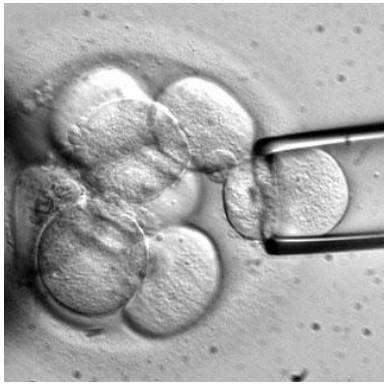
# Introduction of Stem Cell Therapy



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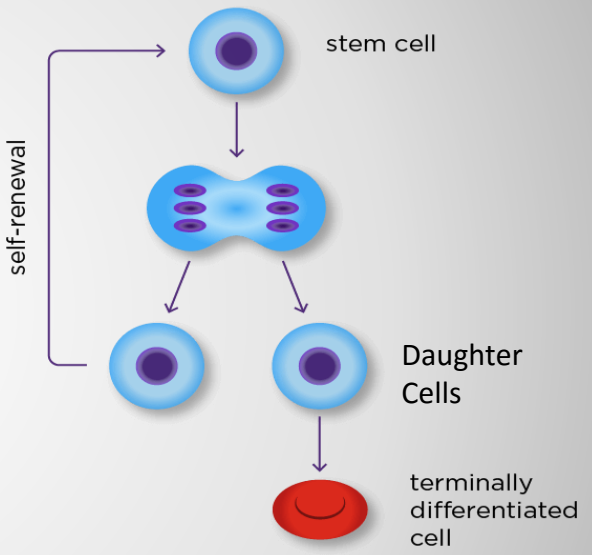
University of Cincinnati

# Stem Cells

# Stem Cells

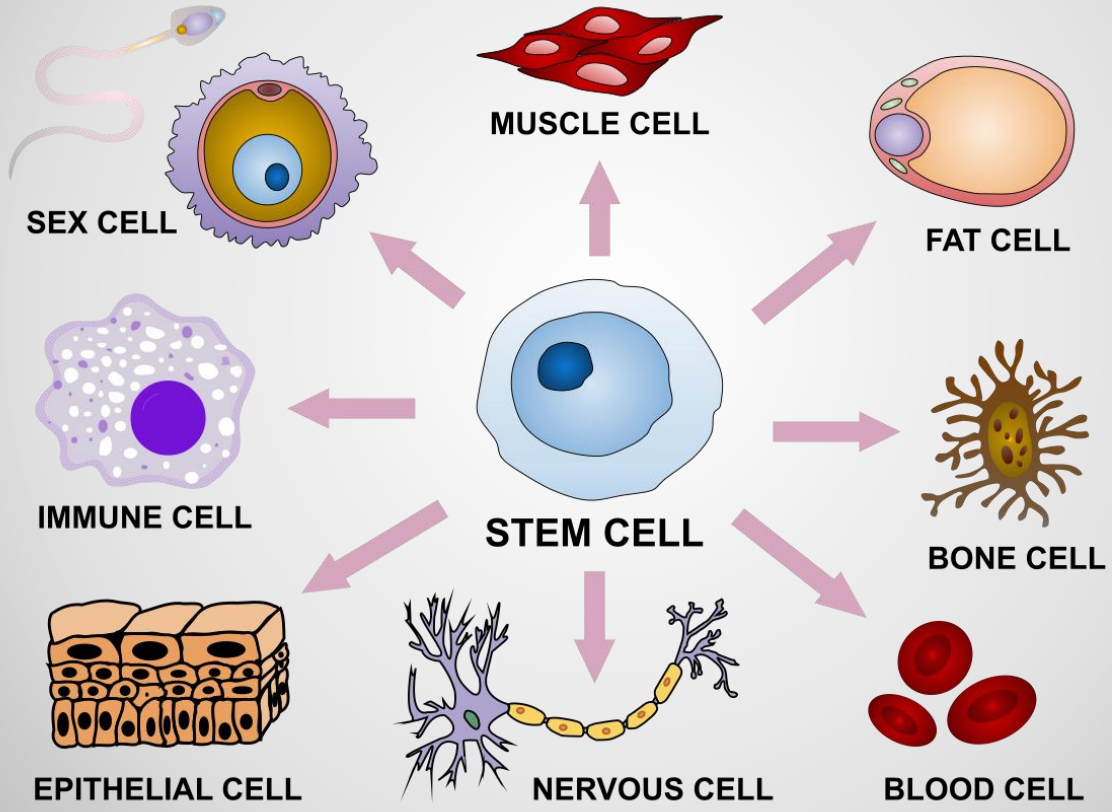
Stem cells are the cells that do not have a specific role under the right conditions in the body.

They can renew themselves by dividing, even after they have been inactive for a long time. Stem cells divide to form more cells called daughter cells. These daughter cells become new stem cells (self-renewal).




The daughter cells also have the ability to develop into specialized cell types (terminally differentiated) with a more specific function, such as blood cells, brain cells, heart muscle cells or bone cells. No other cell in the body has the natural ability to generate new cell types.

# Differentiation of Stem Cells



# Differentiation Potential

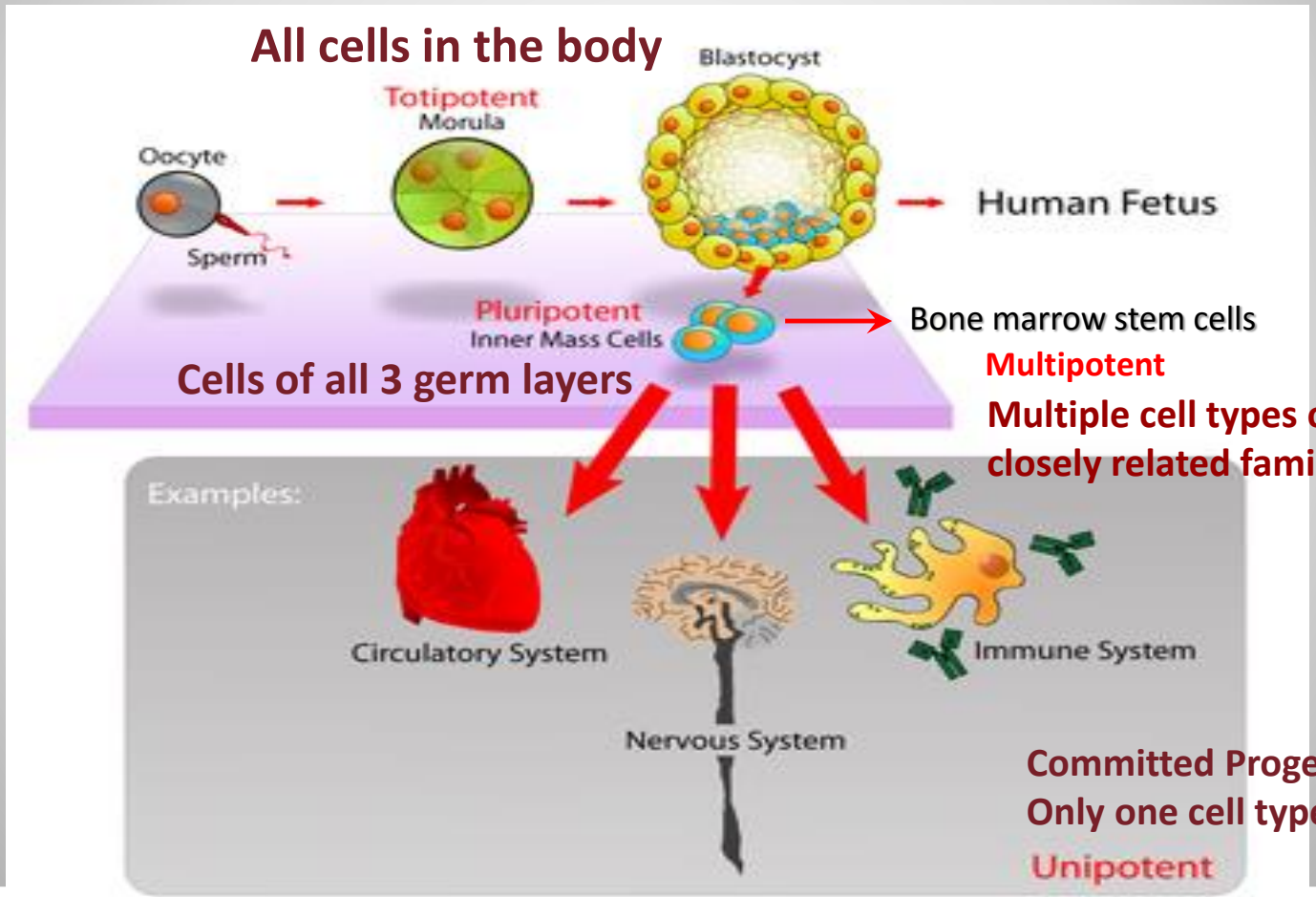
**Totipotent** can differentiate into all possible cell types. These cells are produced from the fusion of an egg and sperm cell, e.g. zygote formed at egg fertilization.

**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



# Types of Stem Cells

Three types of stem cells may be considered to be used in stem cell therapy:

1) Embryonic stem cells;
2) Adult stem cells;
3) Induced pluripotent stem (iPS) cells.

