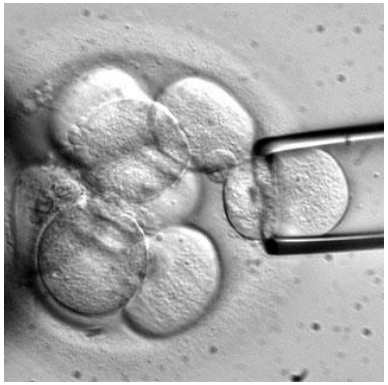
Introduction of Stem Cell



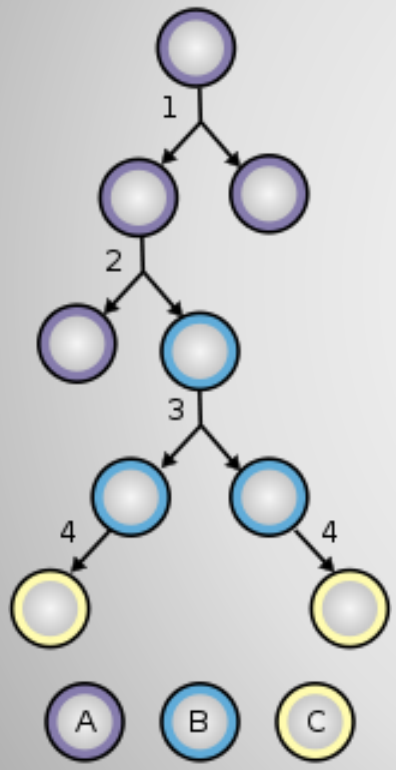
Meifeng Xu, MD, PhD

Department of Pathology
and Laboratory Medicine

Stem Cells

Stem cells are unspecialized and capable of dividing and renewing themselves for long periods through cell division. In addition, they can give rise to specialized cell types through differentiation.

Unique Properties of Stem Cells



1. *Self-renewal*: the ability to go through numerous cycles of cell division to produce more stem cells.
2. *Differentiation:* these cells can differentiate into specialized cell types.

Stem cell self-renew and differentiation

A: stem cells

B: progenitor cells

C: differentiated cells

Progenitor Cells

A progenitor cell likes a stem cell, has a tendency to differentiate into a specific type of cell. They are in the “intermediate status” between stem cells and fully differentiated cells.

1) progenitor cell is already more specific than a stem cell and is pushed to differentiate into its "target" cell (e.g. endothelial progenitor cells).
2) stem cells can replicate indefinitely, progenitor cells can divide only a limited number of times.
3) they can be activated by damaged or dead cells. Growth factors or cytokines can mobilize them toward the damaged tissue.

Differentiation Potential

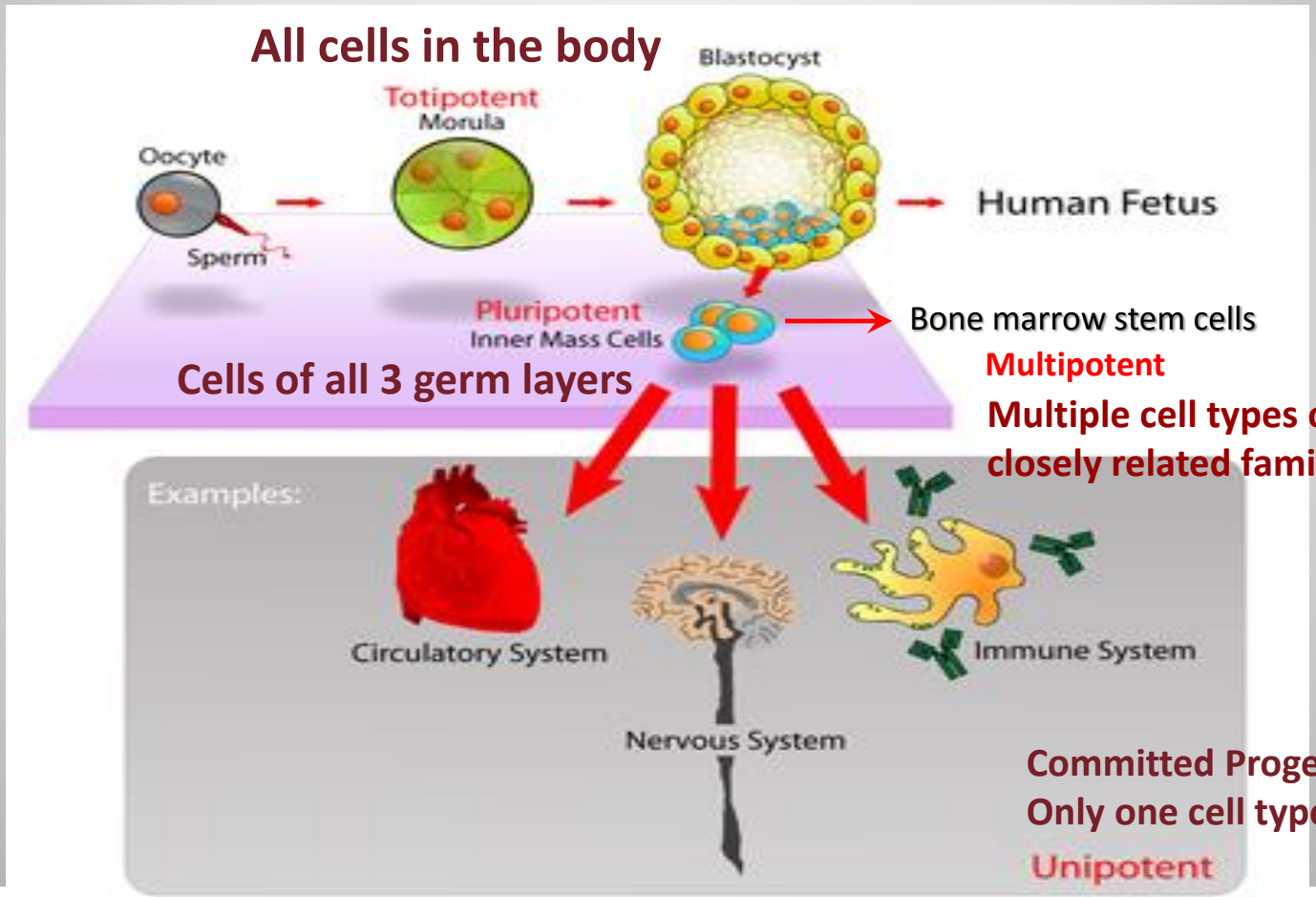
Totipotent can differentiate into all possible cell types. These cells are produced from the fusion of an egg and sperm cell, e.g. zygote formed at egg fertilization.

Pluripotent are the descendants of totipotent cells and can differentiate into nearly all cell types, e. g., embryonic stem cells.

Multipotent can differentiate into those of a closely related family of cells, i.e. bone marrow stem cells.

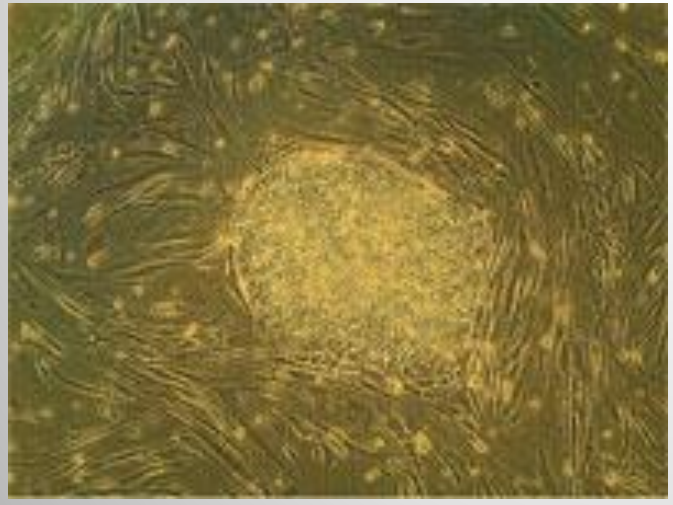
Unipotent can produce only their own, but have the property of self-renewal, which distinguishes them from non-stem cells, e.g., muscle stem cells.

Differentiation Potential



Stem Cells

In mammals, there are two broad types of stem cells: embryonic stem cells which are isolated from the inner cell mass of blastocysts, and adult stem cells which are found in various tissues.



Human embryonic stem cell colony

Bone marrow stem cells

Embryonic Stem Cells

Embryonic stem (ES) cells are the cells of the inner cell mass of a blastocyst, an early-stage embryo.

