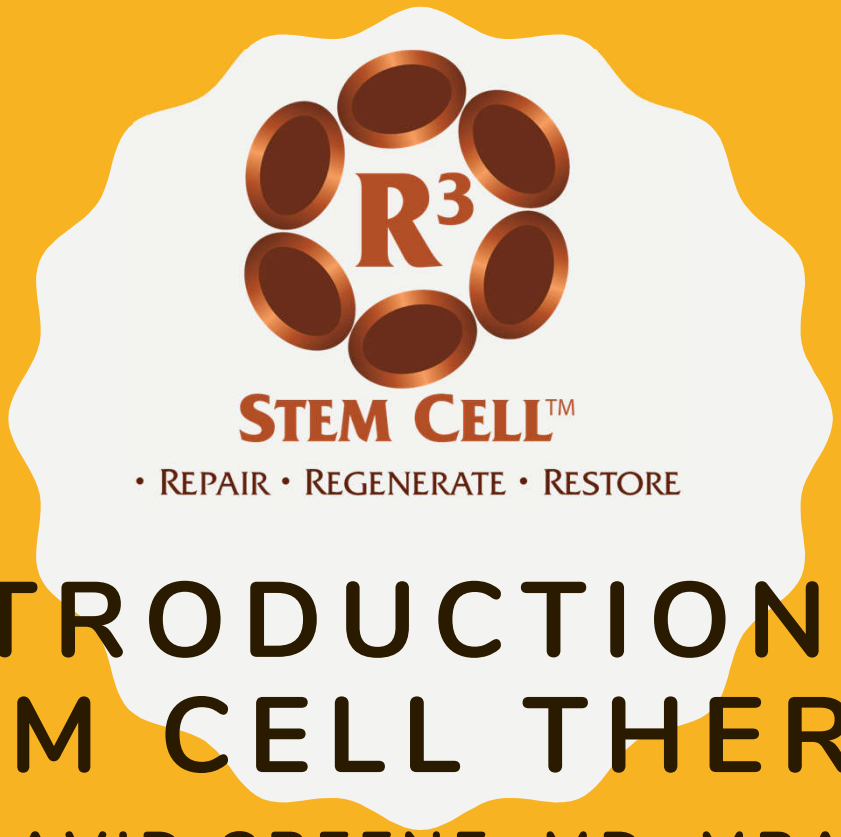
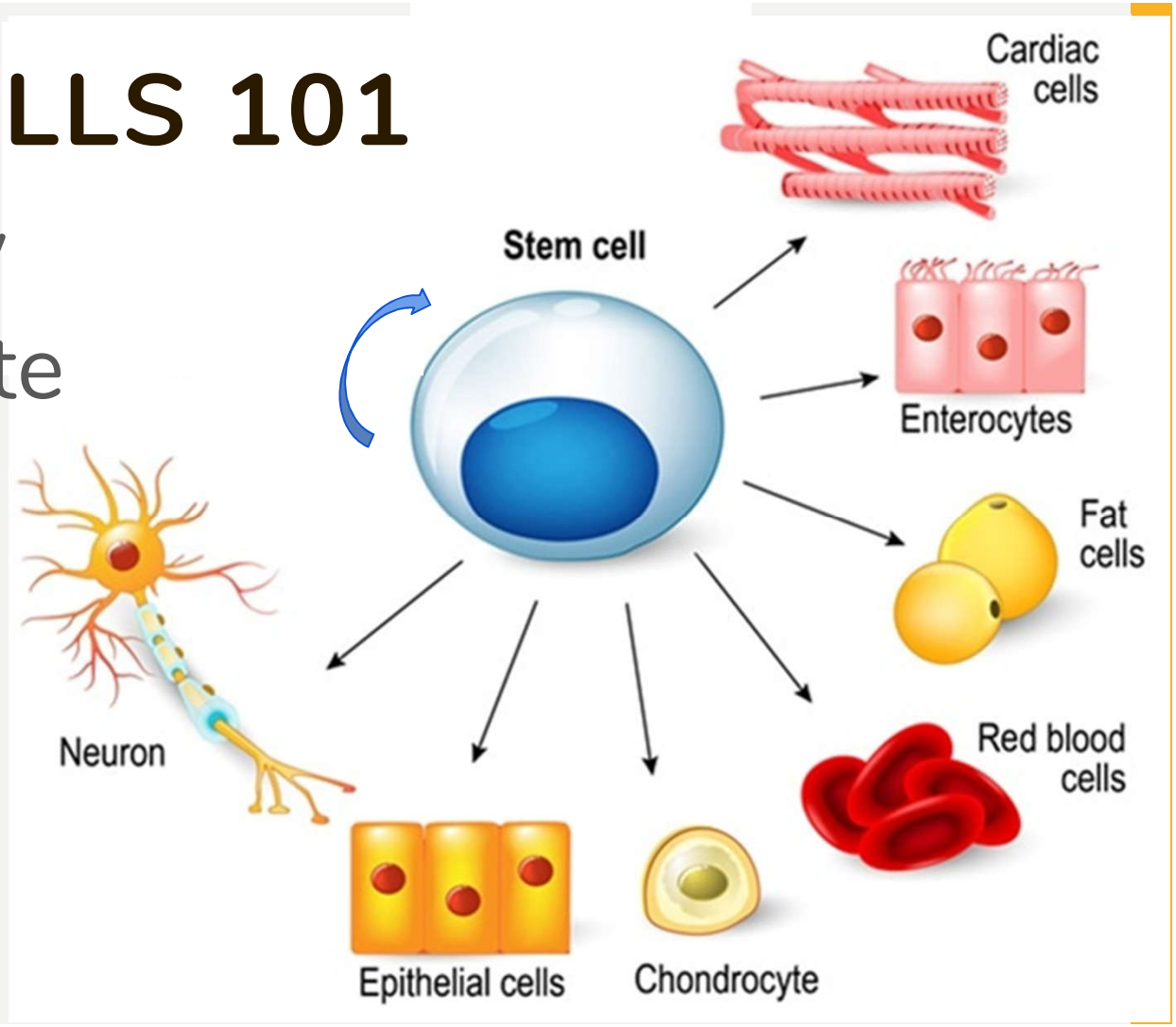