# Embryonic Stem Cells

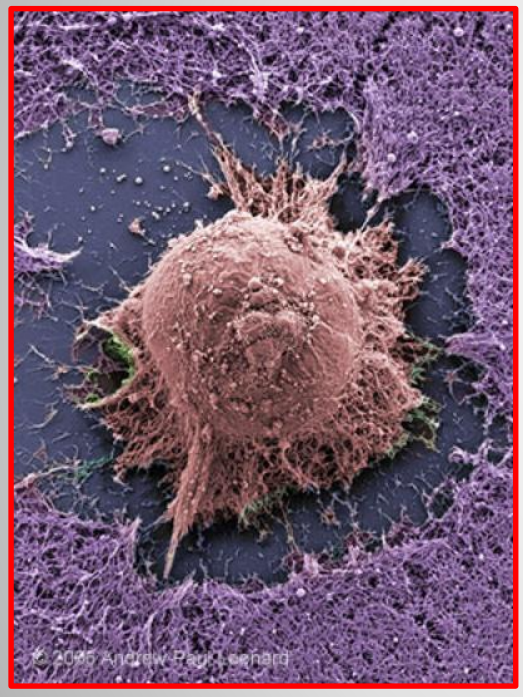
- Embryonic stem (ES) cells come from embryos that are three to five days old. At this stage, an embryo is called a blastocyst and has about 150 cells.
- These cells are pluripotent. They can divide into more stem cells or can become any type of cell in the body. This versatility allows embryonic stem cells to be used to regenerate or repair diseased tissue and organs.

Derivation of ES cells from mouse blastocysts (1981)

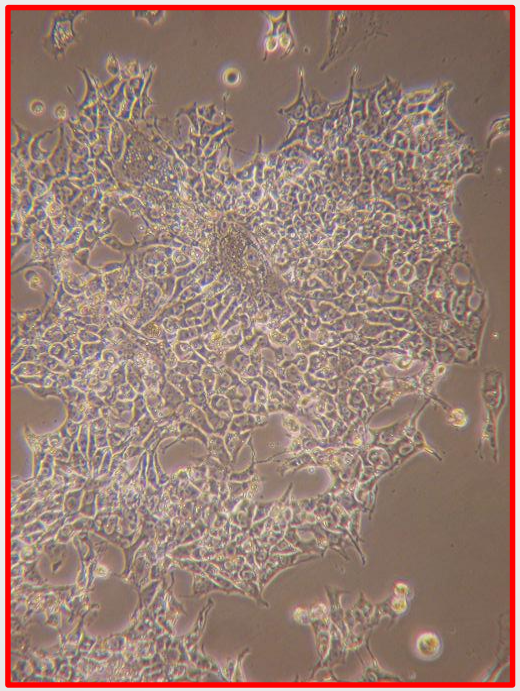
Human ES cells (Thomson, 1998)

Blastocysts produced by in vitro fertilization (IVF)

# Embryonic Stem Cells




Single Cell



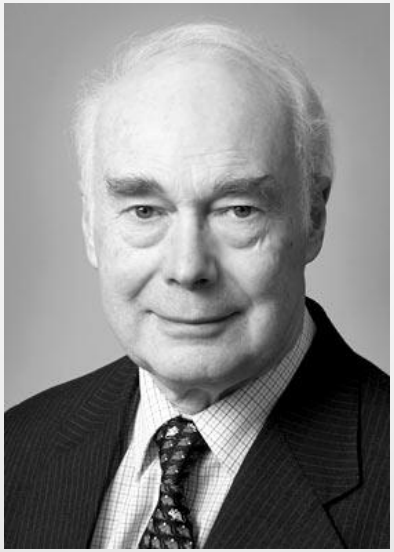
Culture (early)

Culture (later - colony)

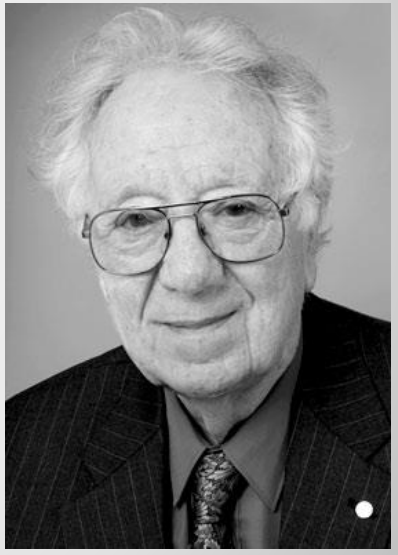
# The Nobel Prize in Physiology or Medicine 2007

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

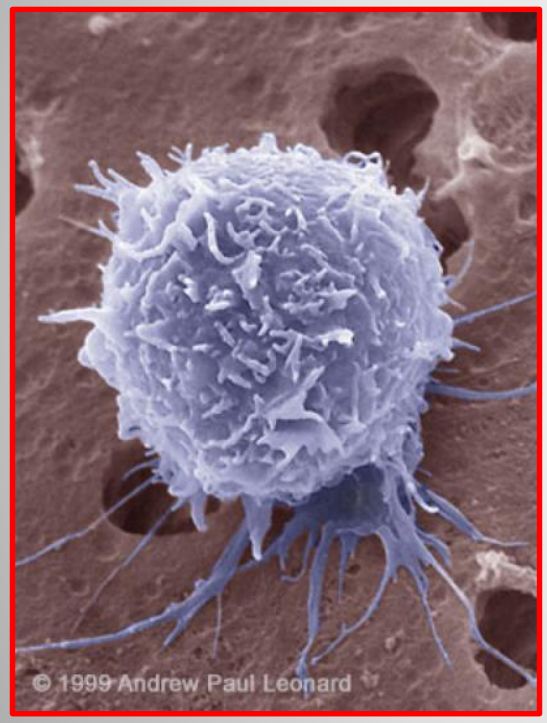
# Adverse Effect of ES Cells

1. The ES cell controversy is the ethical debate centered only with research involving the creation, usage, and destruction of human embryos.
2. The embryos being used in embryonic stem cell research come from eggs that were fertilized at in vitro fertilization clinics but never implanted in a woman's uterus.
3. The major concern with the possible transplantation of ES cells into patients as therapies is their ability to form tumors including teratoma.

# Adult Stem Cells

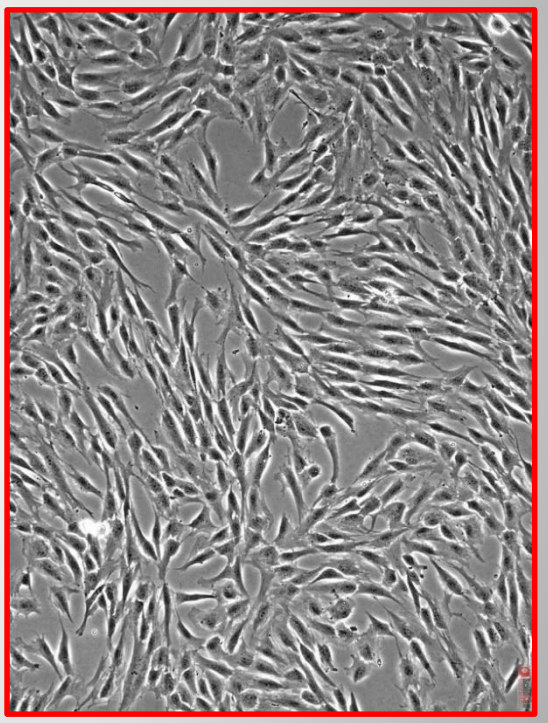
- Adult stem cells are undifferentiated cells in most adult tissues or organs, such as bone marrow or fat tissue. They remain in a quiescent or non-dividing state for years until activated by disease or tissue injury.
- They can divide or self-renew to generate a range of cell types from the originating organ to replenish dying cells and regenerate damaged tissues or entire original organ.
- Most adult stem cells are generally referred to by their tissue origin, but they can also transdifferentiate into other cell types. For instance, stem cells residing in the bone marrow could give rise to blood cells, but also create bone or heart muscle cells.