Pluripotent

- Can contribute to all three germ layers

Derived from blastocysts

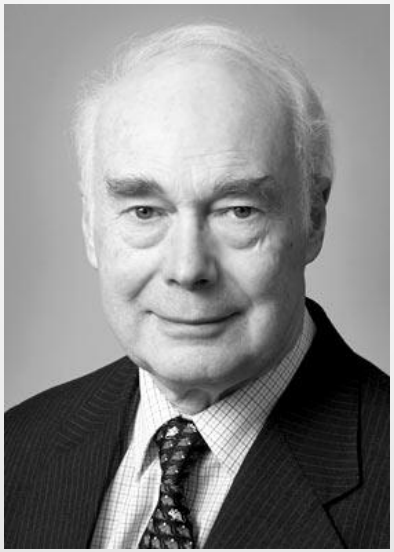
- Derivation of ES cells from mouse blastocysts (1981)
- Human ES cells (Thomson, 1998)

  Blastocysts produced by in vitro fertilization (IVF)

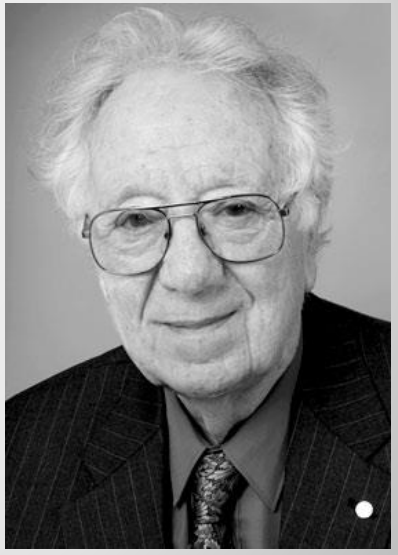
The Nobel Prize in Physiology or Medicine 2007

