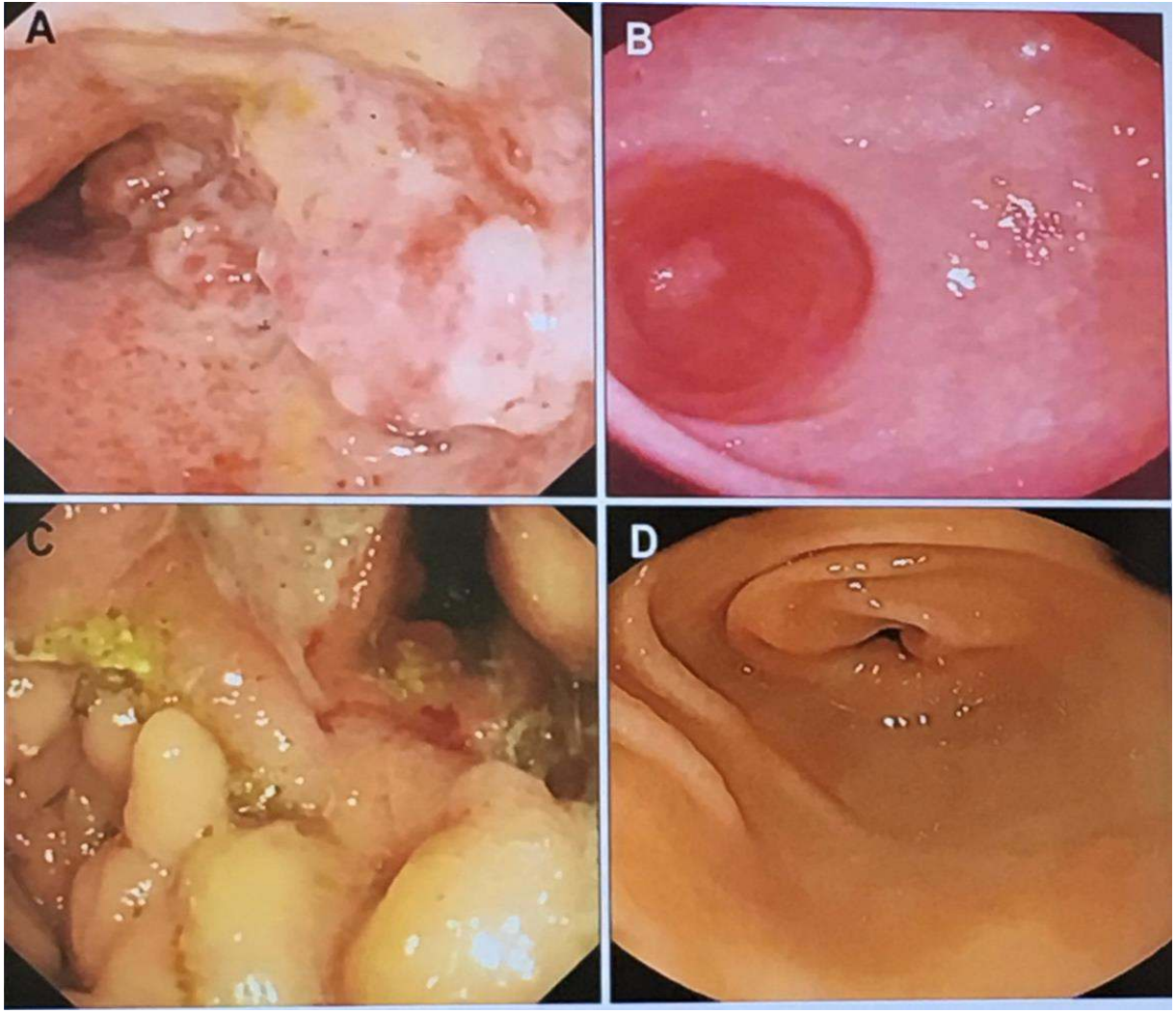
- Local BM-MSC for perianal fistula achieved 85.7% successful fistula healing at week 12, 33% with improvement only
- IV BM-MSC for moderate/severe Crohn's
  - 207 patients
  - 148 reached 28 day endpoint with CDAI score < 150 (measure of remission)

Duran et al. *Therapeutic Advances in Gastroenterology*, 2016.

Crohn's Disease

- 82 patients treated with steroid maintenance x 6 months
  - 41 randomly selected to receive IV UC-MSC weekly for 4 infusions
  - CDAI score decreased by 62.5 vs 23.6 in control group
  - Steroid dose decreased by 4.2mg/d compared to 1.2mg/d in control group

Zhang et al. *Gut and Liver*, 12:1 Jan 2018



Mucosal Recovery at 12 months

A → B
C → D

Cardiovascular Disease (animal models)

- 33% reduction in cholesterol
- 33% reduction in vascular lesion development (aortic root)
- Reduced infarct volume
- 20% increased capillary density around the infarct
- Infarcted myocardium was colonized by hUC-MSC
- IV infused hUC-MSC effectively migrated to area of injury and induced autologous differentiation for repair

Frodermann et all. Scientific Reports, Oct 2015.
Acosta et al. J of Stem Cell Research & Therapy, 2013.