# Adult (Bone Marrow) Stem Cells




Single cell


culture (early)



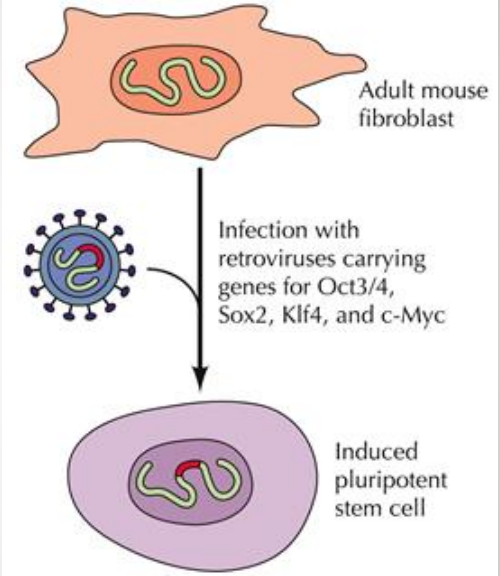
Culture (later)

# Sources of Adult Stem Cells

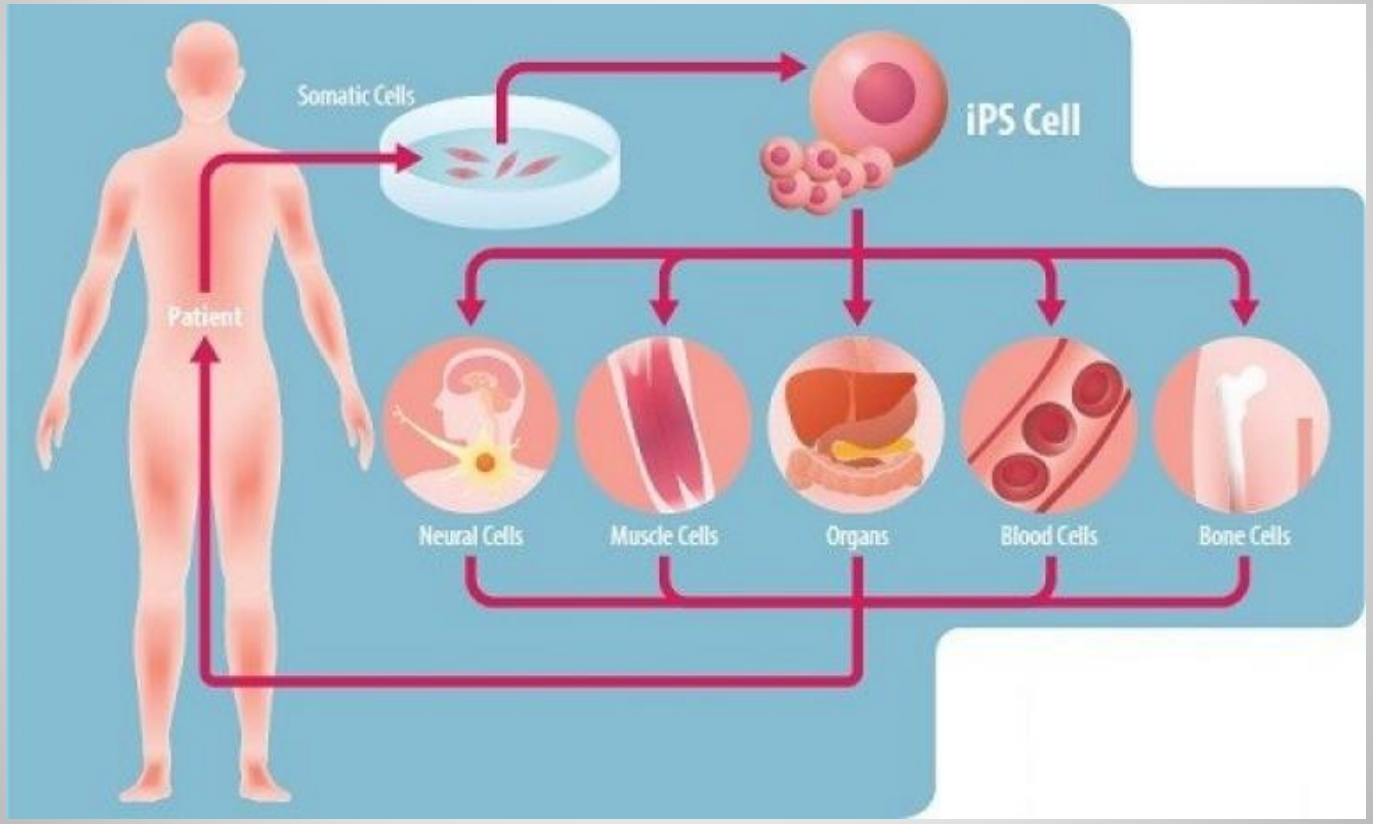
1) The bone marrow stroma contain mesenchymal stem cells (MSCs).
2) Adipose tissue (lipid cells), which requires extraction by liposuction.
3) Blood is drawn from the donor (similar to a blood donation), passed through a machine that extracts the stem cells.
4) In addition, stem cells can also be taken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

# Induced Pluripotent Stem (iPS) Cells

iPS cells are developed stem cells from other mature cells, using genetic "reprogramming" techniques. This new technique may allow researchers to use reprogrammed cells instead of embryonic stem 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.



# Differentiation of iPS Cells



# 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 (Oct3/4, Sox2, Klf4, and c-Myc). 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.

# Aims of Stem Cell Research

- Increase understanding of how diseases occur by watching stem cells mature into cells in bones, heart muscle, nerves, and other organs and tissues.
- Generate healthy cells to replace diseased cells (regenerative medicine). Stem cells can be guided to become specific cells that can be used to replace and/or repair diseased or damaged tissues in people.
- Test new drugs for safety and effectiveness. Before using investigational drugs in people, researchers can use some types of stem cells to test the drugs for safety and quality. This type of testing will most likely have a direct impact on drug development.

# Stem Cell Therapy

Replacement of damaged cells to regenerate tissues and organs by stem cells or the therapeutic molecules carried by stem cells.

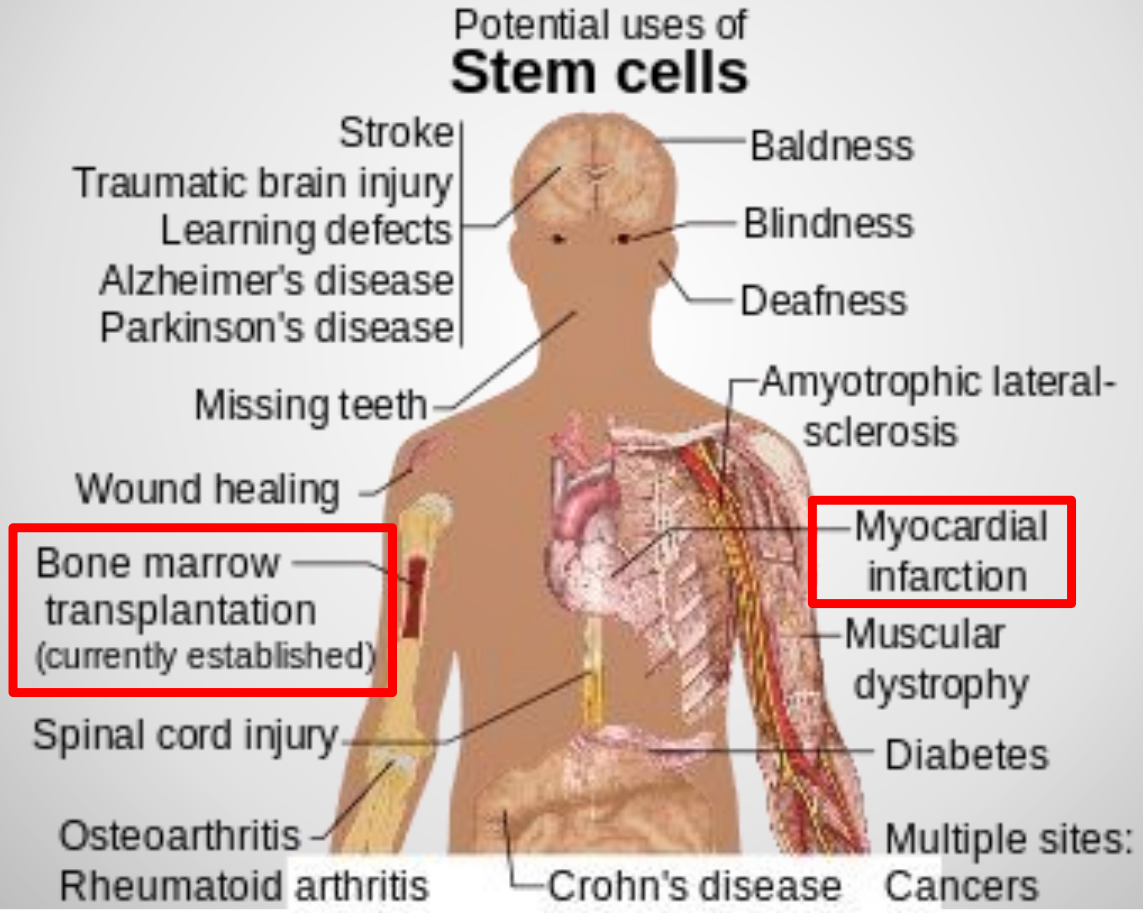
# Stem Cell Therapy

Transplantation of bone marrow gives patients with radiation damage or blood cancers a chance to make new, healthy blood cells using the donor's bone marrow stem cells.

There is a shortage of donor organs but, stem cells differentiation helps to grow a specific tissue type or organ. For example: in cases of severe burn, when a patient does not have a sufficient amount of undamaged skin for skin graft treatment.

If the person has heart disease, the cells could be injected into the heart muscle. The healthy transplanted heart muscle cells could then contribute to repairing defective heart muscle.

# Stem Cell Therapy




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

# Hematopoietic SC Transplantation

Hematopoietic stem cells (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.