for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells

Mario R. Capecchi
Born: 6 October 1937,
Verona, Italy

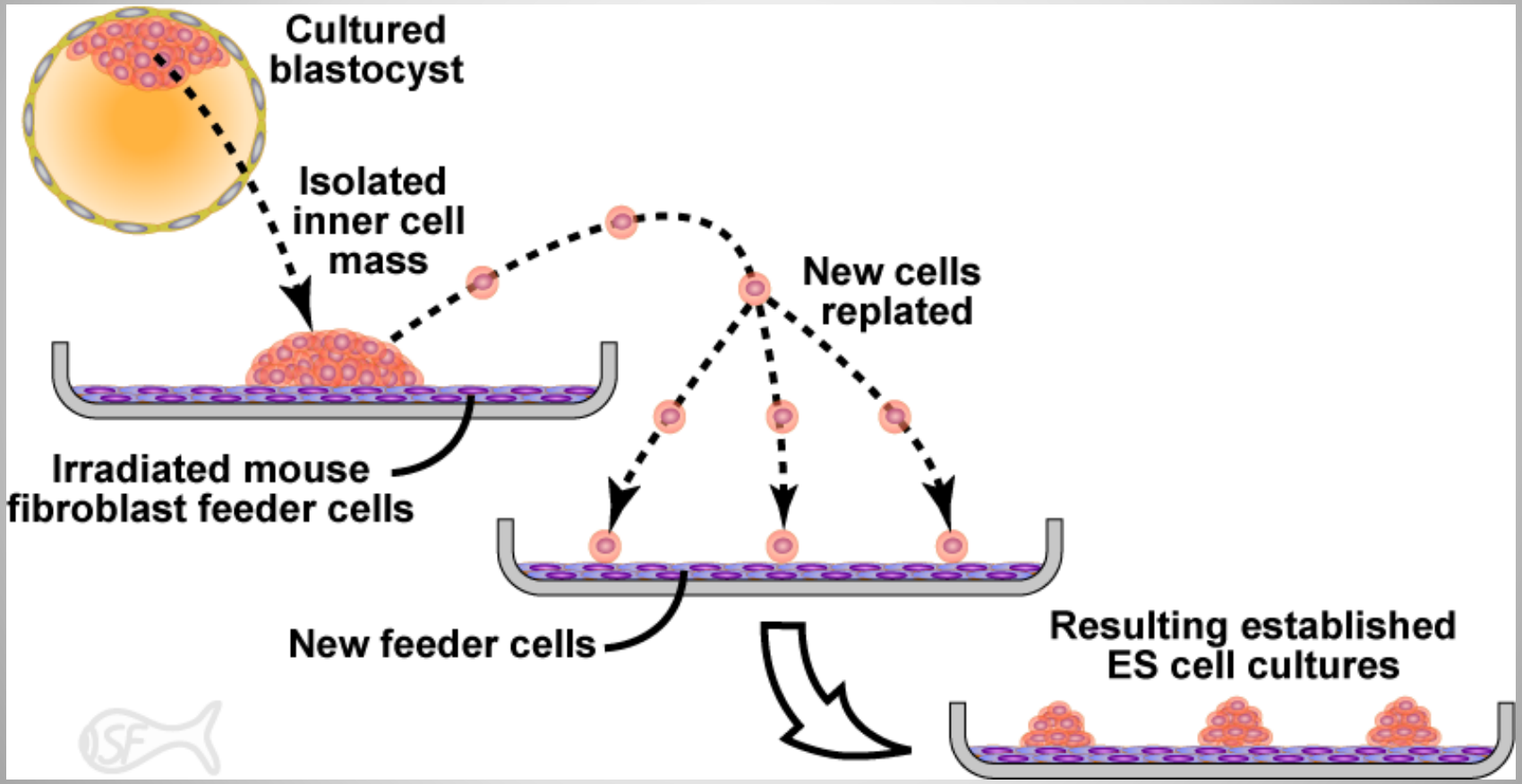


Sir Martin J. Evans
Born: 1 January 1941,
Stroud, United Kingdom



Oliver Smithies
Born: 23 June 1925,
Halifax, United Kingdom

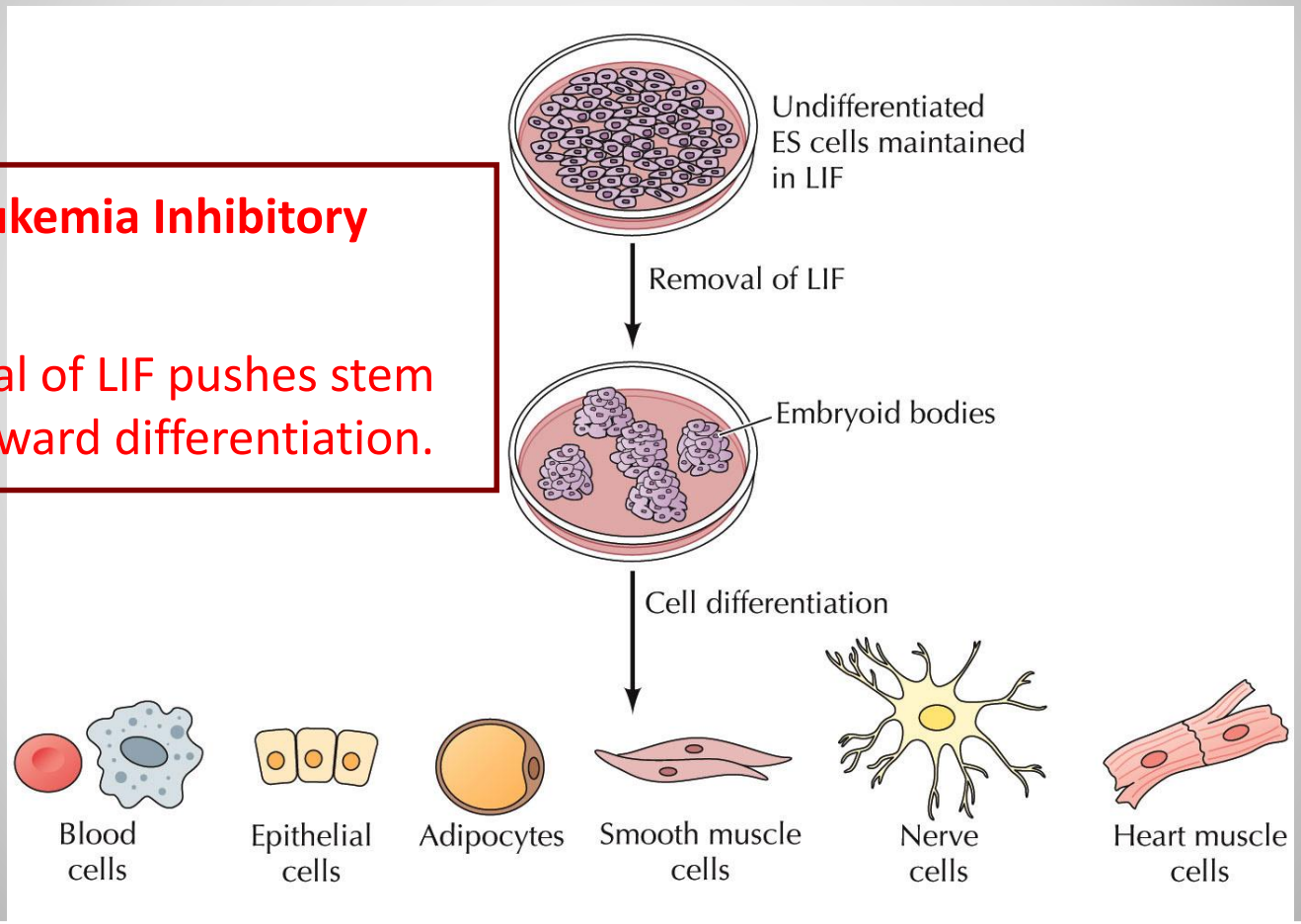
ES Cells (human) culture



ES Cells (mouse) Culture

LIF: Leukemia Inhibitory Factor

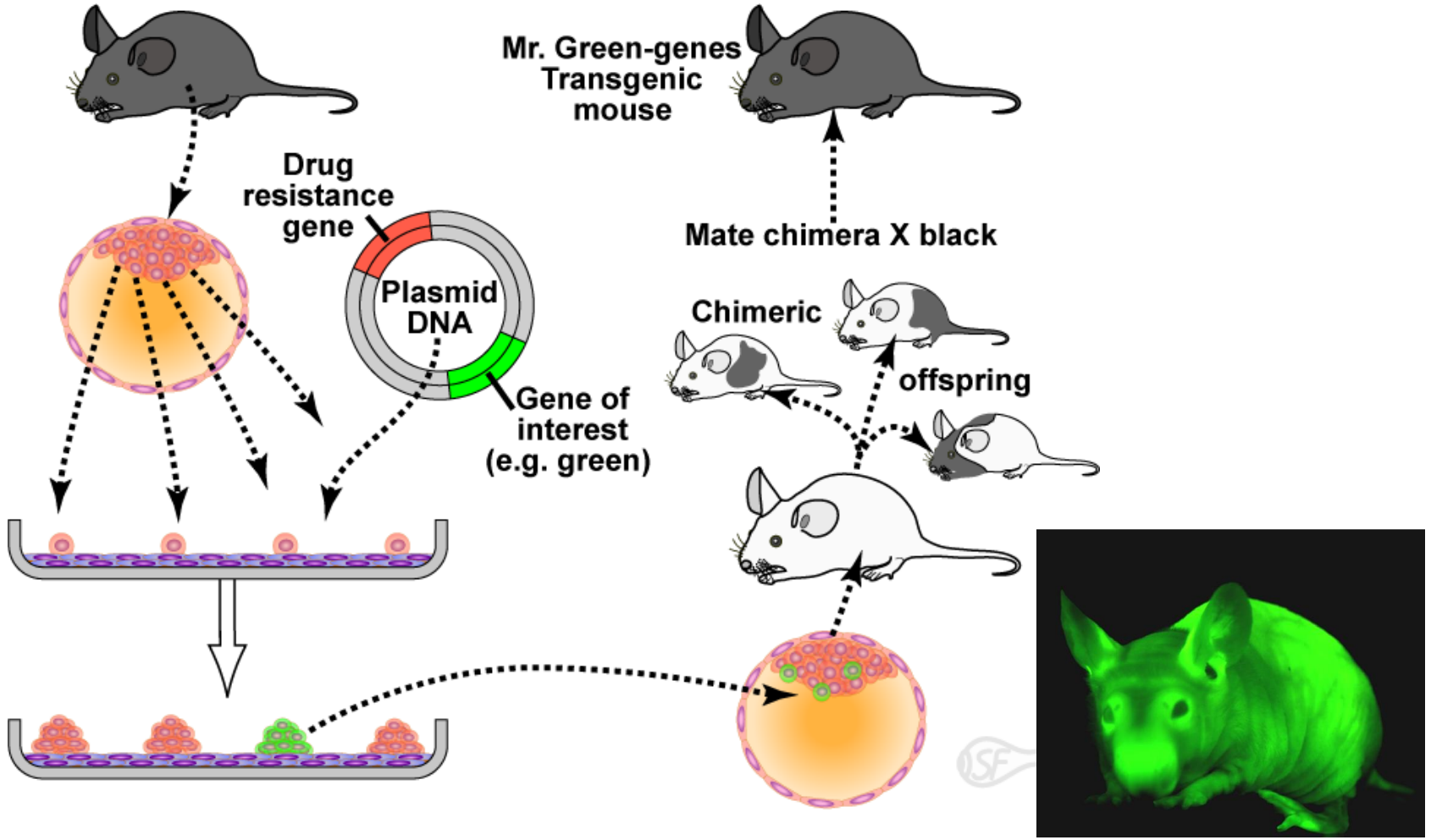
Removal of LIF pushes stem cells toward differentiation.



Utilizations of ES Cells

- Pluripotent stem cells have shown potential in treating a number of varying conditions, including but not limited to: spinal cord injuries, age related macular degeneration, diabetes, neurodegenerative disorders (such as Parkinson's disease), AIDS, etc.
- Tissue/organs derived from ESCs can be made immunocompatible with the recipient.
- Embryonic stem cells can also serve as tools for the investigation of early human development, study of genetic disease and as in vitro systems for toxicology testing.

eGFP Transgenic Mice



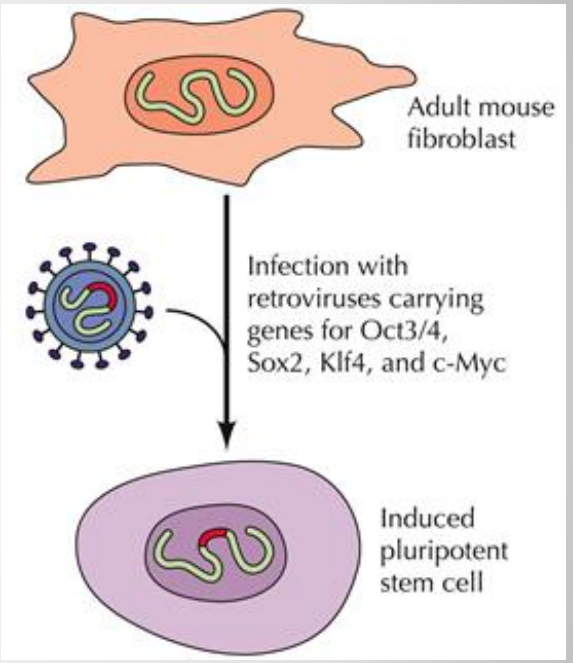
Adverse Effect of ES Cells

1. The ES cell controversy is the ethical debate centered only with research involving the creation, usage, and destruction of human embryos.
2. The major concern with the possible transplantation of ESC into patients as therapies is their ability to form tumors including teratoma.

Induced Pluripotent Stem Cells

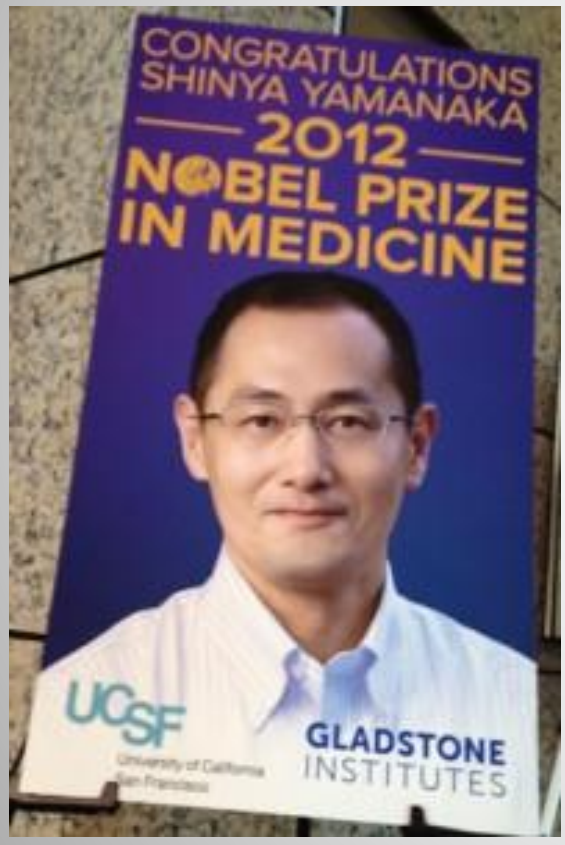
Induced pluripotent stem cells (iPS cells or iPSCs) are artificially derived from the non-pluripotent cells by forcing to express the specific genes (Oct-4, c-Myc, Kif4, and Sox2).

iPS cells are first produced in 2006 (Yamanaka *et al. Cell*) from mouse cells and in 2007 (Yu *et al, Science*) from human cells.



iPS cells are similar to natural pluripotent stem cells (e.g., ES cells), such as the expression of certain stem cell genes and proteins, doubling time, embryoid body formation, potency of differentiation, and teratoma formation.

The Nobel Prize in Physiology or Medicine 2012



In 2012, Dr. Yamanaka was awarded the Nobel Prize in Physiology or Medicine for his discovery that adult somatic cells can be reprogrammed into pluripotent cells. By introducing the genes for four factors, he induced the skin cells of adult mice to become like embryonic stem cells, which he called induced pluripotent stem (iPS) cells. This iPS cell technology represents an entirely new platform for fundamental studies of developmental biology.

Adult Stem Cells

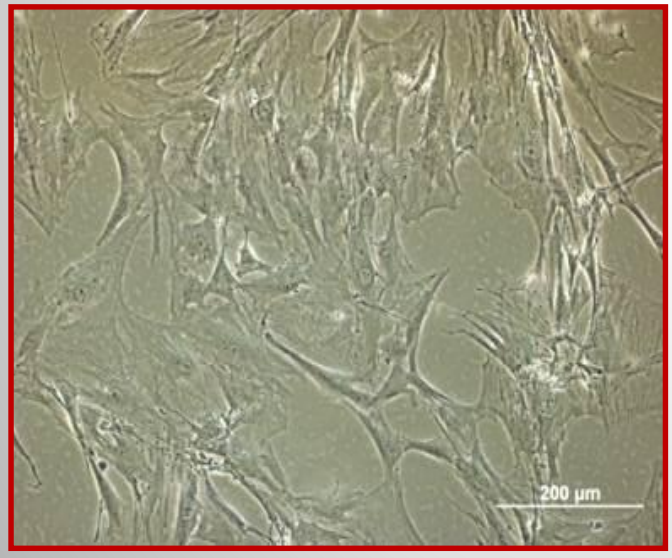
Adult stem cells are undifferentiated cells, found throughout the body among differentiated cells in a tissue or organ after development.

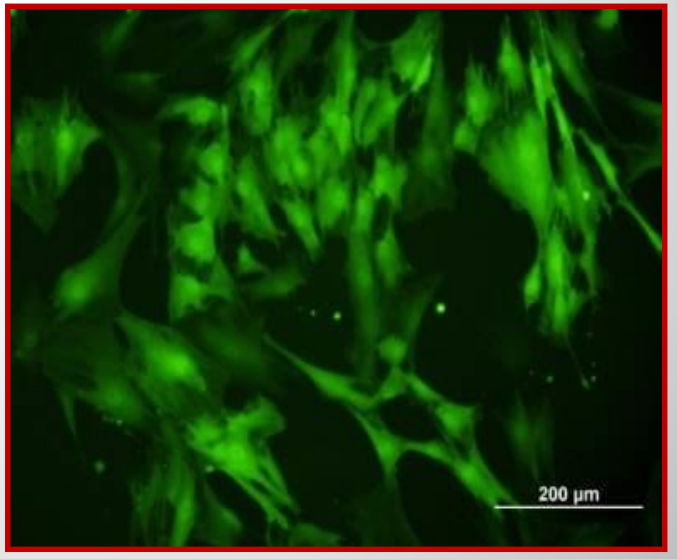
They remain in a quiescent or non-dividing state for years until activated by disease or tissue injury. They can divide or self-renew to generate a range of cell types from the originating organ to replenish dying cells and regenerate damaged tissues or entire original organ.

Most adult stem cells are lineage-restricted and are generally referred to by their tissue origin, but they can also differentiate to become other cell types.

Adult Stem Cells

Adult stem cells are undifferentiated cells which are found throughout the body after development. Most adult stem cells are multipotent or progenitors.