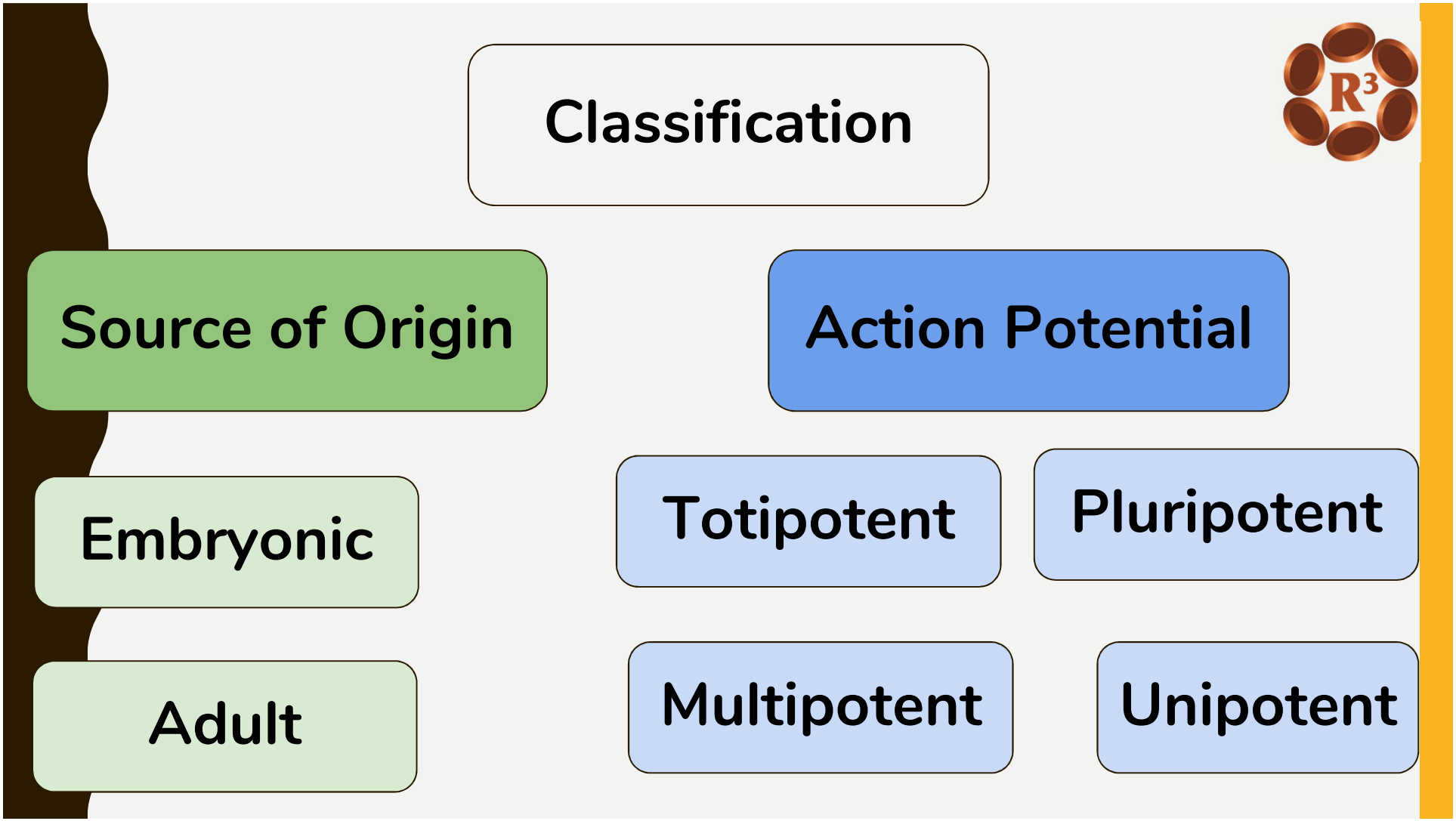
INTRODUCTION TO STEM CELL THERAPY

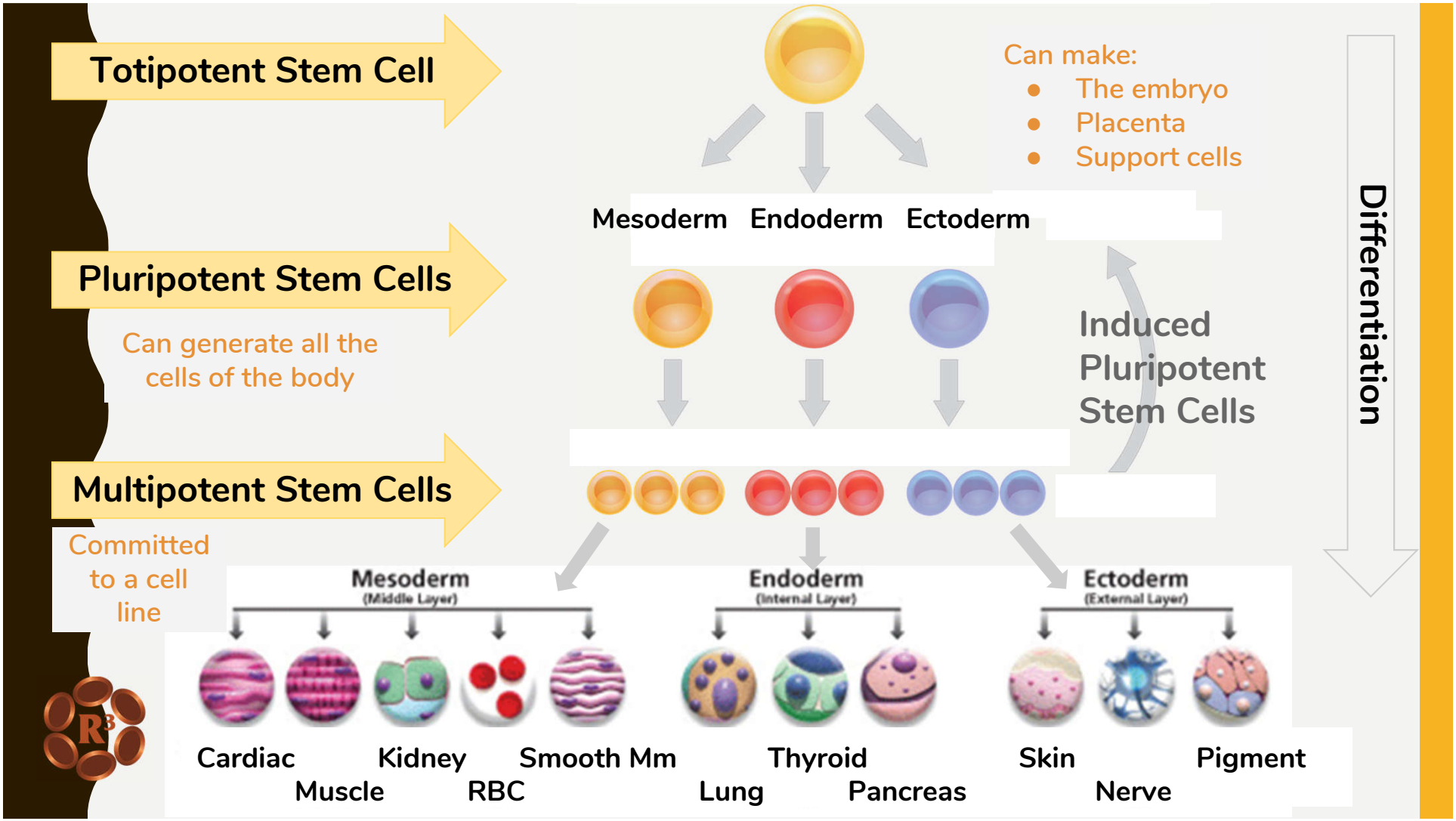
DAVID GREENE, MD, MBA
FOUNDER/CEO

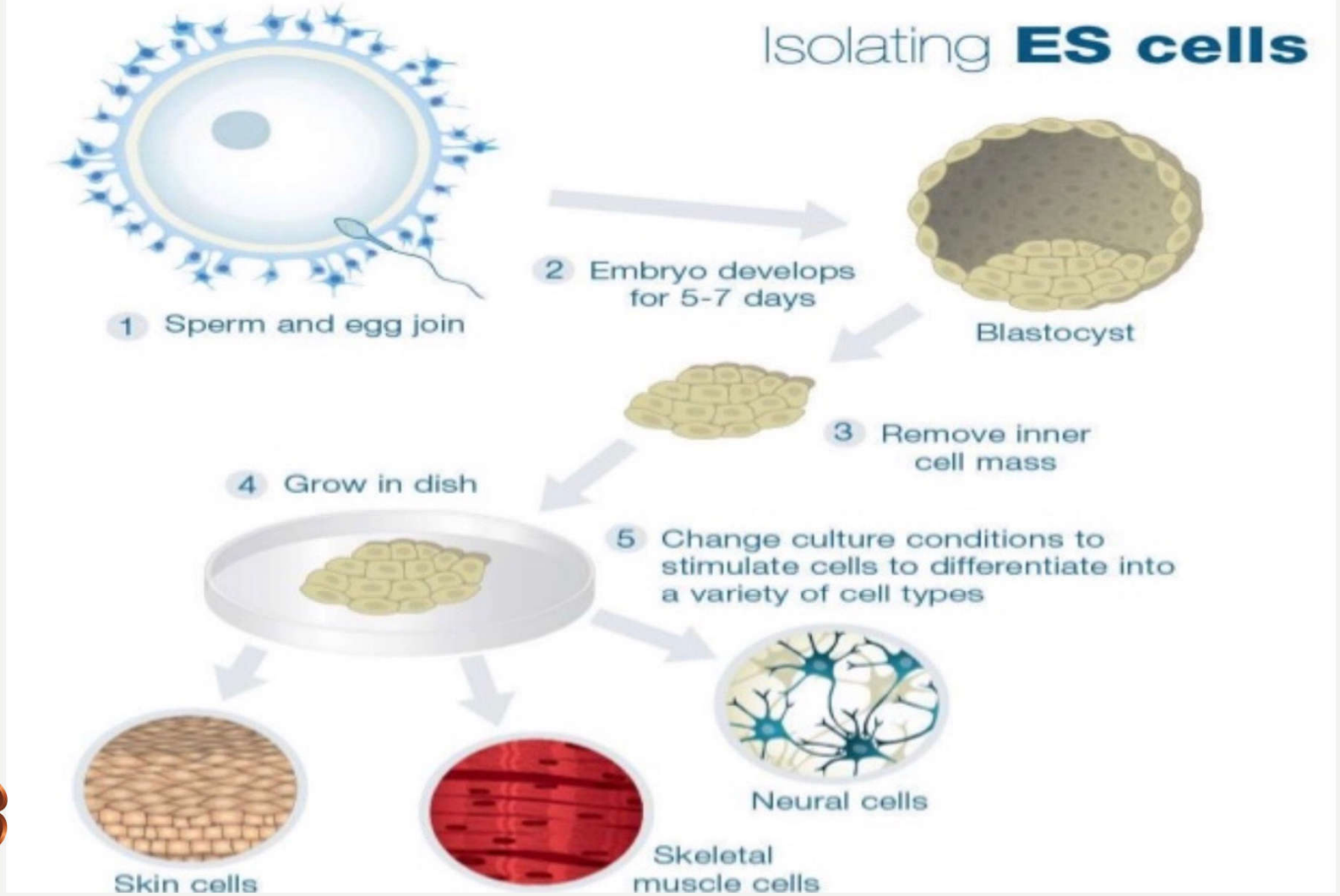
STEM CELLS 101

- 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
Lung
Thyroid
Pancreas
Skin
Nerve
Pigment


Isolating ES cells
1 Sperm and egg join
2 Embryo develops for 5-7 days
Blastocyst
3 Remove inner cell mass
4 Grow in dish