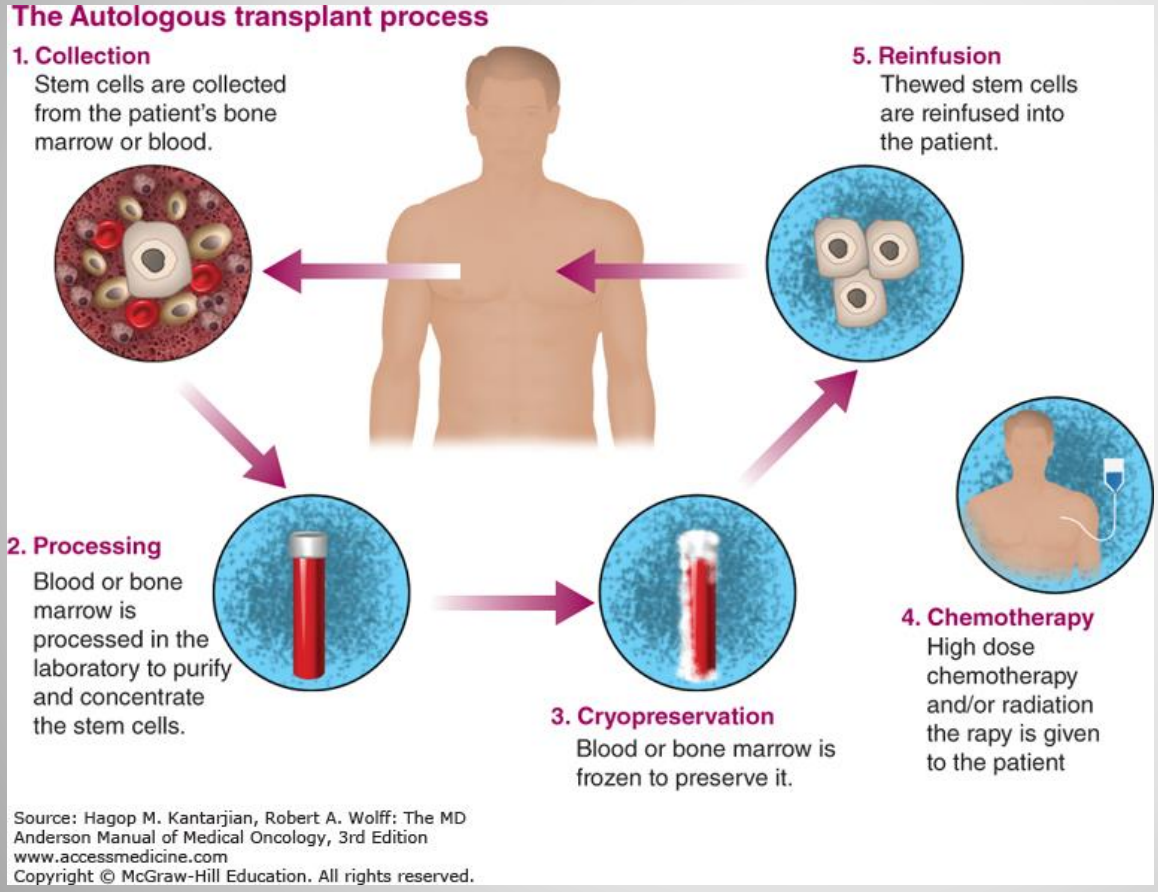
It is most often performed for patients with certain cancers of the blood or bone marrow, such as multiple myeloma or leukemia.

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

# Source of Stem Cells

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

# Source of Stem Cells--Autologous




1. Mobilization
2. Collection of stem cells
3. Procession: to purify and concentrate the stem cells; or culture cells to reprogram to stem cells
4. Cryopreservation
5. (Chemotherapy)
6. Reinfusion

# Source of Stem Cells -- Autologous

Stem cells are collected from patient.

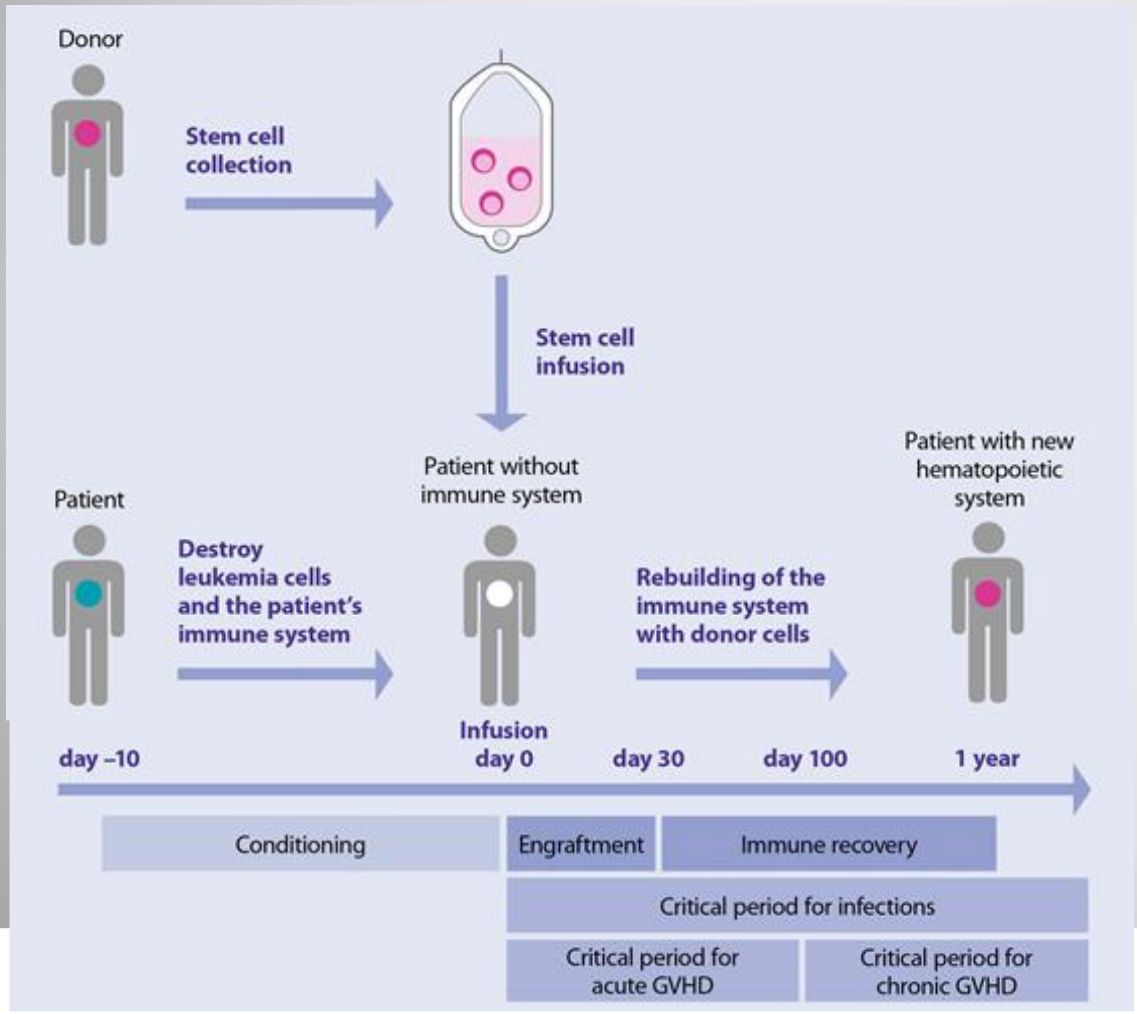
Mobilization of stem cells: patient will receive injections of a medication that makes an increase in production of stem cells.

Stem cells are collected from patient. Several harvesting procedures (between one and five) are usually needed to get enough stem cells.

The patient will undergo high doses of chemotherapy or a combination of chemotherapy and radiation therapy to kill the cancer cells and to get rid of the stem cells that are left in bone marrow.

Reinfusion stem cells back to patient. This process is similar to having a blood transfusion. Now these bone marrow stem cells can make new blood cells.

# Source of Stem Cells -- Allogenic




1. Collecting stem cells from donor (bone marrow or blood)
2. Processing: to purify and concentrate the stem cells; or reprogram these cells
3. Cryopreservation
4. Patient treatment
5. Reinfusion

# Source of Stem Cells -- Allogenic

Stem cells are collected from donor's bone marrow or bloodstream.

Several harvesting procedures (between one and five) are usually needed to get enough stem cells.

The patient will undergo high doses of chemotherapy or a combination of chemotherapy and radiation therapy to kill the cancer cells and get rid of the stem cells that are left in bone marrow.

Stem cells collected will be infused to patient. Meanwhile, the patient will be treated with immune regulators.