Sources of Adult Stem Cells

1) The bone marrow stroma contain mesenchymal stem cells (MSCs), also called *marrow stromal cells*.
2) Adipose tissue (lipid cells), which requires extraction by liposuction.
3) Blood is drawn from the donor (similar to a blood donation), passed through a machine that extracts the stem cells and returns other portions of the blood to the donor.
4) In addition, stem cells can also be taken from umbilical cord blood, amniotic fluid, adult muscle or the dental pulp of deciduous baby teeth.

Stem Cell Therapy

Regeneration of damaged tissue and organ by stem cells or the therapeutic genes carried by stem cells.

Selection of Stem Cells

1. **The capacity of differentiation**: Embryonic stem cells can become all cell types of the body. Adult stem cells are limited to differentiating into the cell types of their tissue of origin.
2. **Grow in culture**: Embryonic stem cells can be grown relatively easily in culture. Adult stem cells are rare in mature tissues, so isolating these cells from an adult tissue is challenging.
3. ES cells, injected directly into another body, ES cells will differentiate into many different types of cells, causing a **teratoma**.
4. **Immune rejection**: Identical matches between donor and recipient must be made for successful transplantation treatments, but matches are uncommon.
5. **Ethical considerations**: many nations currently have limitations on either human ES cell research or the production of new human ES cell lines. The use of adult stem cells does not require the destruction of an embryo.

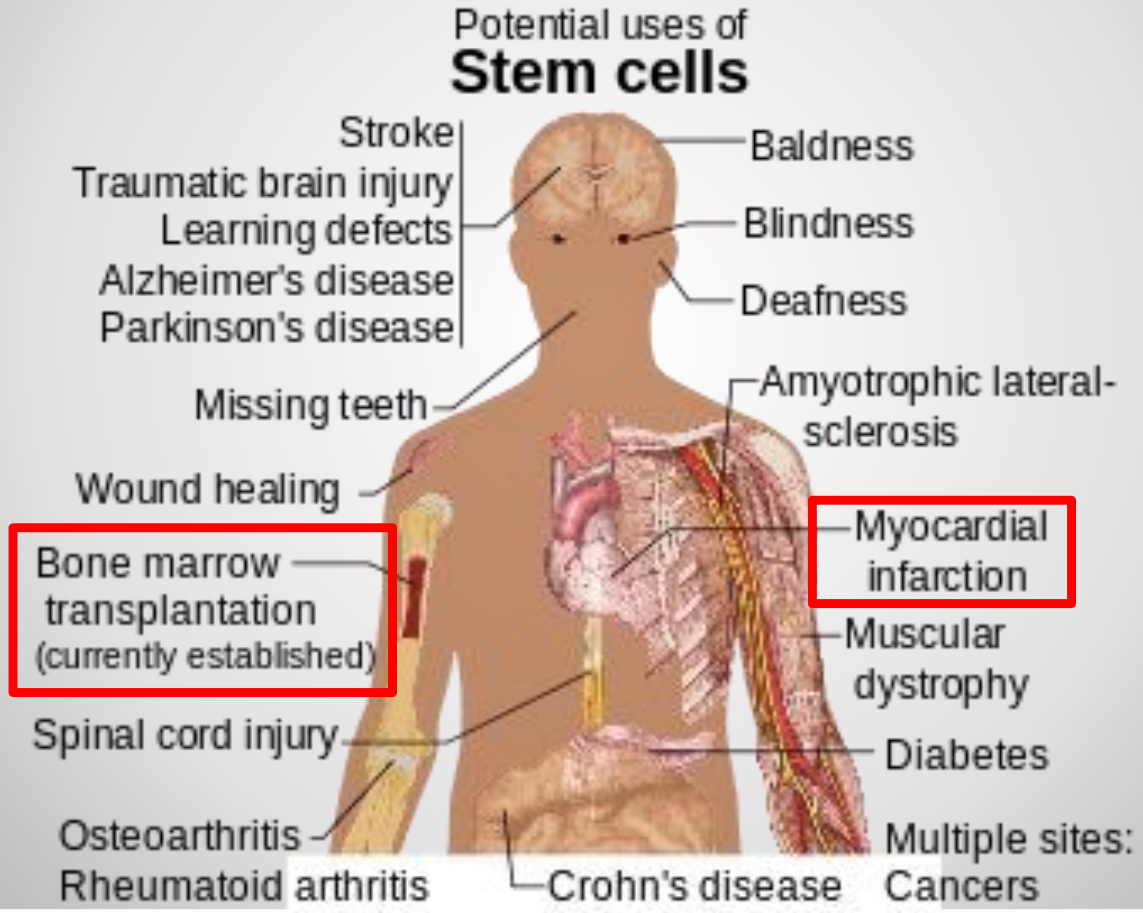
Clinic Sources of Stem Cell Therapy

Cell type	Abbreviation	Origin
Bone marrow derived stem cell	BMSC	Bone marrow
Skeletal myoblast	SM	Adult skeletal muscle
Cardiomyocyte progenitor cell/Cardiac stem cell	CMPC	Adult or fetal heart
Endothelial progenitor cell/endothelial precursor cell	EPC	Bone marrow/peripheral blood
Embryonic stem cell	ESC	Blastocyst stage embryos
Induced pluripotent stem cell	iPSC	Any somatic cell

Source of Stem Cells

- **Allogenic**
  - Donor with matching tissue type
  - Immune system suppression therapy
  - Risk of graft-versus-host disease & infection
- **Autologous**
  - Extraction and storage of stem cells from patient (bone marrow or adipose tissue)
  - Lower risk of infection/rejection

Stem Cell Therapy



http://en.wikipedia.org/wiki/Stem_cell_treatments

Stem Cell Therapy

The effect of stem cells includes: protecting risky cells, regenerating damaged tissues or organs, replacing lost cells, stimulating growth of new blood vessels to repopulate damaged tissue.

Therapy success is highly dependent on: survival of transplanted cells in the recipient; integration within the targeted tissue and restoration of function; proliferation and differentiation in a site-specific manner.

HSC Transplantation

HSC transplantation is the transplantation of multipotent HSC, usually derived from bone marrow, peripheral blood, or umbilical cord blood. It may be autologous, allogeneic, or syngeneic.

It is most often performed for patients with certain cancers of the blood or bone marrow, such as multiple myeloma or leukemia.

However, HSC transplantation remains a dangerous procedure with many possible complications, including infection and graft-versus-host disease. It is reserved for patients with life-threatening diseases.

Myocardial Regeneration

Several clinical trials targeting heart disease have shown that adult stem cell therapy is safe, effective, and equally efficient in treating old and recent infarcts. Stem cell therapy for treatment of myocardial infarction usually makes use of autologous bone marrow stem cells (a specific type or all).

However other types of adult stem cells may be used, such as adipose-derived stem cells. Adult stem cell therapy for treating heart disease was commercially available in at least five continents at the last count.

Cells Being Tested for Heart Therapy

Cell Type	Advantages	Disadvantages
Embryonic stem cells	Divide for indefinite periods Evolve with cardiomyocyte action potential Become both myocytes and vascular cells	Major ethical opposition Possibility of teratoma formation
Bone marrow stem cells	Feasible and safe in humans Readily prepared in hospitals Possibility of "off-the-shelf" use	Pluripotency uncertain Limited success in clinical trials
Skeletal myoblasts	Readily obtained Low risk of tumor formation Survive and differentiate in human hearts Align parallel with host cardiac cells Resistant to ischemia	Do not form gap junctions Ventricular arrhythmias in clinical trial
Cardiac stem cells	Cardiac origin Differentiation into all cardiac lineages Readily obtained at cardiac biopsy Clinical trials underway	Cardiac stem cells isolated from an aging heart may not sufficiently improve function More invasive because biopsy should be obtained from the septum Long-term outcome not yet known
Induced pluripotent cell	Readily obtained from skin and thus less invasive Closely resemble embryonic stem cells Differentiate into all cell lines Regenerate myocardium in animal studies	Potential for malignant transformation

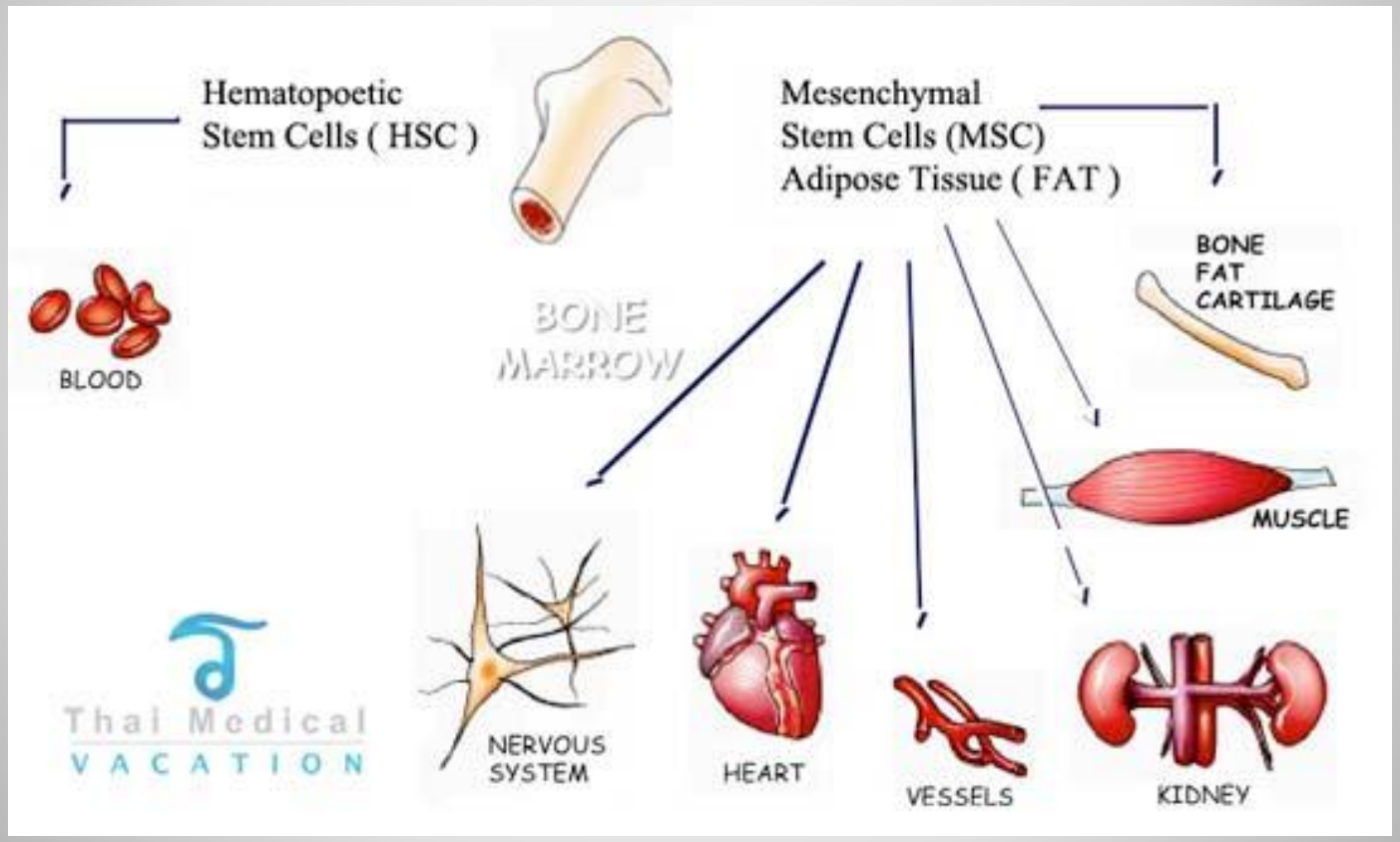
Rangappa, *et al, J Cardiovasc Pharmacol Ther.* 2010, 15:338-43.