5 Change culture conditions to stimulate cells to differentiate into a variety of cell types
Neural cells
Skeletal muscle cells
Skin cells

Adult Stem Cells

Bone Marrow

Adipose Tissue

Post Partum Birth Tissues

Bone Marrow Stem Cells (HSC)

Bone Marrow Aspiration & Concentration- BMAC

FDA Approved

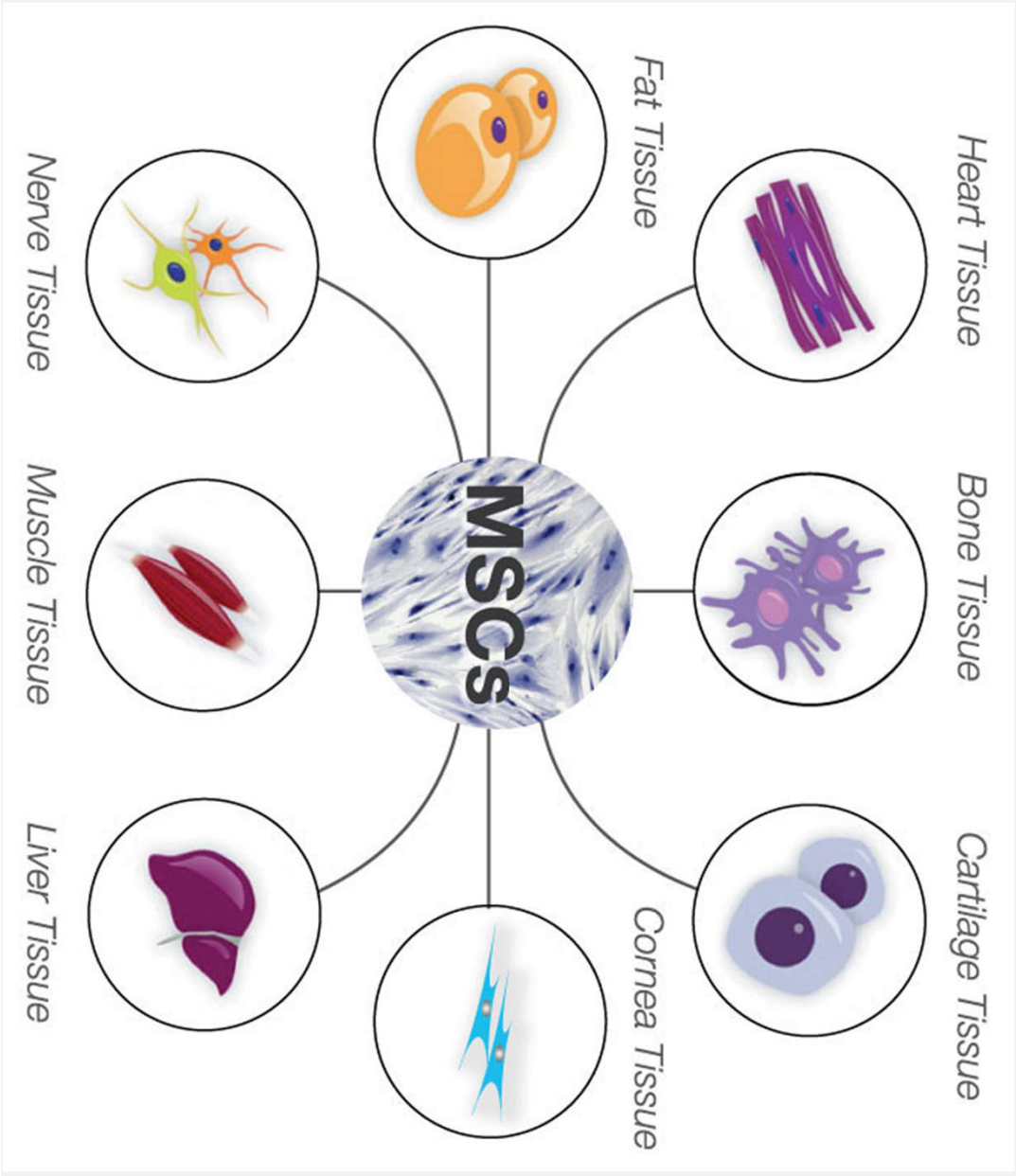
PROS:

- Autologous

CONS:

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

ADULT MULTIPOTENT STEM CELLS (MSCS)