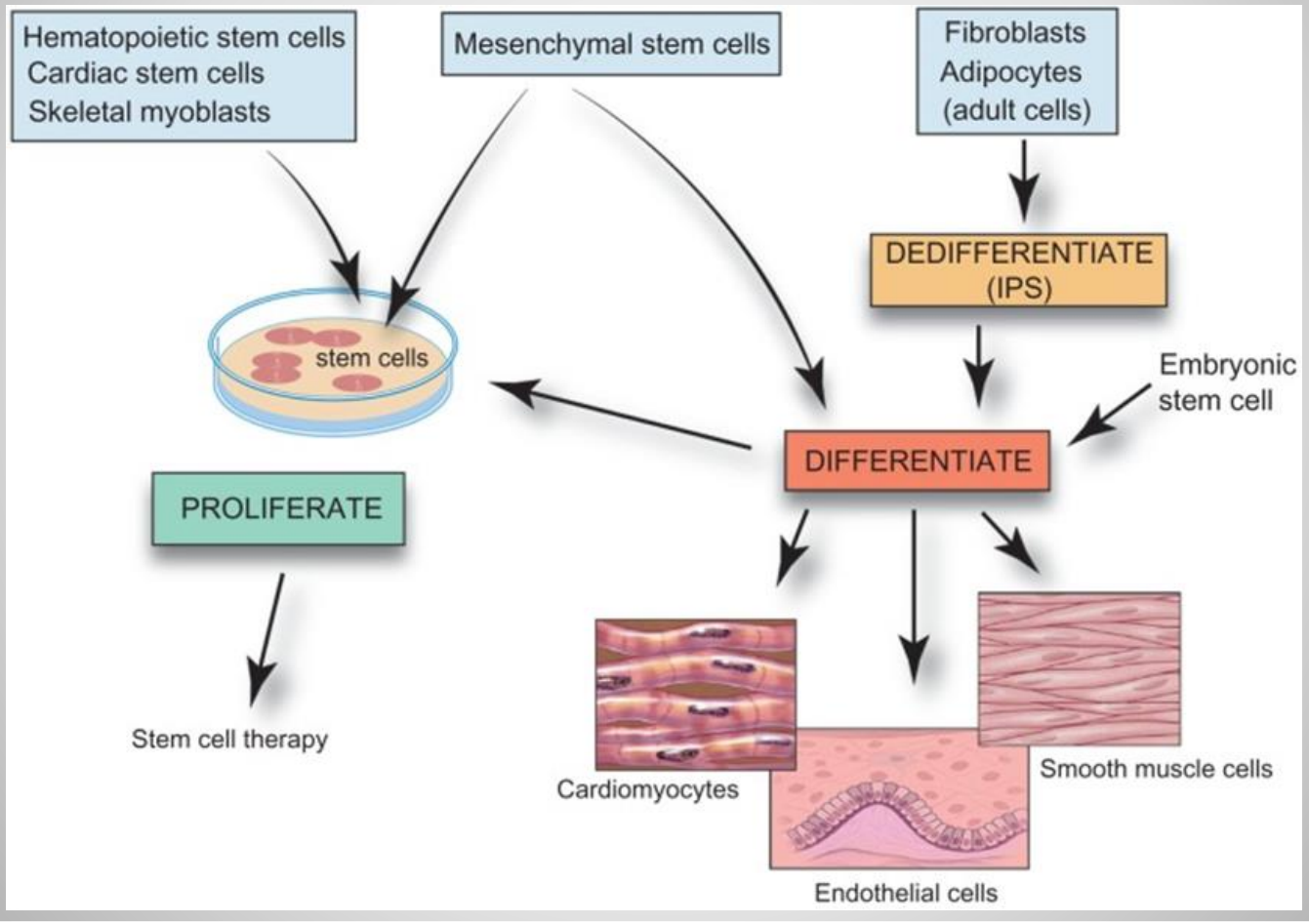
# Myocardial Regeneration

Patient's heart contains damaged tissue, doctors might be able to stimulate healthy tissue to grow by transplanting laboratory-grown stem cells into the person's heart. This could cause the heart tissue to renew itself.

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 or iPS cells.

Other types of adult stem cells may also be used, such as adipose-derived stem cells.

# Expansion of Stem Cells

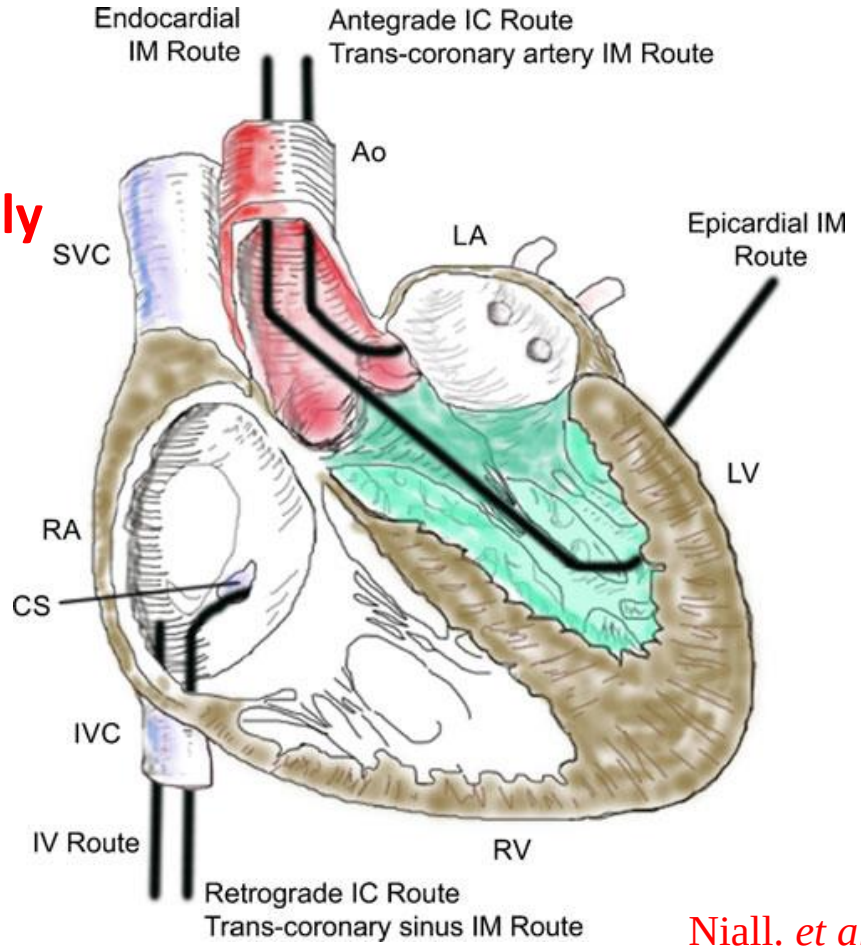



Hoover-Plow. *et al.* *Vasc Health Risk Manag*. 2012; 8: 99–113.