Cardiovascular Disease (human study)

- Chile study – Randomized, Double Blinded study in 15 patients.

- Intravenous infusion of UC-MSC was safe in this group of patients with stable heart failure and reduced ejection fraction under optimal medical treatment.

- Improvements in left ventricular function, functional status, and quality of life were observed in patients treated with UC-MSCs.

Safety and Efficacy of the Intravenous Infusion of Umbilical
Cord Mesenchymal Stem Cells in Patients With Heart Failure, Circ Res. 2017

Type 1 Diabetes

- 29 patients new onset T1DM
  - Treatment group- WJ-MSC by IV 2 infusions, 4 weeks apart
  - Control group- saline
- No side effects
- HbA1c and C peptide were significantly better than pre-treatment and controls

Hu et al. *Japan Endocr Soc*, 2012

Type 2 Diabetes

22 patients with T2DM, WJ-MSC IV followed 5 days later by an intrapancreatic endovascular injection

- Decreased fasting blood glucose, HbA1c, C-peptide, and beta cell function
- Reduced systemic markers of inflammation
- HbA1c lowest at 3 months post treatment
- Fasting glucose, C-peptide and beta cell function best at 6 month, but sustained for 12 month follow up

Liu et al. *Stem Cell Research & Therapy* 2014

Osteoarthritis

- MSC treatment increased regeneration of medial meniscus and decreased cartilage destruction
  - Likely induction of endogenous progenitor cells

Chen et al. Arthritis Research & Therapy, 2008

- Rabbit model of induced disc degeneration, intra disc injection of MSCs embedded in ECM preserved annular structure, re-established the disc nucleus, improved height and hydration

Leung et al. *European Spine Journal* 2006

Avascular Necrosis

189 hips (116 patients) injected with BM-MSC

- Excellent results at 5 year follow up in early disease
- 25/44 patients with Stage III+ required THA
- Patients with fewer stem cells, history of organ transplant, or corticosteroid exposure had increased risk of progression

Houdek et al. *Stem Cells and Cloning Adv and Applications* 2014

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Houdek et al. *Stem Cells and Cloning Adv and Applications* 2014

Erectile Dysfunction

7 men with T2DM, mean age 69.5 with no erection for 6 months despite medications, awaiting prostheses

- UC-MSC injected in corpus cavernosum
- 3 participants regained morning erections
- Maintained erections for 6 months
- Increased rigidity requiring PDE5 for penetration
- 1 maintained erection sufficient for coitus for 11 months
- HbA1c improvement for 3-4 months

Bahk et al. *Experimental and Clinical Transplantation* 2010

Renal Failure: CASE REPORT

62 yo woman paraplegia due to thoracic spinal cord entrapment, with diabetic nephropathy in CRF for 2 years on HD. Cre 11. No urination for 2 years.

- UC-MSC IV an intrathecal
- 3 weeks: toe movement, improved renal function
- 8 months: raise legs, Cre 2, normal urination

Rahussalim et al. J Med Case Reports 2017

Cirrhosis

51 patients with ESLD, life expectancy of 6 months. ⅔ had ascites. ⅓ encephalopathy. UC-MSC injected into liver parenchyma

- All survived 6 months
- 2 lived more than 5 years
- Most encephalopathy resolved
- ⅔ ascites improved

Bahk et al. *Stem Cells in Clinical Research* 2011

Anti-Aging

20 patients with chronic inflammatory conditions also desiring anti-aging treated with UC-MSC IV.

- 10 had significantly improved inflammatory markers
- Significant improvement in
  - Hair and nail growth
  - Skin
  - Libido
  - Energy, mood, sleep and pain

Mehling et al. Journal of Stem Cell Research & Therapy 2015

Anti-Aging

30 patients, average age 75 with frailty treated with IV MSCs

- Improved 6 min walk test
- Improved FEV1
- Improved markers of immunity (serum TNF-a, B cell intracellular TNF-a)
- No adverse events

Tompkins et al. Gerontological Society of America 2017.

Modes of Delivery

- **IV**- animal studies show MSC in lungs at 1hr, decreased at 24 hours, a lot still detectable at 6 weeks.
- **Subcutaneous**- remained at site, undetectable after 30 days
- **Intrathecal**
- **Intranasal**- 3% proliferative at 4.5 months (spinal cord, brainstem, cerebellum, cortex, etc)
- **Direct Injection**
  - **Intraarticular – best we can tell is a few weeks.**
  - **Intramuscular**- detectable for 104 days, metabolically active
- **Nebulized – 72% survive the journey.**

Medications that Affect Stem Cells

(-)

ACE Inhibitors

Steroids

Local Anesthetics

Chemotherapy Agents

Antibiotics

NSAIDS

PPI

(+)

Plavix

Melatonin

Recommendations

1. Intelligent Advertising
2. Proper Consent
3. Use Peer Reviewed Protocols
4. Join an IRB
5. Good Practice Guidelines
6. Create an Appropriate Patient Experience