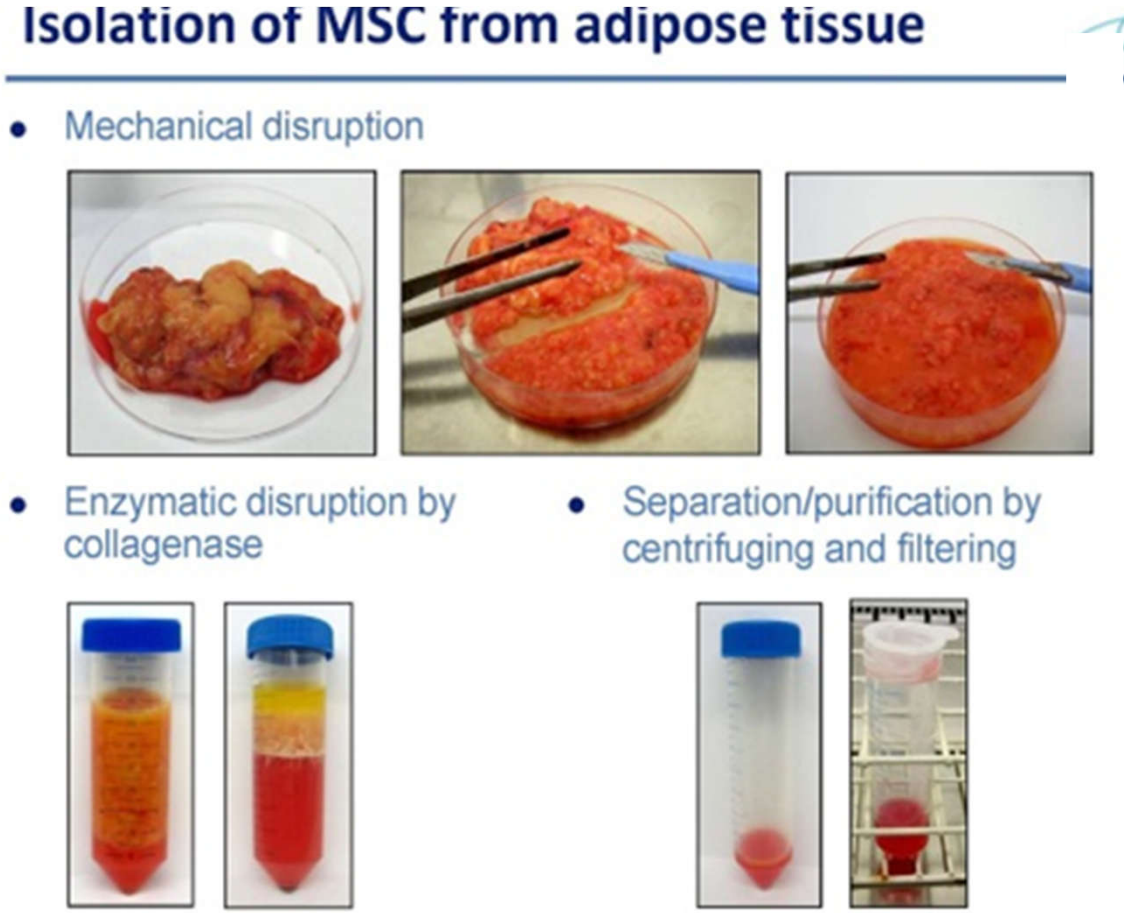


Adipose Stromal Vascular Fraction

Good source of **older** adult MSCs.

PROS:
- Autologous

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



www.impactjournals.com/oncotarget/ Oncotarget, Vol. 6, No. 10

Adipose-derived stem cells promote tumor initiation and accelerate tumor growth by interleukin-6 production

Hong-Jian Wei[1,2], Rong Zeng[3], Jui-Hua Lu[1], Wen-Fu T. Lai[4], Wei-Hong Chen[2], Hen-Yu Liu[2], Ya-Ting Chang[1] and Win-Ping Deng[1,2,3]

[1] Graduate Institute of Biomedical Materials and Engineering, College of Oral Medicine, Taipei Medical University, Taipei, Taiwan

[2] Stem Cell Research Center, Taipei Medical University, Taipei, Taiwan

[3] Department of Orthopedic Surgery, The Affiliated Hospital of Guangdong Medical College, Zhanjiang, China

[4] Graduate Institute of Clinical Medicine, Taipei Medical University, Taipei, Taiwan

In conclusion, we demonstrated that ADSCs play a pro-malignant role in tumor development of breast and colon cancer cells. Interaction of ADSCs and cancer cells stimulated secretion of IL-6 in ADSCs, which in turn acted in a paracrine manner on cancer cells to enhance their malignant properties, including tumor initiation

Published in final edited form as:

Prostate. 2010 November 1; 70(15): 1709–1715. doi:10.1002/pros.21206.

Adipose Tissue Derived Stem Cells Promote Prostate Tumor Growth

Lukas Prantl[1], **Fabian Muehlberg**[1], **Nora M. Navone**[2], **Yao-Hua Song**[1], **Jody Vykoukal**[1], **Christopher J. Logothetis**[2], and **Eckhard U. Alt**[1,*]

[1]Department of Molecular Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

[2]Department of Genitourinary Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

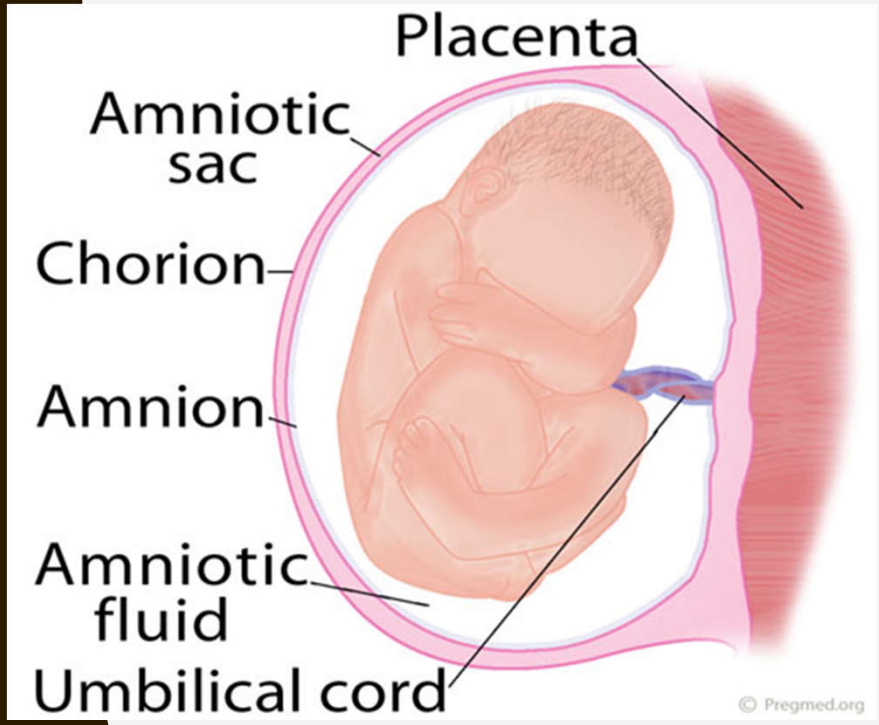
Abstract

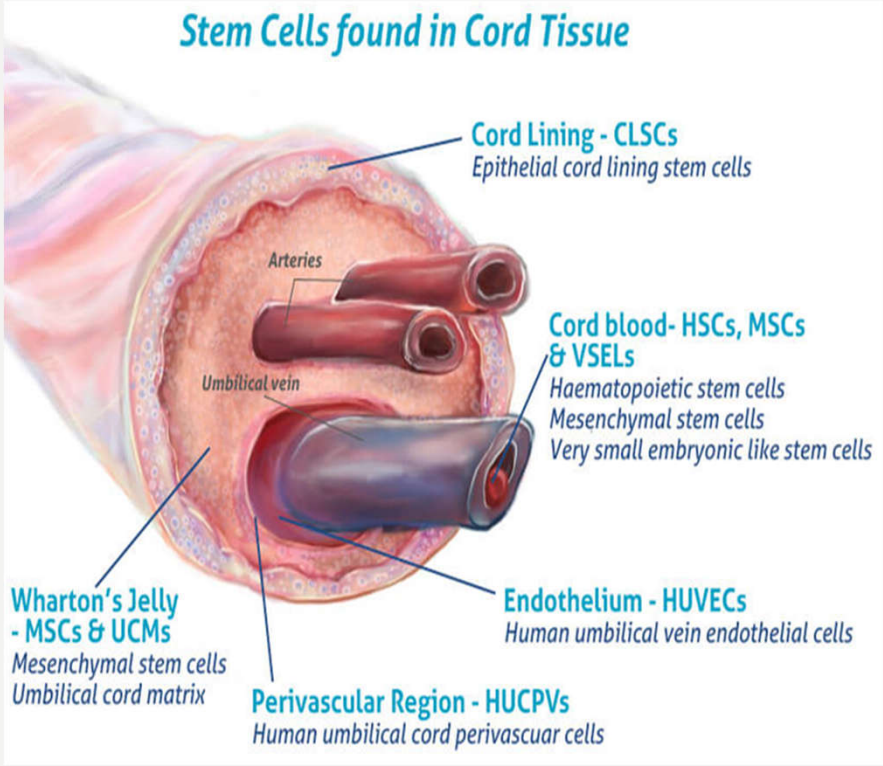
BACKGROUND—Recent evidence indicates that cancer stem cells play an important role in tumor initiation and maintenance. Additionally, the effect of tissue-resident stem cells located in