Mechanism

1. (Trans)-differentiation

2. Paracrine Effect
   - Cytoprotection
   - Angiogenesis
   - Recruiting other cells

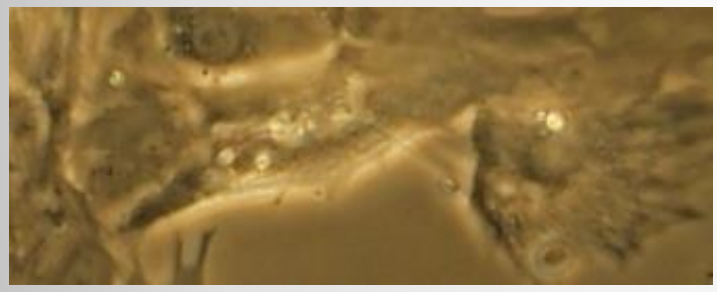
Transdifferentiation of Adult Stem Cells



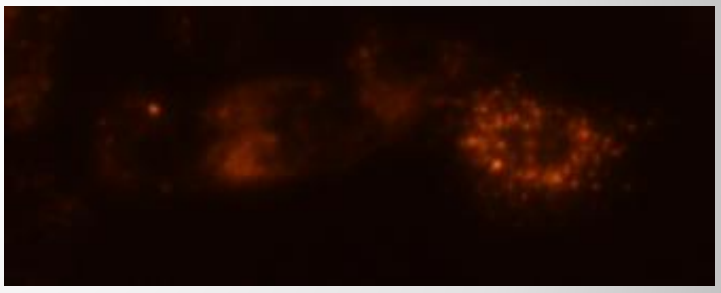
Transdifferentiation of Stem Cells

Stem cells (MSCs) were GFP^+ (green) and co-cultured with cardiomyocytes (CM, red) for 3 days. MSCs are beating with CM.

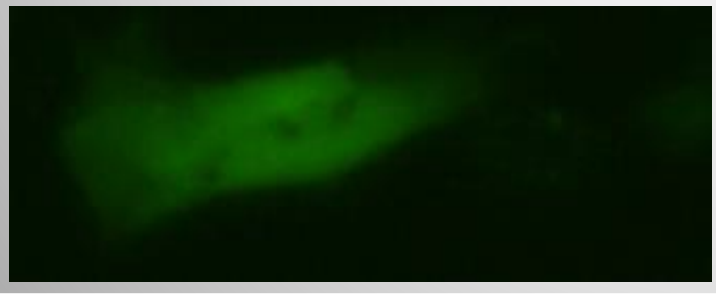
Contrast (MSC + CM)



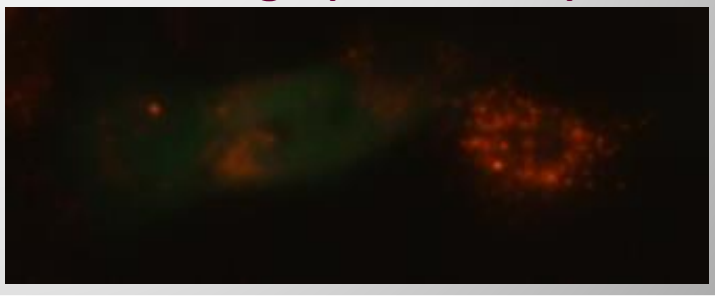
CM (red)



MSC (Green)



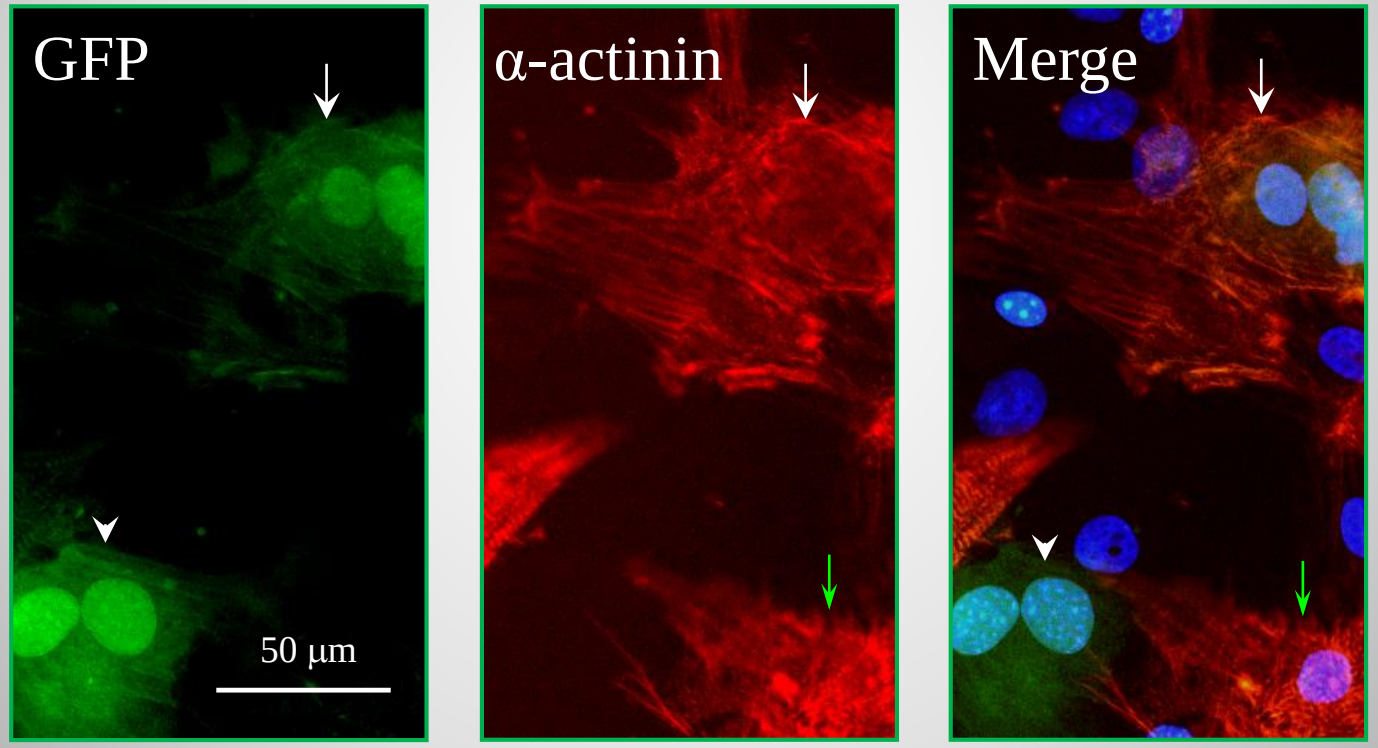
Merge (MSC + CM)



Li. *et al. Cytotherapy*. 2011;13:1057-65

Immunostaining of Co-cultured Cells

Green—stem cells; Red---CM (α-actinin positive)



Xu, *et al*. *Circulation* 2004;110:2658-65

3. 2

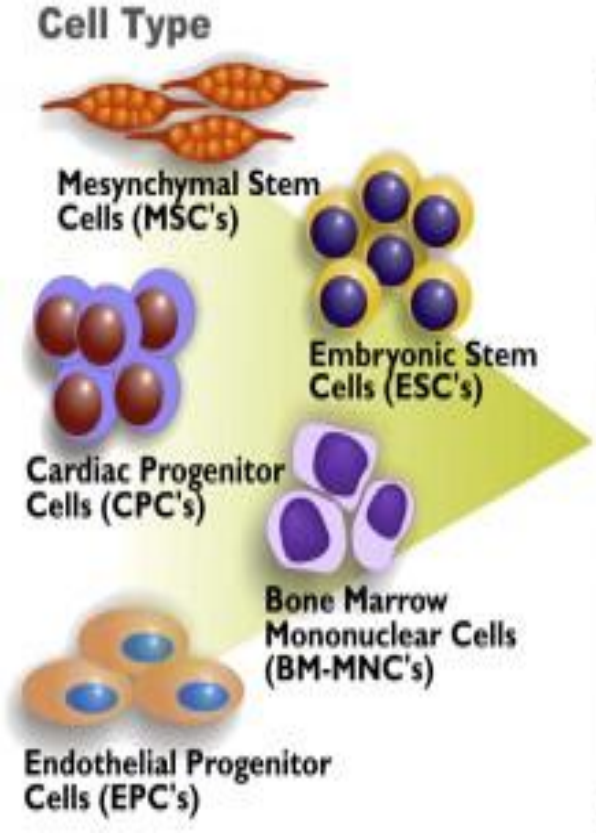
Mechanism--Paracrine Effect

Stem cells secrete paracrine factors into the immediate extracellular environment. These factors then travel to nearby cells to result in a response, through

1) activating a specific receptor, and

2) triggering a biochemical chain of events inside the cell.