# Administration of Stem Cells

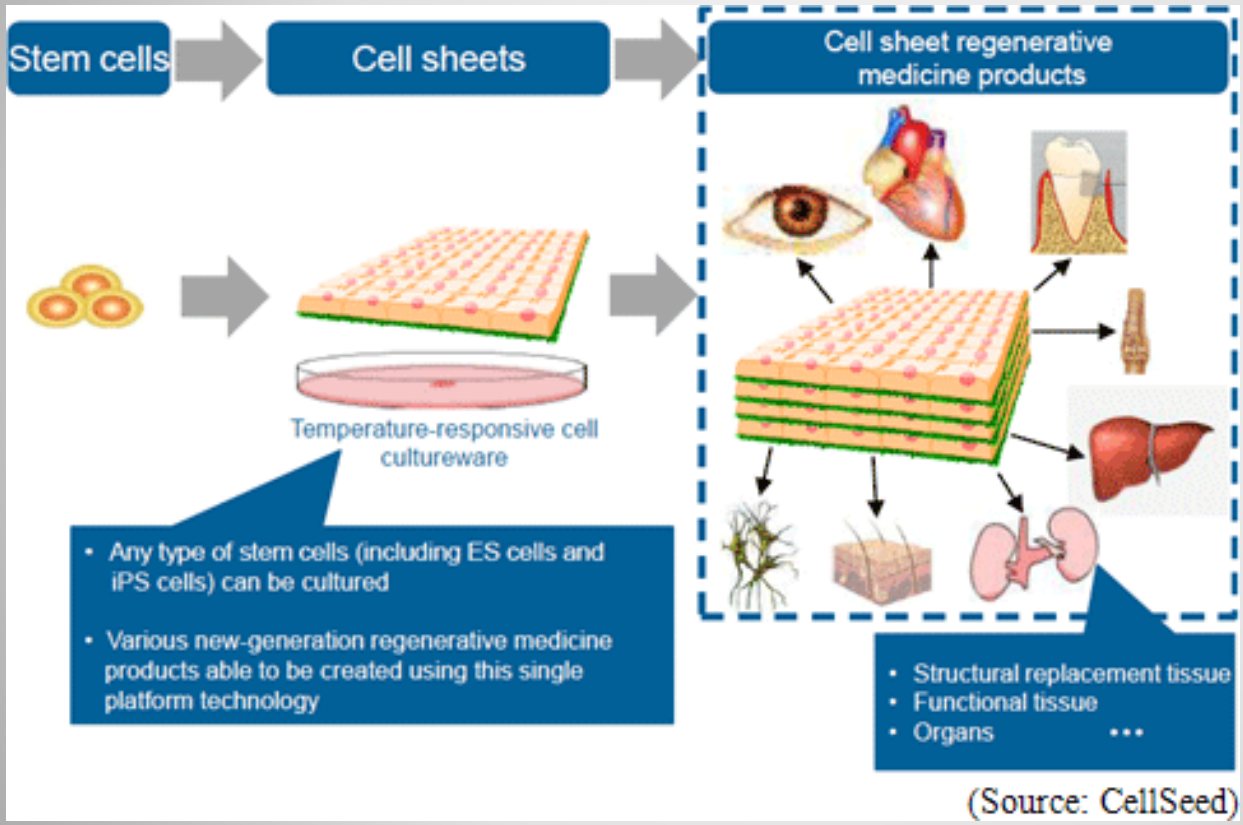
1. **Introduce locally**

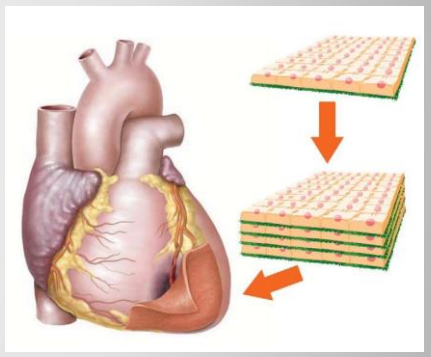
2. **Administration through IV**




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

# Stem Cell Patch



# Effect of Stem Cell Therapy

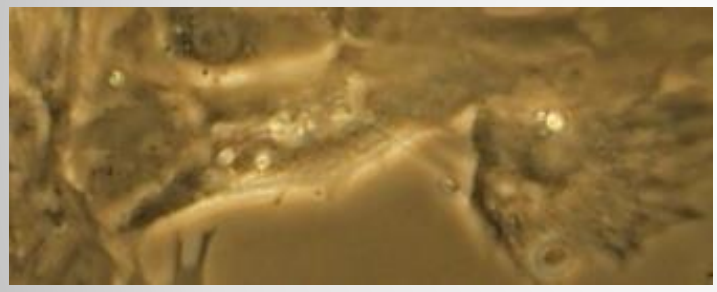
The effect of stem cells includes: protecting risky cells, replacing lost cells, regenerating damaged tissues or organs, stimulating growth of new blood vessels to repopulate damaged tissue.

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.