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

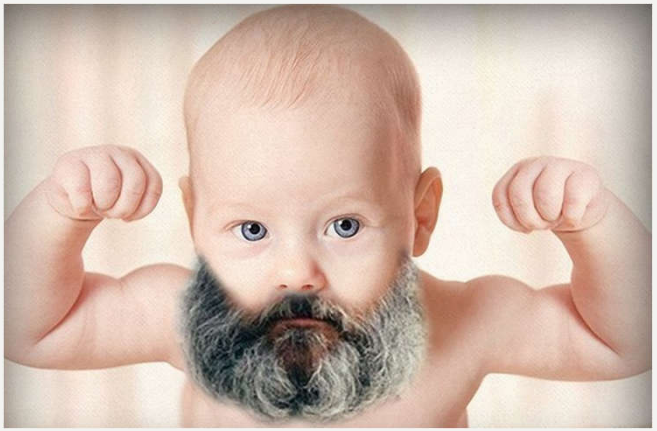
- Robust
- Less likely to have genetic errors
- Not the same as mature adult
- Immune Priviledged
- 1:10,000 (Plentiful)

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.



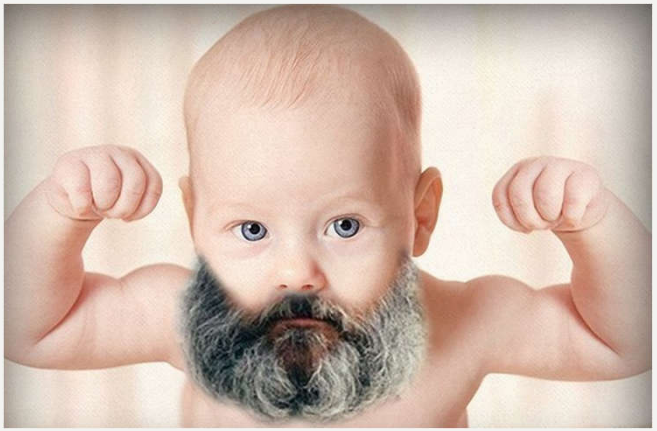
- **Adult MSC are exposed to a lifetime of factors, eg NSAIDS -- may inhibit MSC chondrogenic differentiation and disrupt endochondral bone formation.**

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.

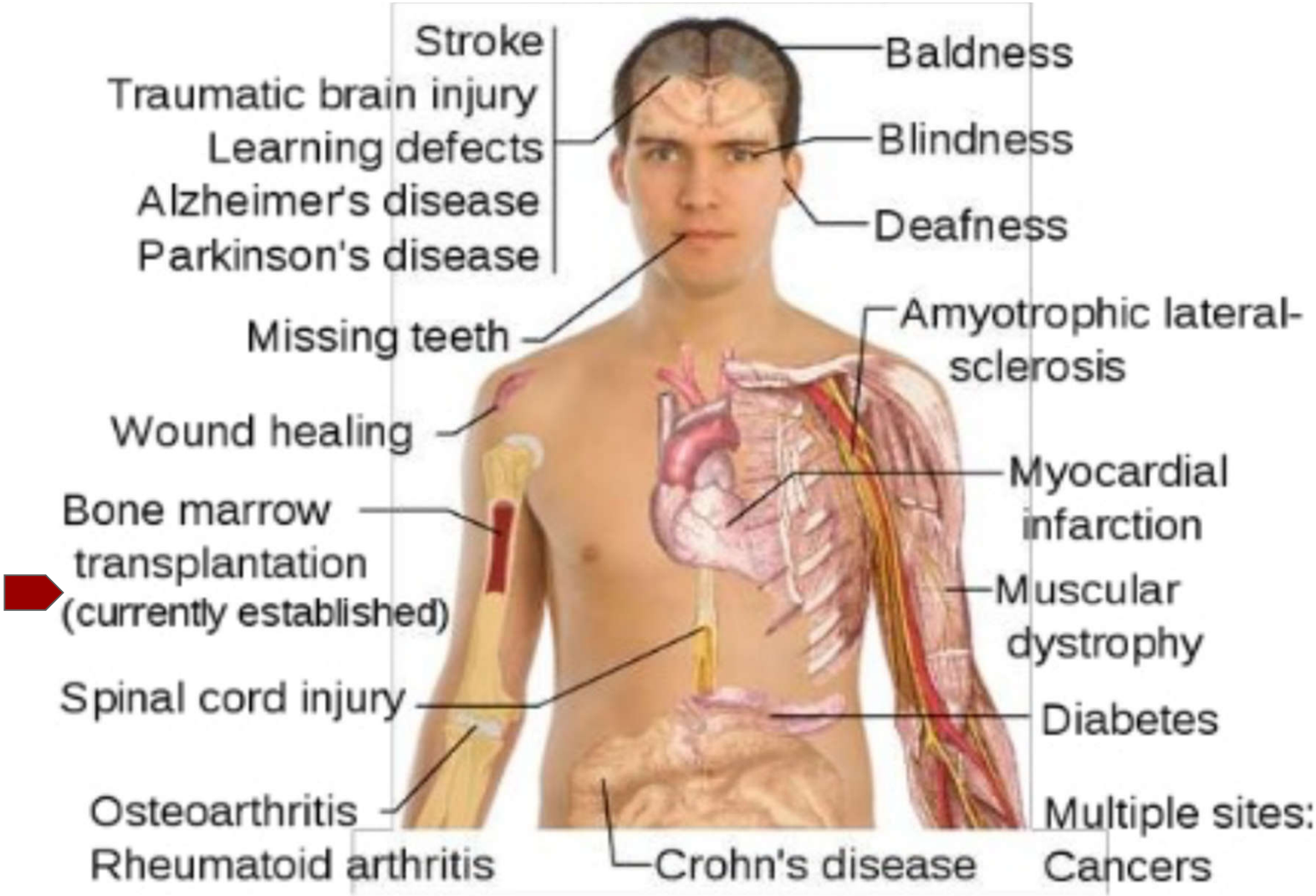
**Baksh D. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow stem cells, 2007.

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.