Stem Cells Secrete Paracrine Factors



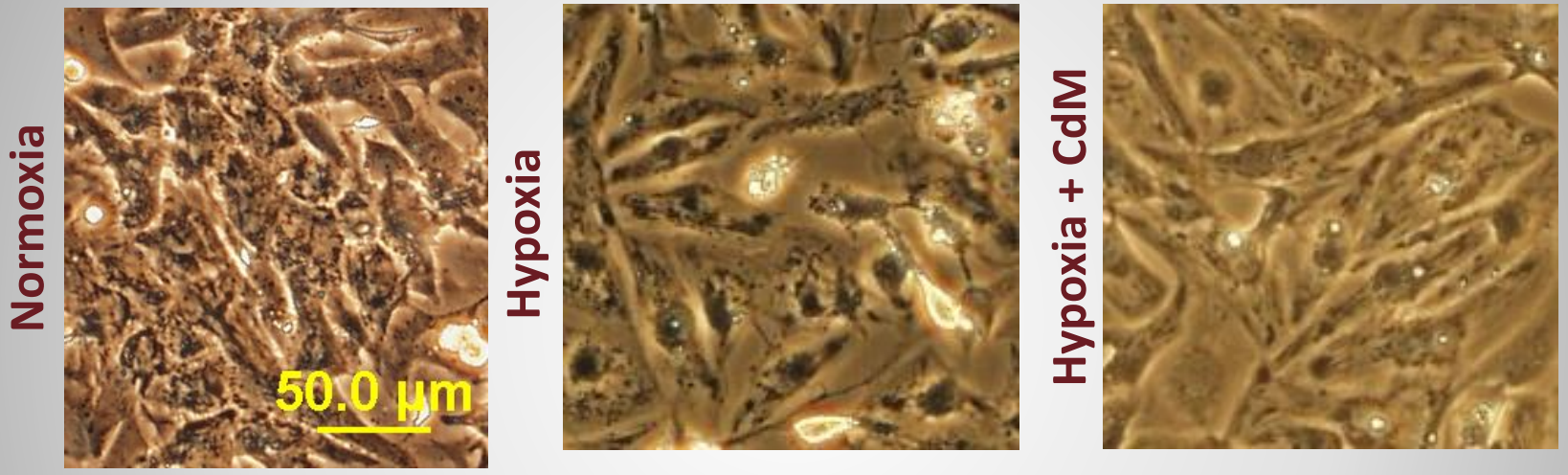
Cell Type	Paracrine Mediators	Mechanisms of Action
Mesynchymal Stem Cells (MSC's)	SFRP2, VEGF, HGF, STC-1 SDF-1, TGF-β, IGF-1, bFGF, TB-4	Survival
Embryonic Stem Cells (ESC's)	VEGF, bFGF, FGF2, HGF, TB-4	Contractility
Cardiac Progenitor Cells (CPC's)	bFGF, VEGF, IL-1, TNF-α HGF, Ang-1, Ang-2, TGF-β, IGF-1 SDF-1, PIGF, MCP-1, PDGF-BB	Neovascularization
Bone Marrow Mononuclear Cells (BM-MNC's)	VEGF, IGF-1, HGF, TNF-α	Differentiation
Endothelial Progenitor Cells (EPC's)	IL-10, TB-4, MMP-2, MMP-9, MCP-1, TSP1, TGF-β, TIMP-1, TIMP-2, TIMP-9, HGF, NGF, ErbB2, tenacin C, IL-1	Remodeling

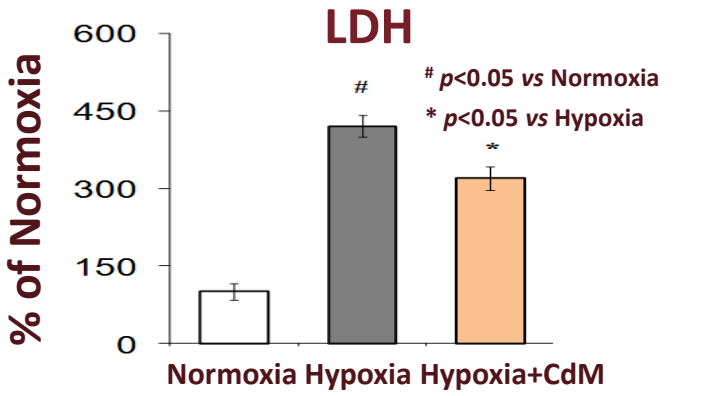
Mirotsou, *et al.*, *JMCC*, 2011; 50:280-9.