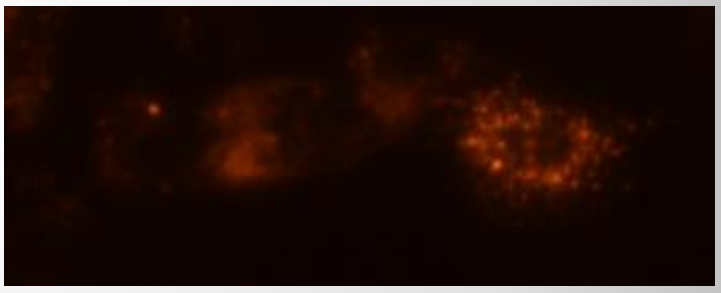
# Differentiation of Stem Cells into CM

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

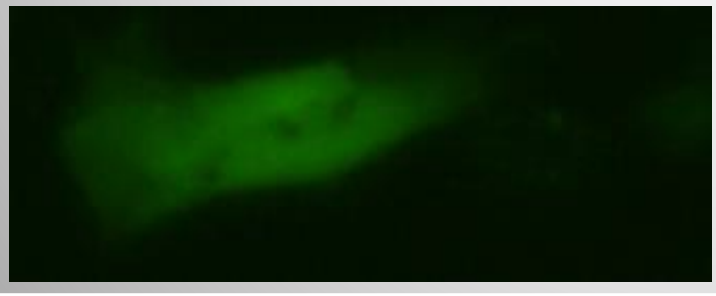
**Contrast (MSC + CM)**



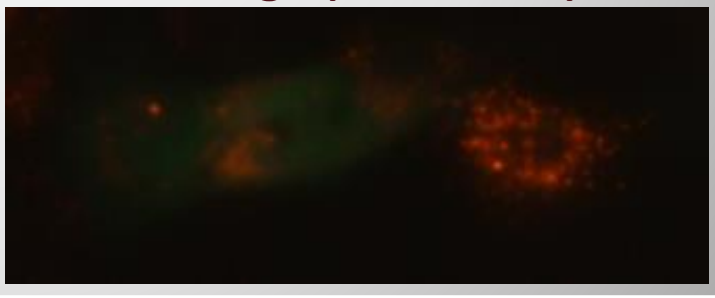
**CM (red)**



**MSC (Green)**



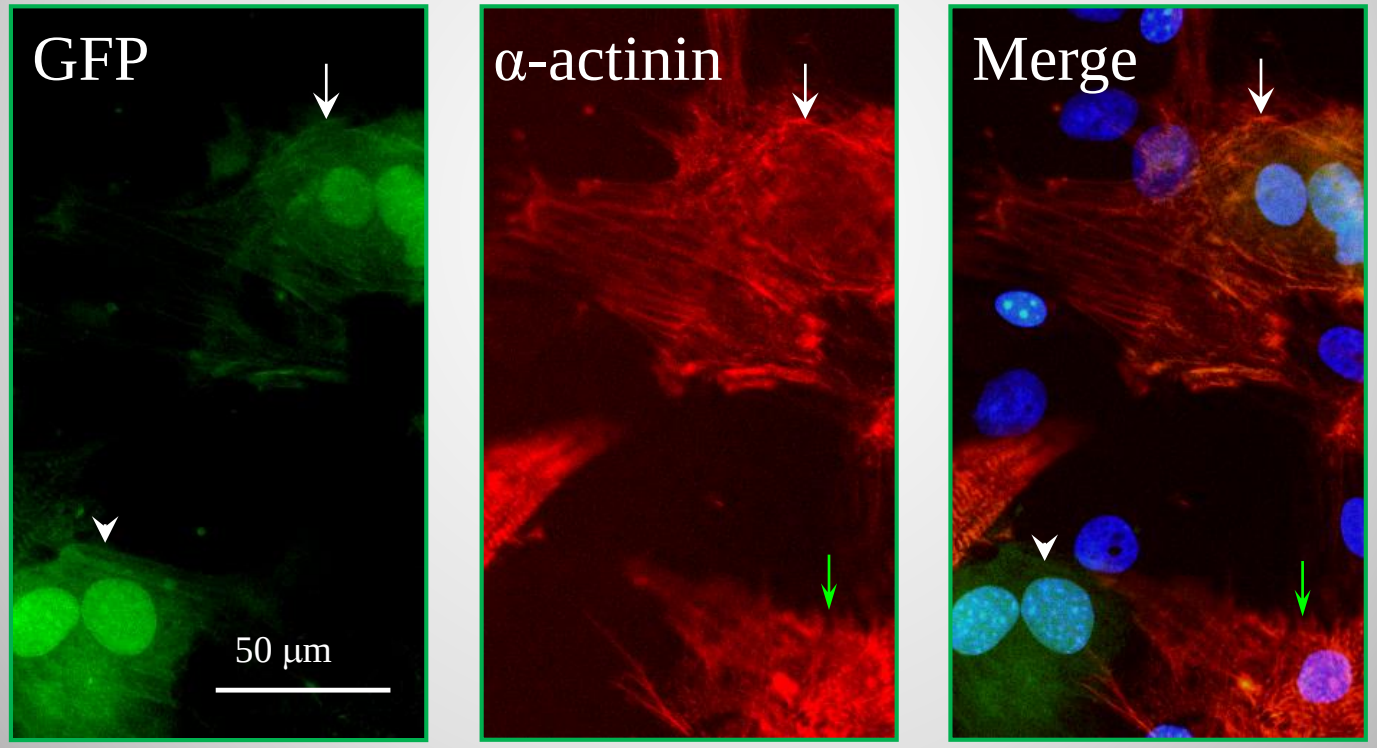
**Merge (MSC + CM)**



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

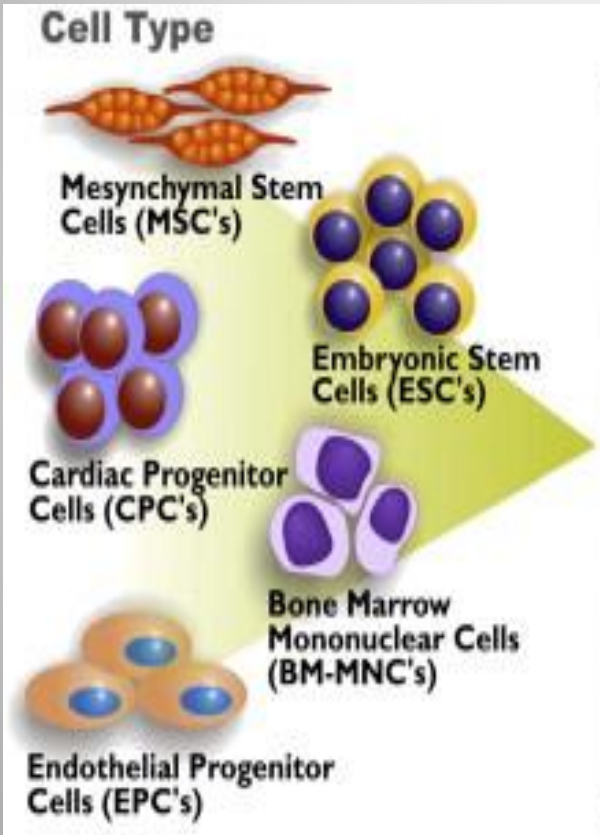
# Immuno-staining of Co-cultured Cells

**Green—stem cells; Red---cardiomyocytes**




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

# Stem Cells Secrete Paracrine Factors



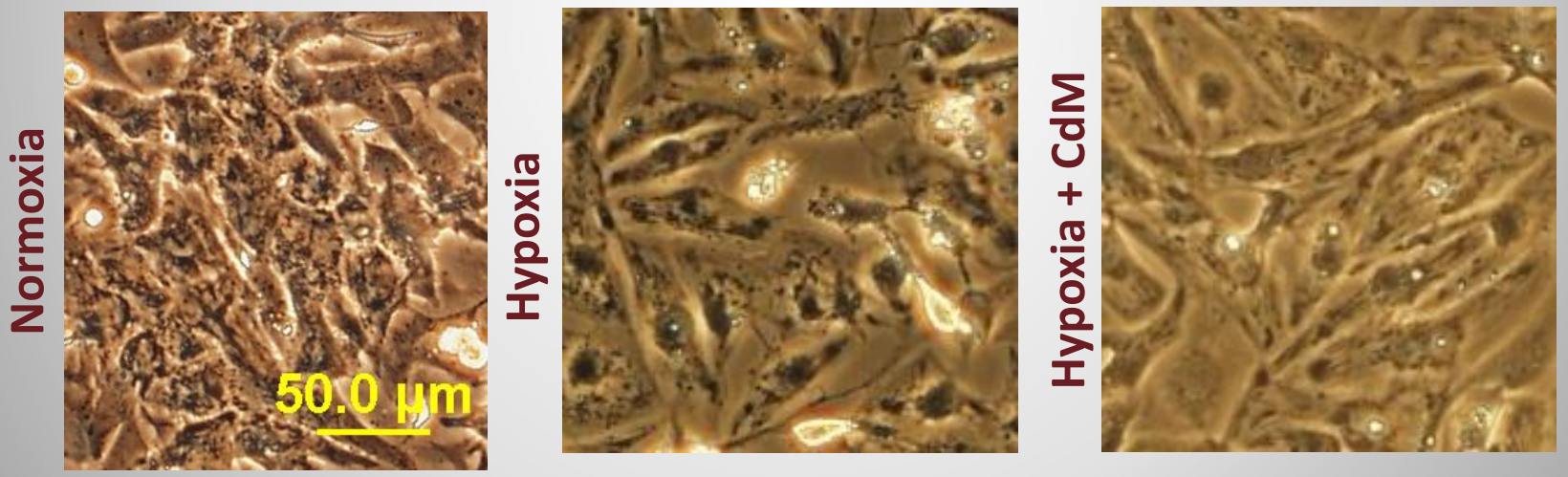

| Cell Type | Paracrine Mediators | Mechanisms of Action |
|---|---|---|
| Mesynchymal Stem Cells (MSC's) | SFRP2, VEGF, HGF, STC-1 SDF-1, TGF-β, IGF-1, bFGF, TB-4 | Survival |
| Embryonic Stem Cells (ESC's) | VEGF, bFGF, FGF2, HGF, TB-4 | Contractility |
| Cardiac Progenitor Cells (CPC's) | bFGF, VEGF, 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.

# Secreted Factors Decrease CM Injury

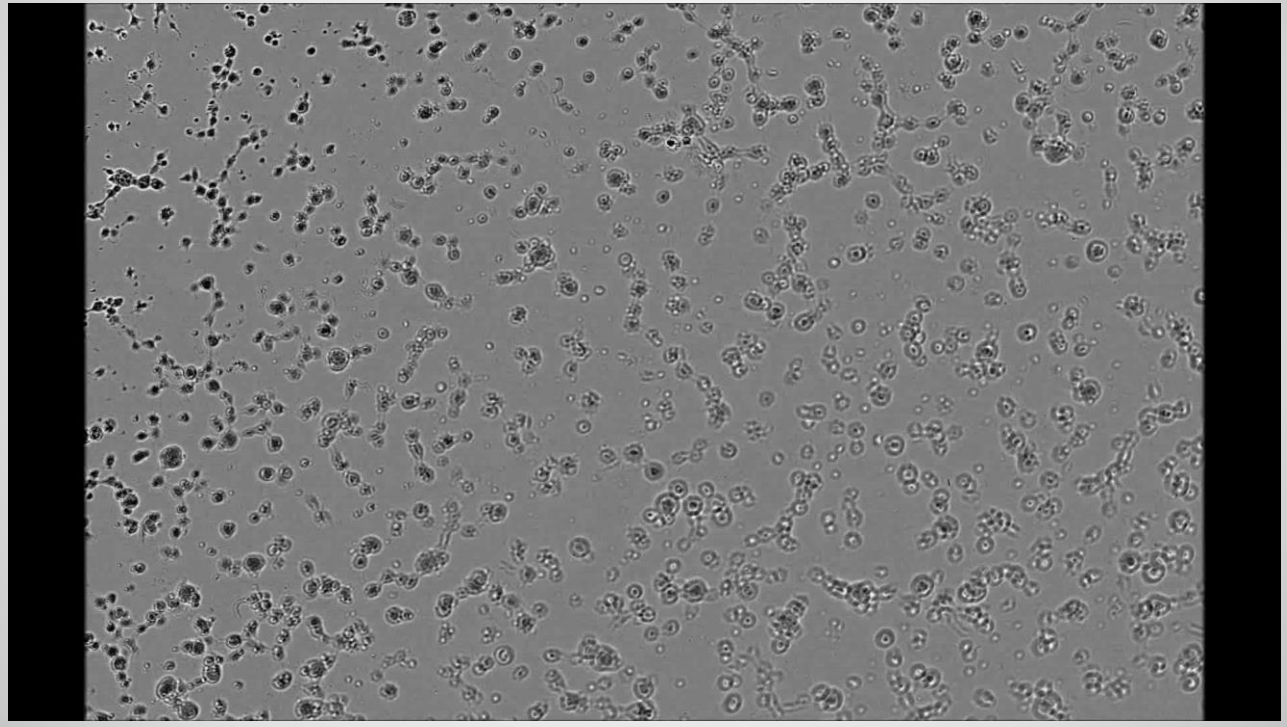
Stem cells secrete cell-protective and angiogenesis factors into the immediate extracellular environment.

(CM= cardiomyocyte; CdM=conditioned Medium)

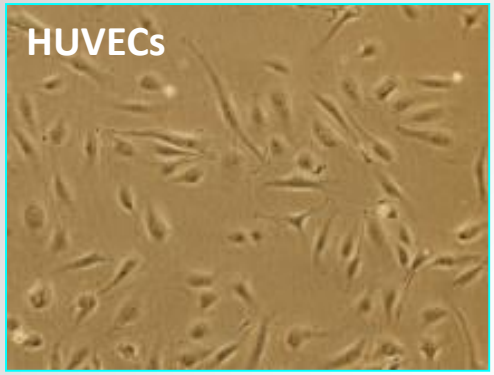


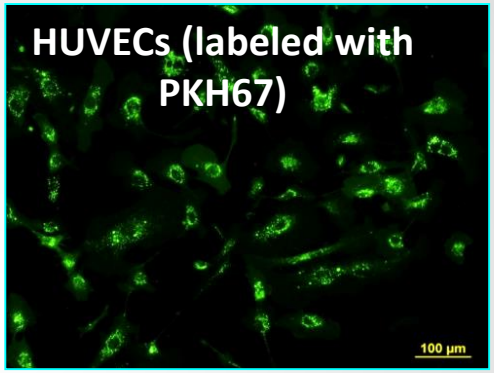

Yu et al.: Plos One

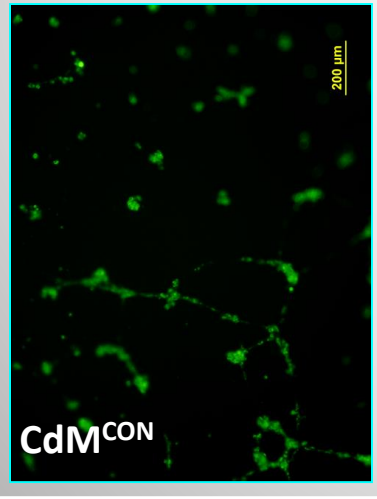
# Capillary-like Tube Formation

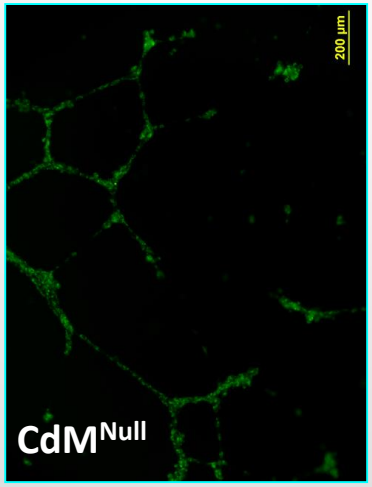


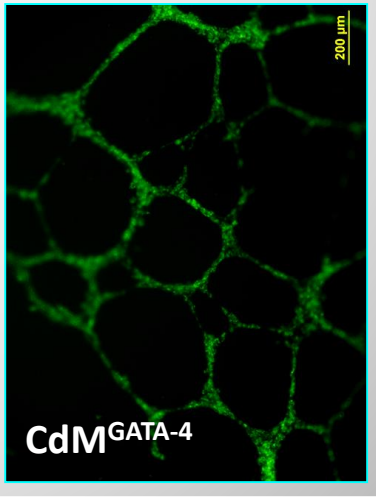
# Secreted Factors Increase Tube Formation




**CdM = Conditioned medium**

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

# Treatment of Type I Diabetes

- Another investigation, published in *Nature Communications* in 2016, has suggested that stem cell therapies could be the basis of personalized diabetes treatment.
- Researchers have successfully produced insulin-secreting cells from stem cells derived from the skin of people with type 1 diabetes.
- The damaged cells in the type 1 diabetes individuals could be replaced with new pancreatic beta cells.
- Some sort of device will be filled with these stem cell-derived beta cells and these device will be placed just beneath the skin of patient. In theory, patients with type 1 diabetes wouldn't need insulin shots anymore.