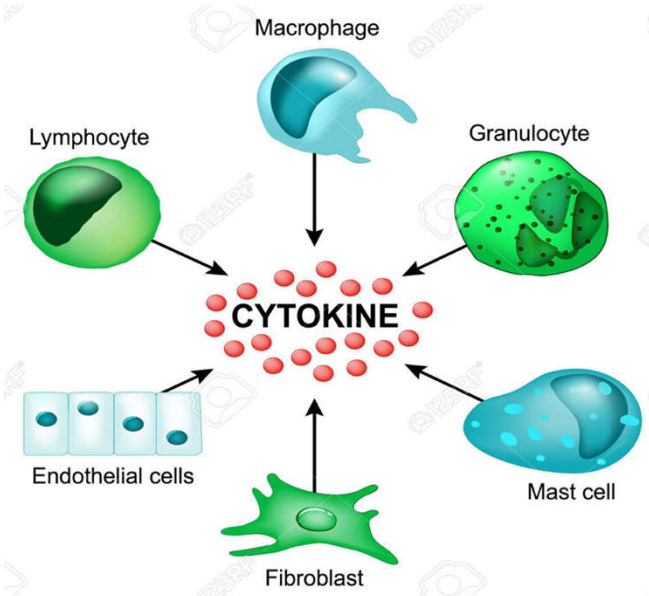
Gauthaman K. Human umbilical cord Wharton's jelly stem cell (hWJSC) extracts inhibit cancer cell growht in vitro. J Cell Biochem, 2012.

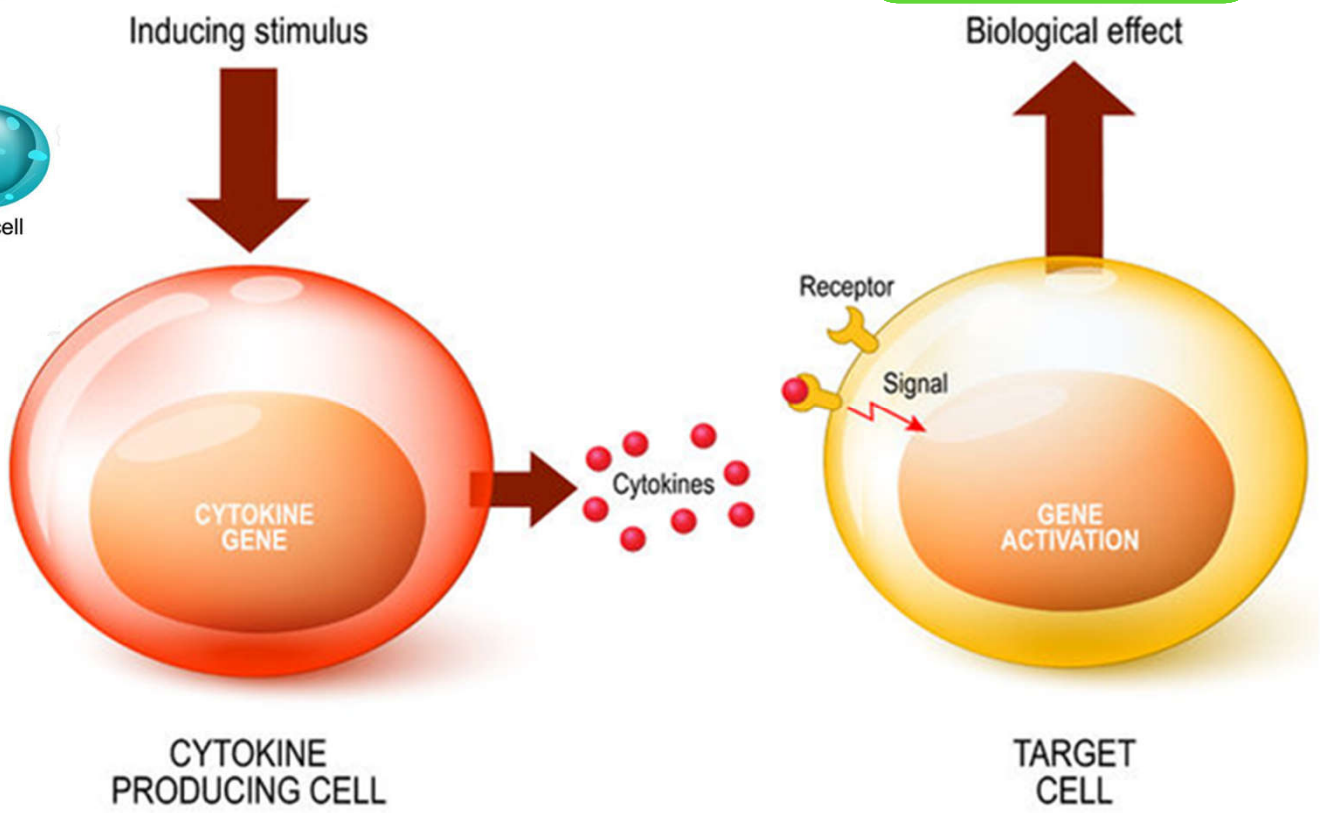
Potential Uses of Stem Cells (MSCs)