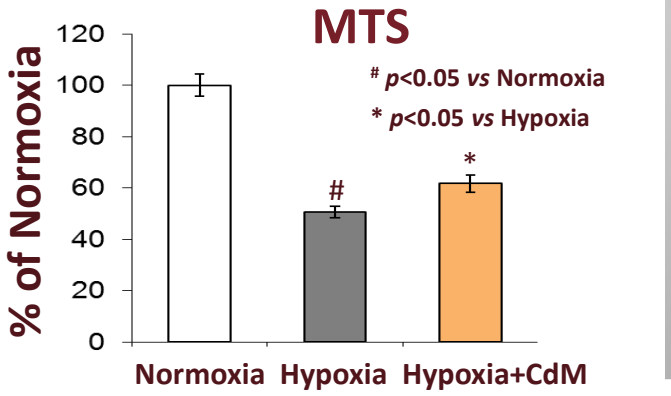
Mechanism of Paracrine Effect

2. Paracrine Effect
   - Cytoprotection
   - Angiogenesis
   - Recruiting other cells

MSC^{CdM} Decrease Hypoxic Injury

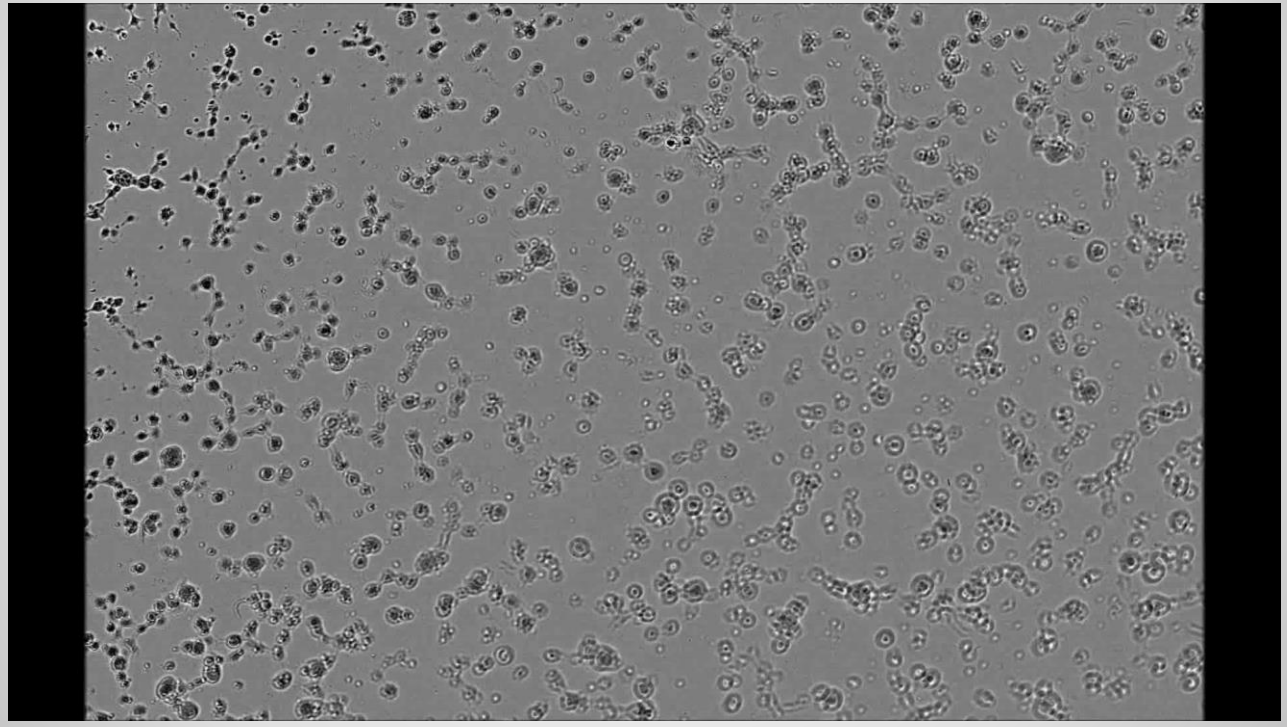




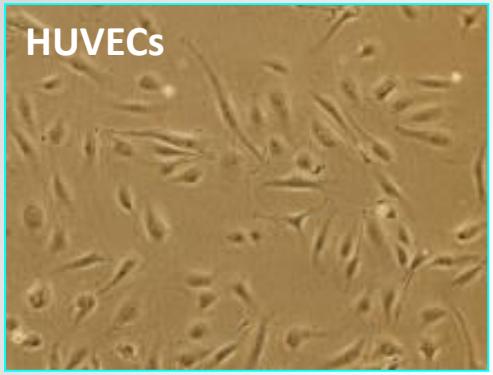


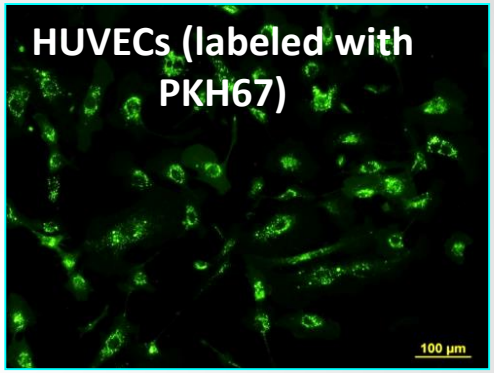
Yu et al.: Plos One

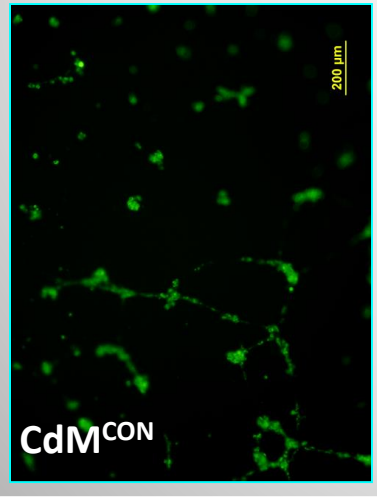
Capillary-like Tube Formation

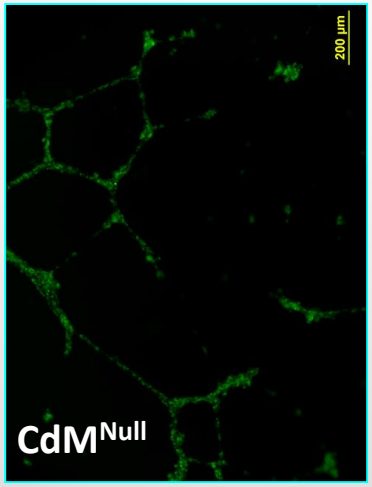


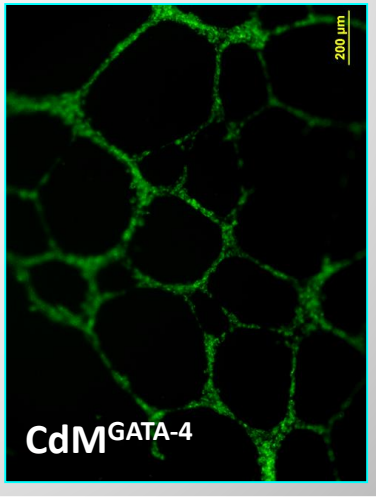
Capillary-like Tube Formation









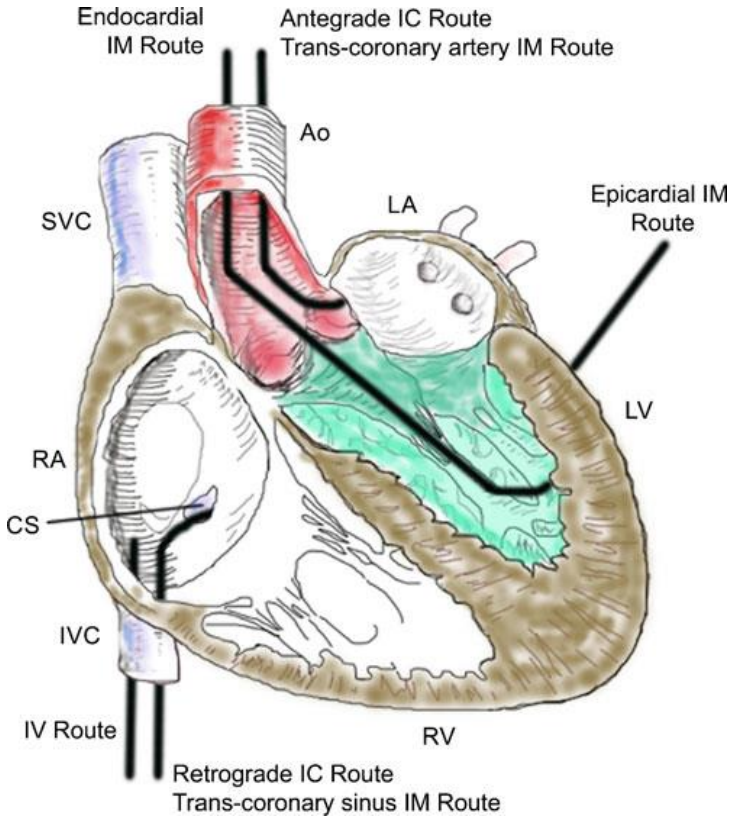


CdM = Conditioned medium

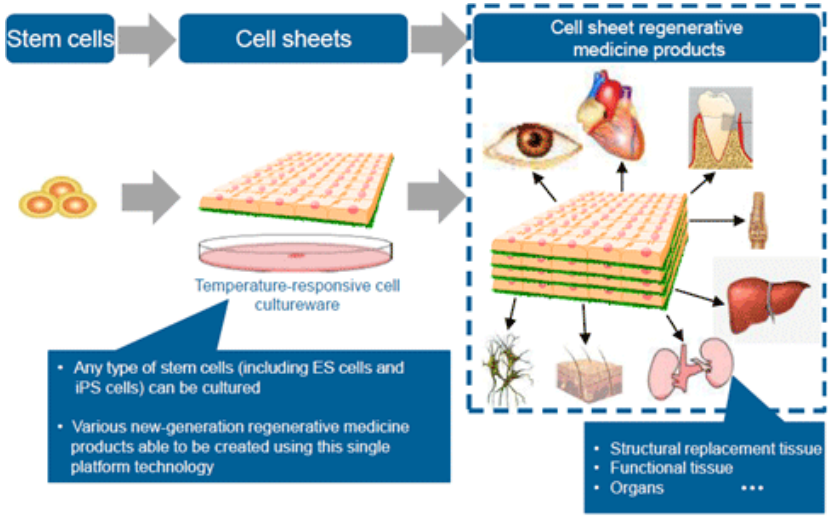
Li, *et al. AJP.* 2010;299:H1772-81

Administration of Stem Cell Therapy

1. Introduce locally



2. Cell Patch



3. Intravenously

4. Mobilization

Niall. *et al. J. of Cardiovasc. Trans. Res*. 2012; 5:713–26

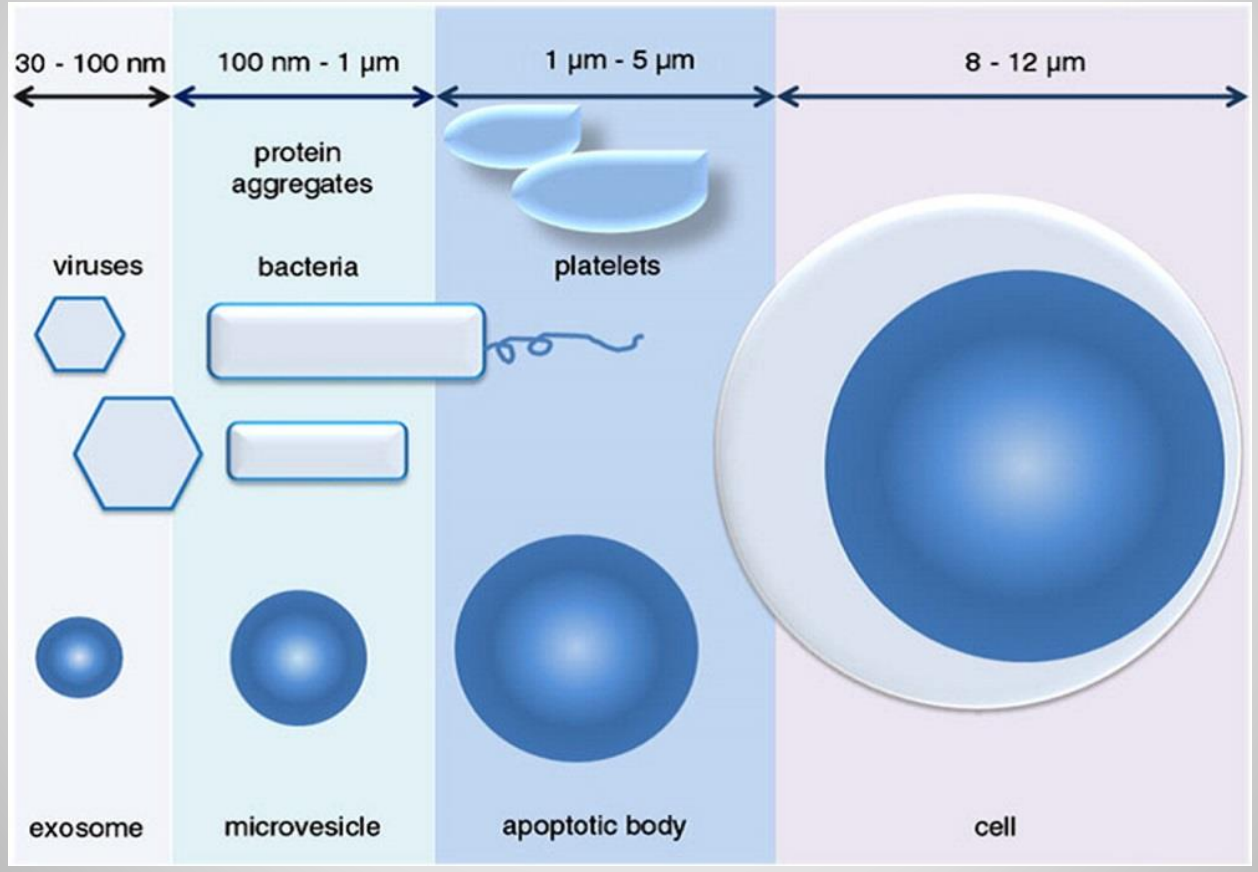
Stem Cell Mobilization

1. Some cytokines and chemokines can promote stem cells mobilization.
2. The damaged cells or tissues release some cytokines or chemokine which can mobilize stem cells to start repair procession.
3. Mobilized stem cells highly expressed cardiac transcription factors, e.g. GATA-4 and anti-apoptotic proteins. Therefore, mobilized cells are more beneficial for repair of damaged tissue.
4. Mobilizing stem cells is an optimal way for repairing the tissues or organs which are difficult to directly transplant stem cells.

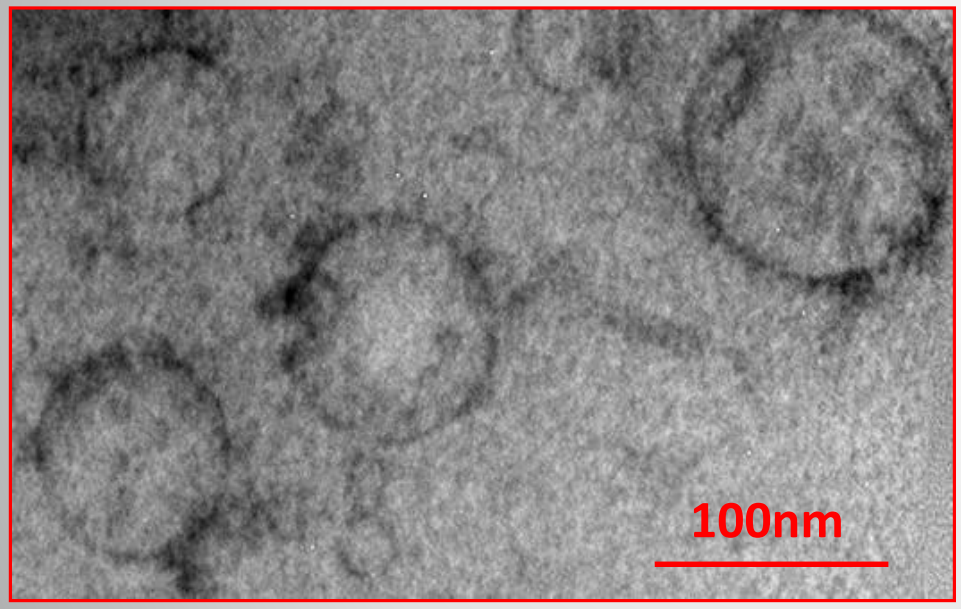
Extracellular Vesicles (EVs) Therapy

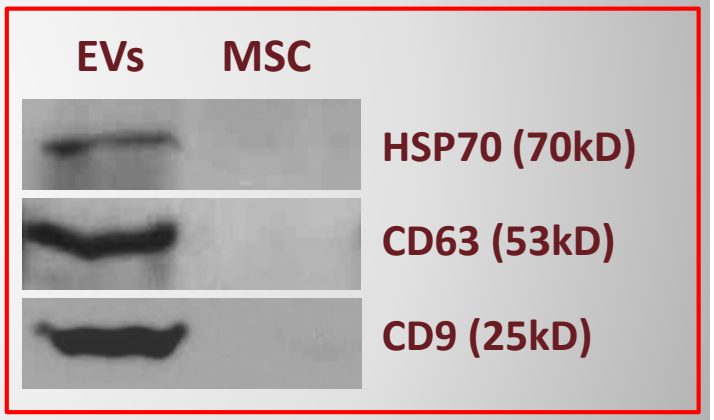
- EVs are ideal therapeutic agents because their complex cargo of proteins and genetic materials. These bioactive molecules participate in multiple biochemical and cellular processes;
- Their bi-lipid membranes can protect their biologically active cargo allowing for easy storage, which allows a longer shelf-life and half-life in patients;
- Their secretion profiles can be altered by preconditioning or genetic manipulation of the parent MSCs;
- Their membranes can be modified to enhance cell-specific targeting;
- Although EV-based therapy cannot replace lost cells, it can prevent or delay the loss of cells, and recruit stem cells.

Intracellular Vesicles



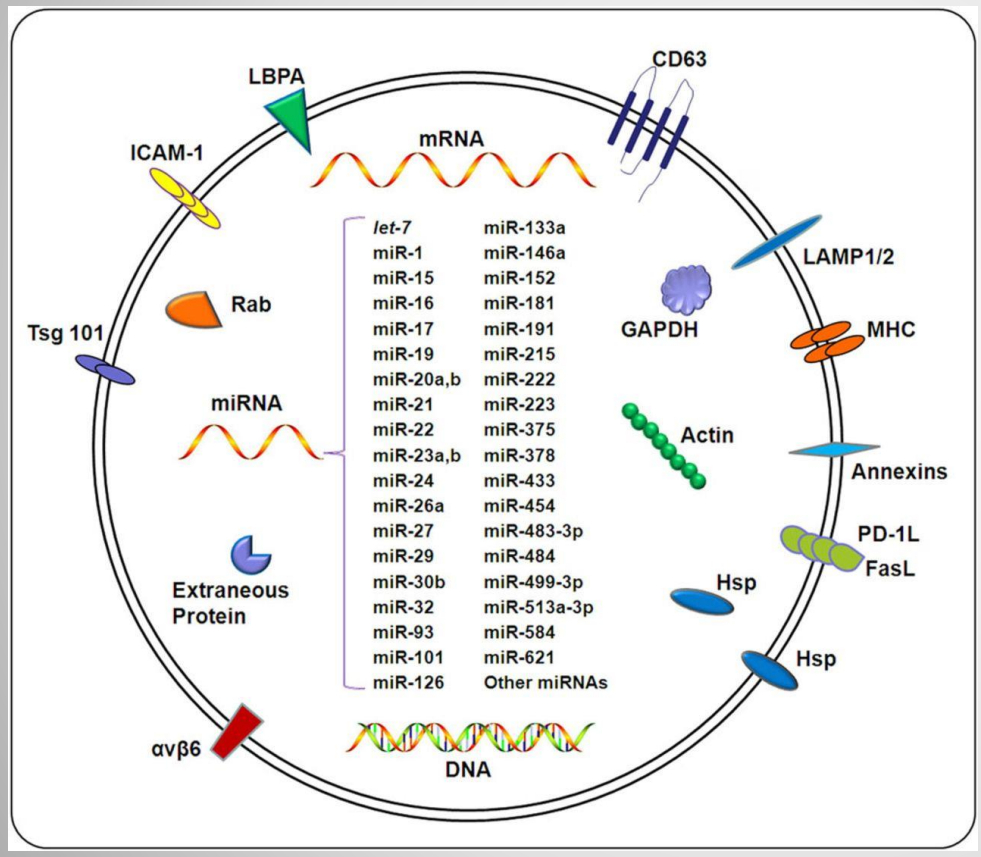
Characterization of Exosomes





Yu, *et al.* doi:10.1371/journal.pone.0073304.g008

Exosomes as the Carriers



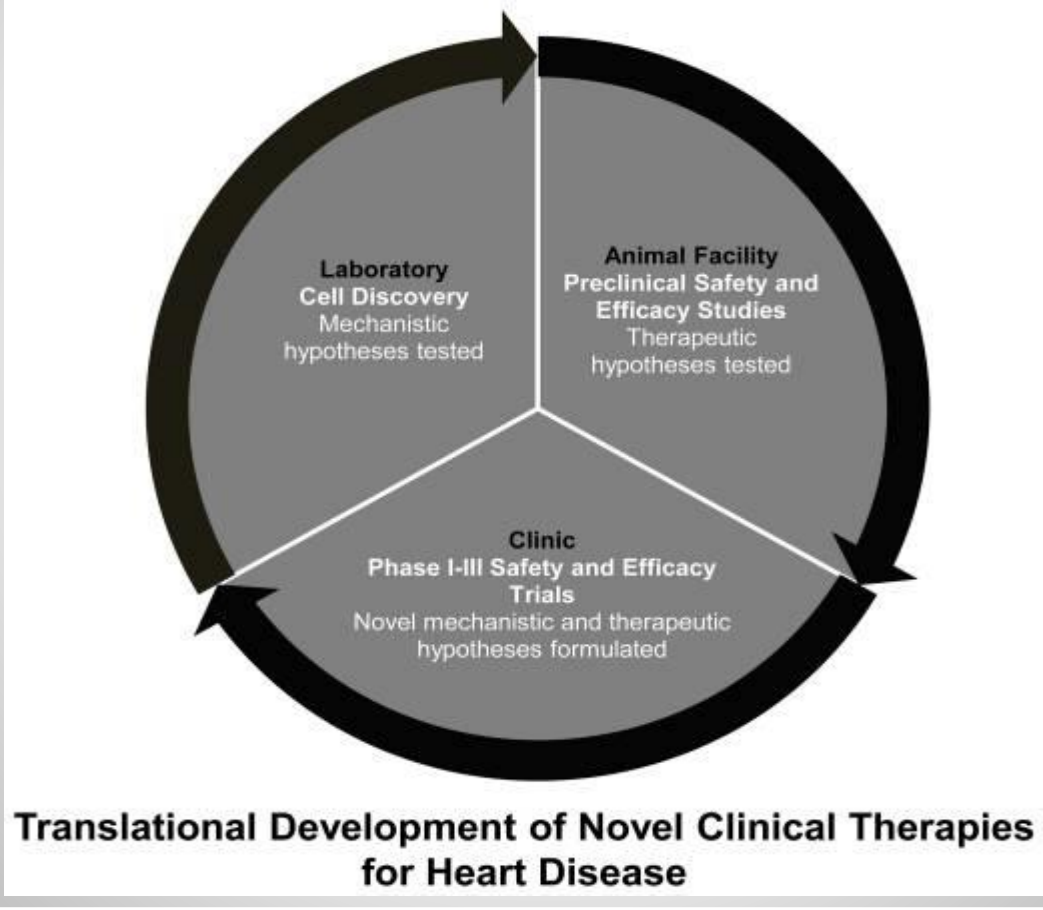
- **mRNA**
- **DNA**
- **microRNA**
- **Protein**

  Enzymes
  Growth factors
  Cytokines
  Cytoskeletal proteins
  Transmembrane proteins

Exosomes carry specific proteins and miRNAs from their parental cell type.

Waldenström A , and Ronquist G *Circulation Research*. 2014;114:315-324

Three Research Stages



Translational Development of Novel Clinical Therapies for Heart Disease

Thank you very much!