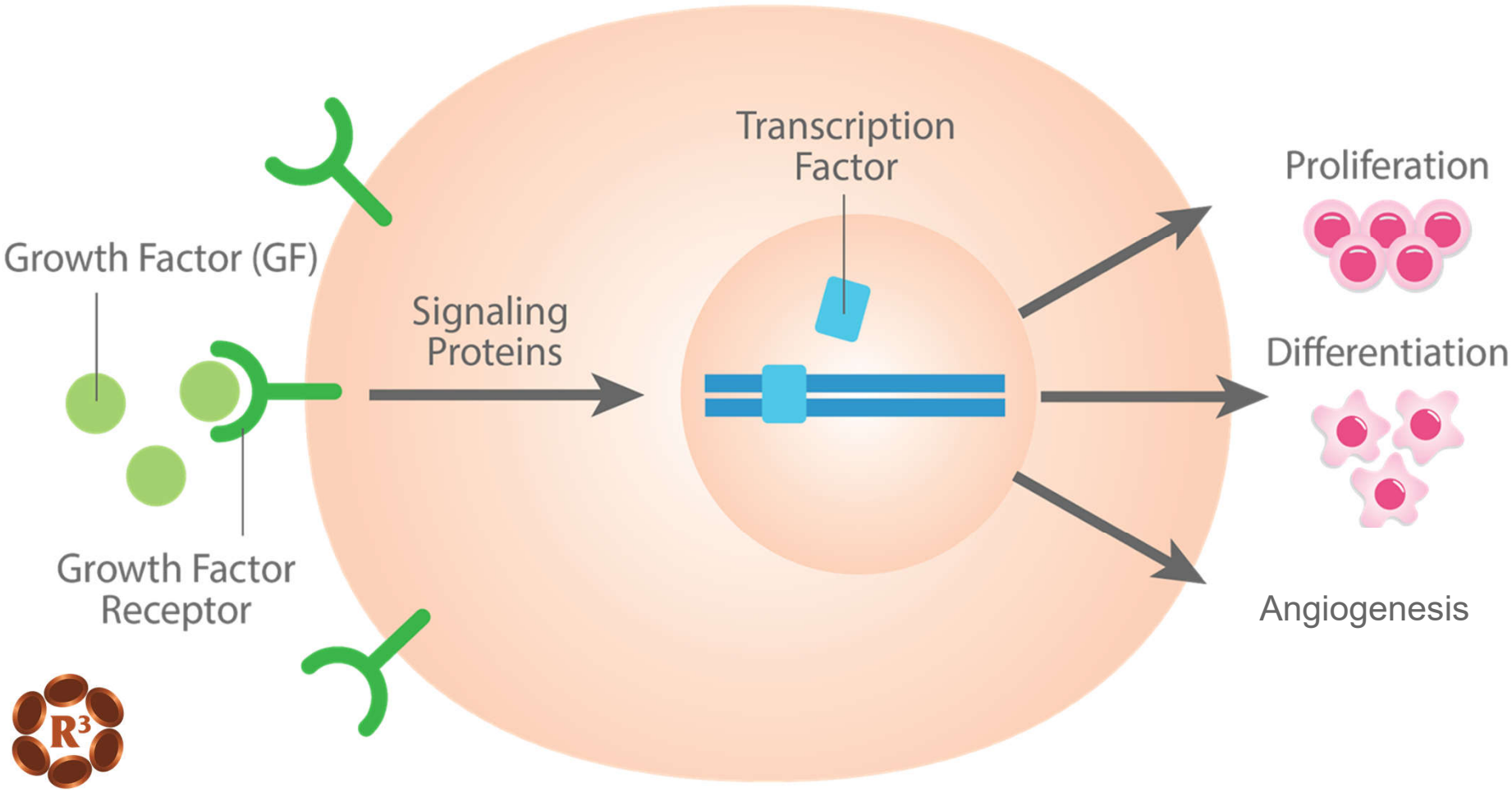


Cytokines

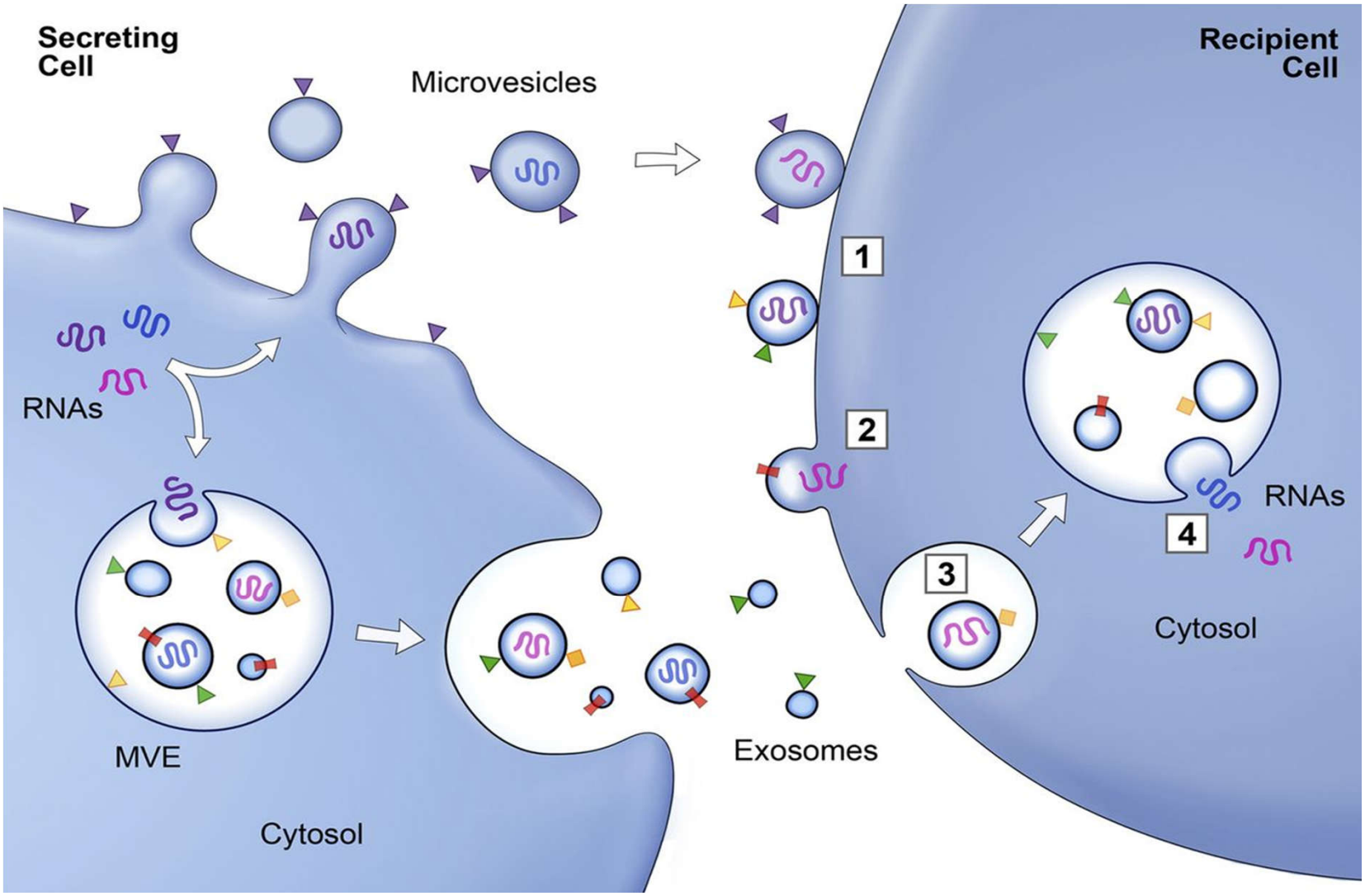
Regulate Cellular Differentiation



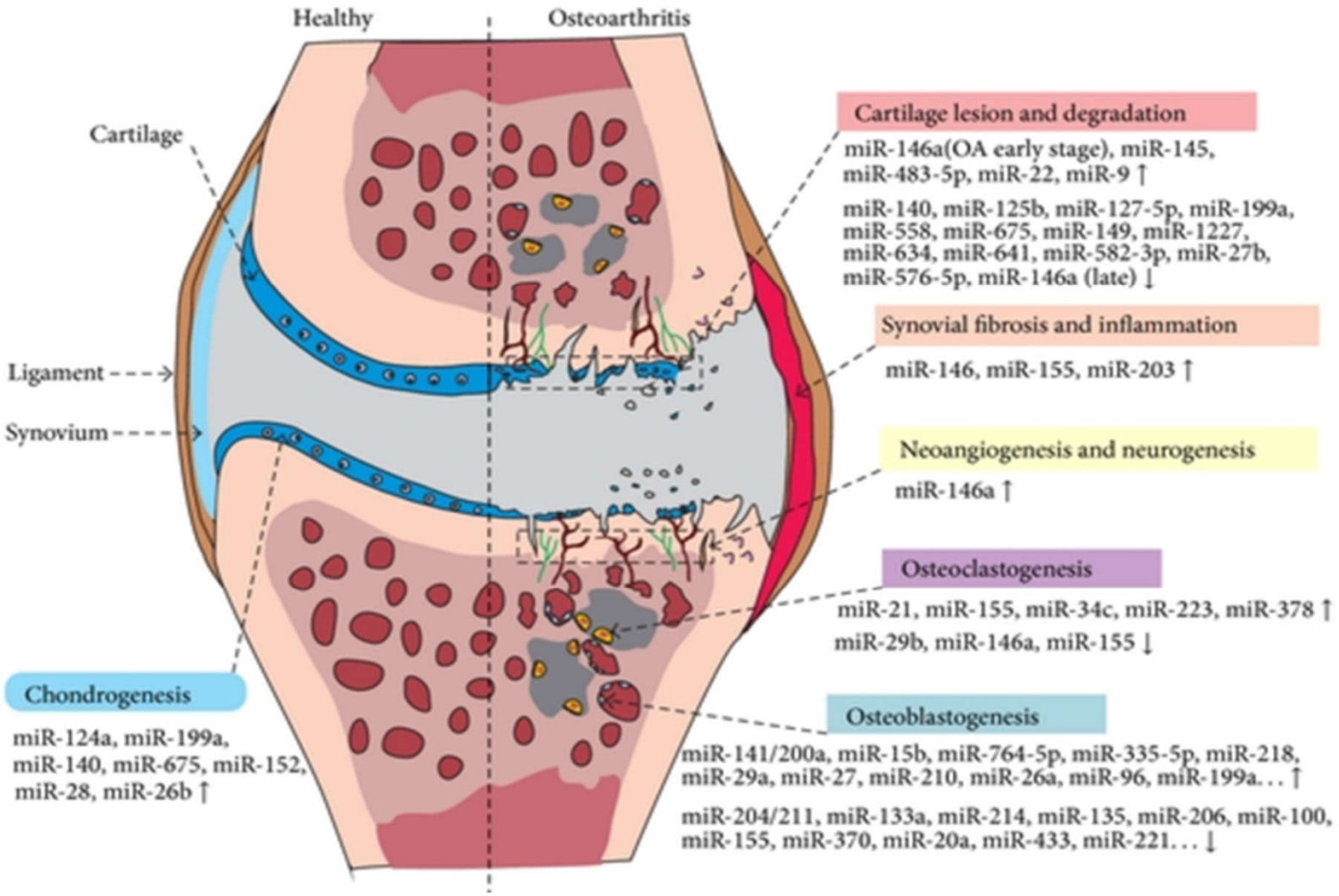



Growth Factor (GF)
Growth Factor Receptor
Signaling Proteins
Transcription Factor
Proliferation
Differentiation
Angiogenesis
R3

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

- 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)

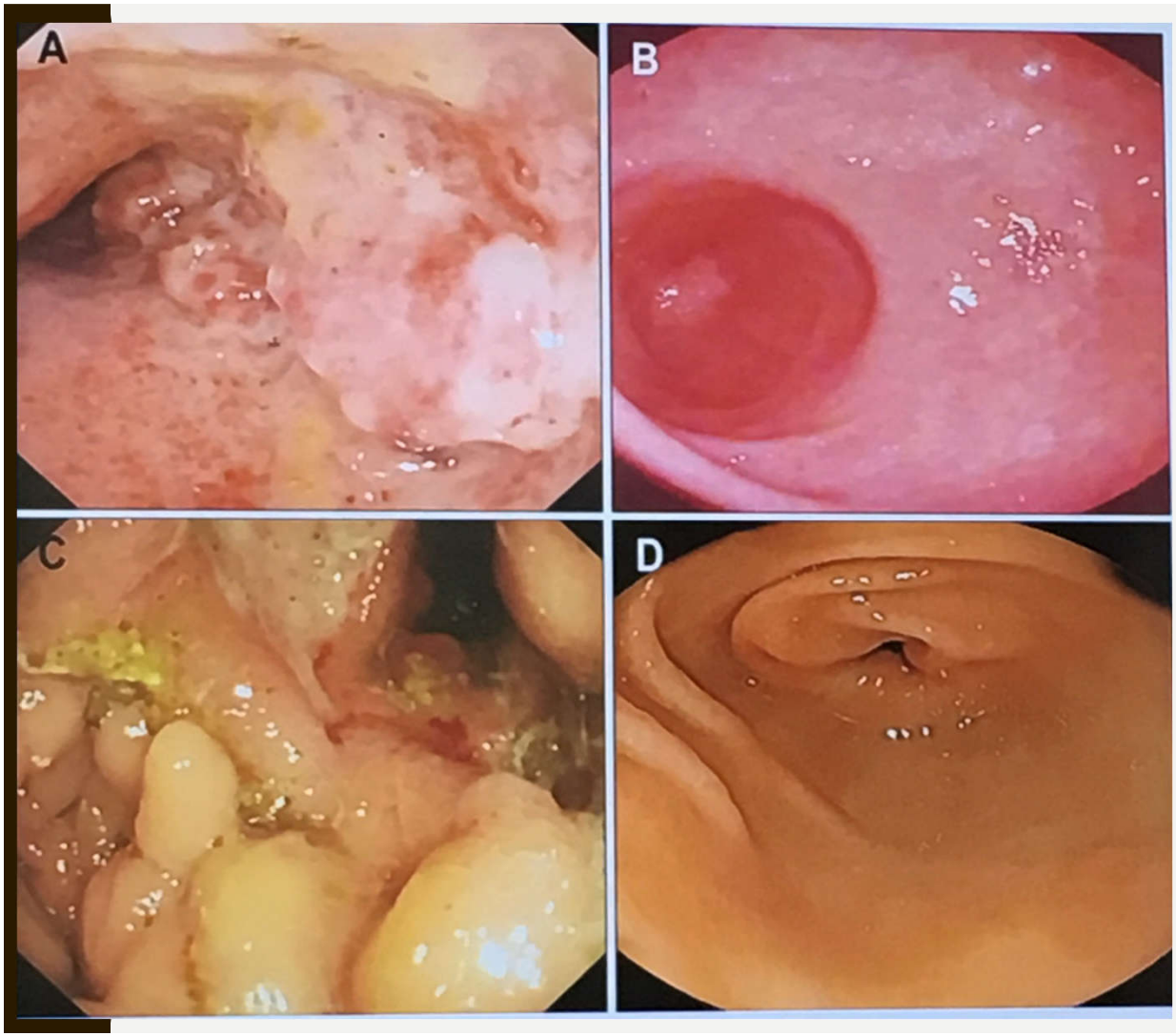
Duran et al. *Therapeutic Advances in Gastroenterology*, 2016.

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 (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

CARDIOVASCULAR DISEASE

Conclusions

Intravenous infusion of UC-MSCs was feasible and safe in this group of patients with HFrEF under otherwise optimal medical therapy. Allogenic UC-MSC treatment induced no humoral immune response in tested individuals. The intervention resulted in a significant improvement in left ventricular function, functional status, and quality of life. These findings suggest that UC-MSCs could have an impact on clinical outcomes, supporting further testing through large clinical trials.

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

OSTEOARTHRITIS - EXOSOMES

Sci Rep. 2017; 7: 16214.
Published online 2017 Nov 24. doi: 10.1038/s41598-017-15376-8
PMCID: PMC5701135
PMID: 29176667

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

Stella Cosenza,[1] Maxime Ruiz,[1] Karine Toupet,[1] Christian Jorgensen,[#1,2] and Danièle Noël[#1,2]

- In conclusion, we provided evidence that MPs and Exos from murine bone marrow BM-MSCs exerted similar functional effect in vitro by re-establishing chondrocyte homeostatic state, protecting chondrocytes from apoptosis and stimulating macrophage polarization towards anti-inflammatory phenotype.
- All of these functions displayed by BM-MSC-derived MPs and Exos might explain their beneficial effect in the CIOA model where treated mice were partly protected from cartilage and bone degradation.

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

AVASCULAR NECROSIS

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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

COLLAGEN FORMATION - EXOSOMES

J Cosmet Dermatol. 2019 Nov 21. doi: 10.1111/jocd.13215. [Epub ahead of print]

Prospective application of exosomes derived from adipose-derived stem cells in skin wound healing: A review.

Qiu H[1], Liu S[1], Wu K[1], Zhao R[1], Cao L[1], Wang H[1].

RESULTS: Most studies have shown the possible roles of ASCs-derived exosomes (ADSCs-Exos) in cutaneous wound healing through regulation of the inflammatory response and promotion of cell proliferation, migration, differentiation, angiogenesis and matrix reconstruction to provide a new perspective strategy for the use of ASCs-Exos in skin wound healing.

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.

ANTI-AGING

PERSPECTIVE ARTICLE

Front. Cell Dev. Biol., 22 November 2016 | https://doi.org/10.3389/fcell.2016.00135

Amniotic Epithelial Cells: A New Tool to Combat Aging and Age-Related Diseases?

- Persistent low grade inflammation accelerates aging process – "inflammaging"
- Following transplantation, exogenous stem cells can graft and replace dysfunctional tissues or improve the tissue milieu, possibly through anti-inflammatory and anti-fibrotic mechanisms.

MODES OF DELIVERY

- **IV**- animal studies show MSC in lungs at 1hr, decreased at 24 hours, some 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

STEM CELL PROGRAM VS PROCEDURE

1. Intelligent Advertising
2. Proper Consent
3. Use Peer Reviewed Protocols
4. Join an IRB
5. Offer ancillary therapies to help activate the biologic cells to the max.
6. E.G. Supplements, acupuncture, electrical stimulation, PT