# 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 |

# Challenges and Solutions

# Current Status of Cell Therapy

- In recent years, clinics have opened that provide stem cell treatments.
- Scientists are carrying out most of the current research in mice or a petri dish.
- Very few stem cell treatments have even reached the earliest phase of a clinical trial. A 2016 study published in *Cell Stem Cell* counted 570 of these clinics in the United States alone. They offer stem cell-based therapies for disorders ranging from sports injuries to cancer.
- However, stem cell therapies are still mostly theoretical rather than evidence-based.

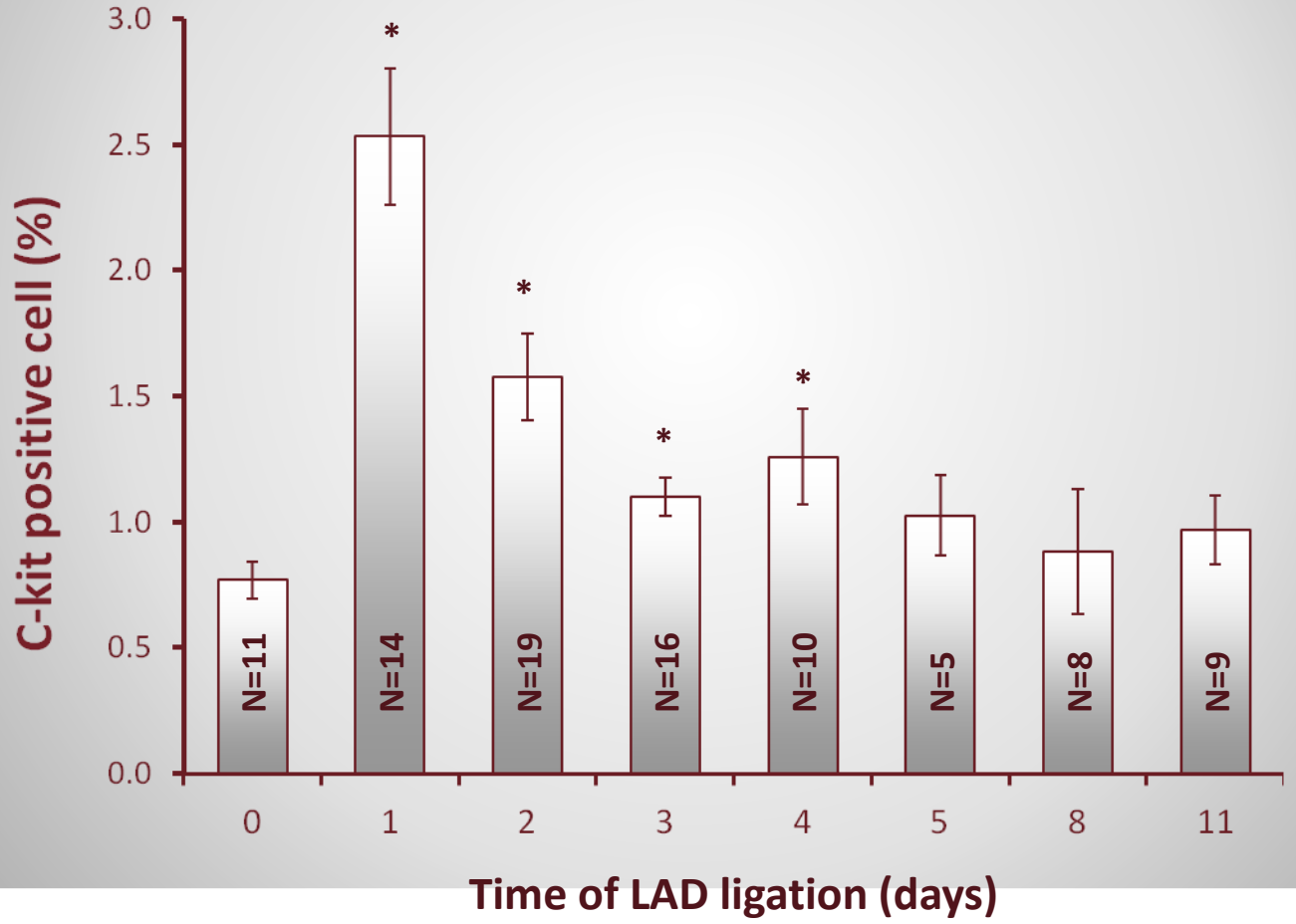
# Limitation of Stem Cell Therapy

1. Human clinical trials have shown that the initial improvement in left ventricular function performance is not sustained in some patients with myocardial infarction.
2. Several studies have sparked intense debate over the ability of stem cells to differentiate into target phenotypes.
3. Poor survival of transplanted stem cells in the acidotic and hypoxic microenvironment of the infarcted myocardium or damaged tissues is another of the major challenges.

# Mobilization of Stem Cells

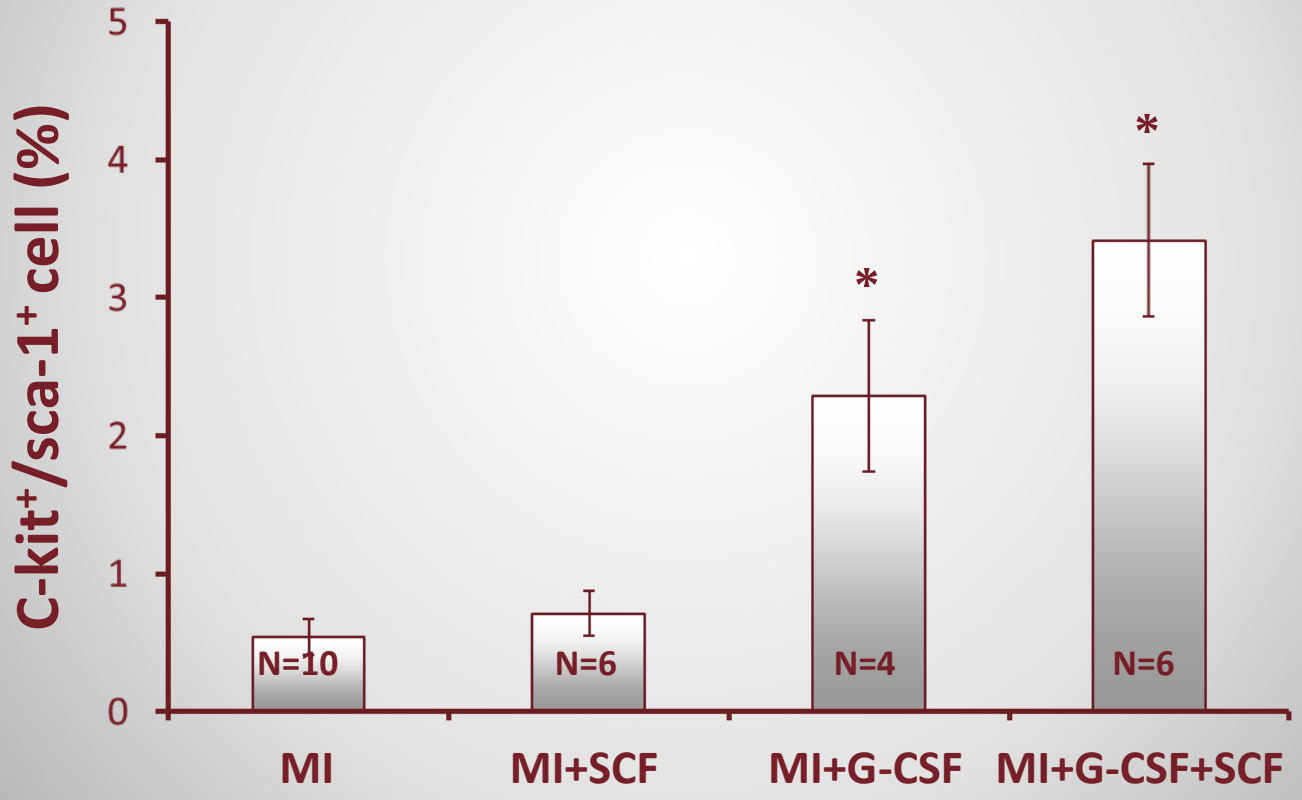
1. The damaged cells or tissues release some cytokines or chemokine which can mobilize stem cells to the damaged area to start repair procession.
2. Some cytokines and chemokines can promote stem cells mobilization.
3. Mobilizing stem cells is an optimal way for repairing the tissues or organs which are difficult to directly transplant stem cells.

# Ischemic Injury Mobilizes Stem Cells




Dai, *et al. JMCC* 2008;44:607-617

# G-CSF Mobilizes Stem Cells

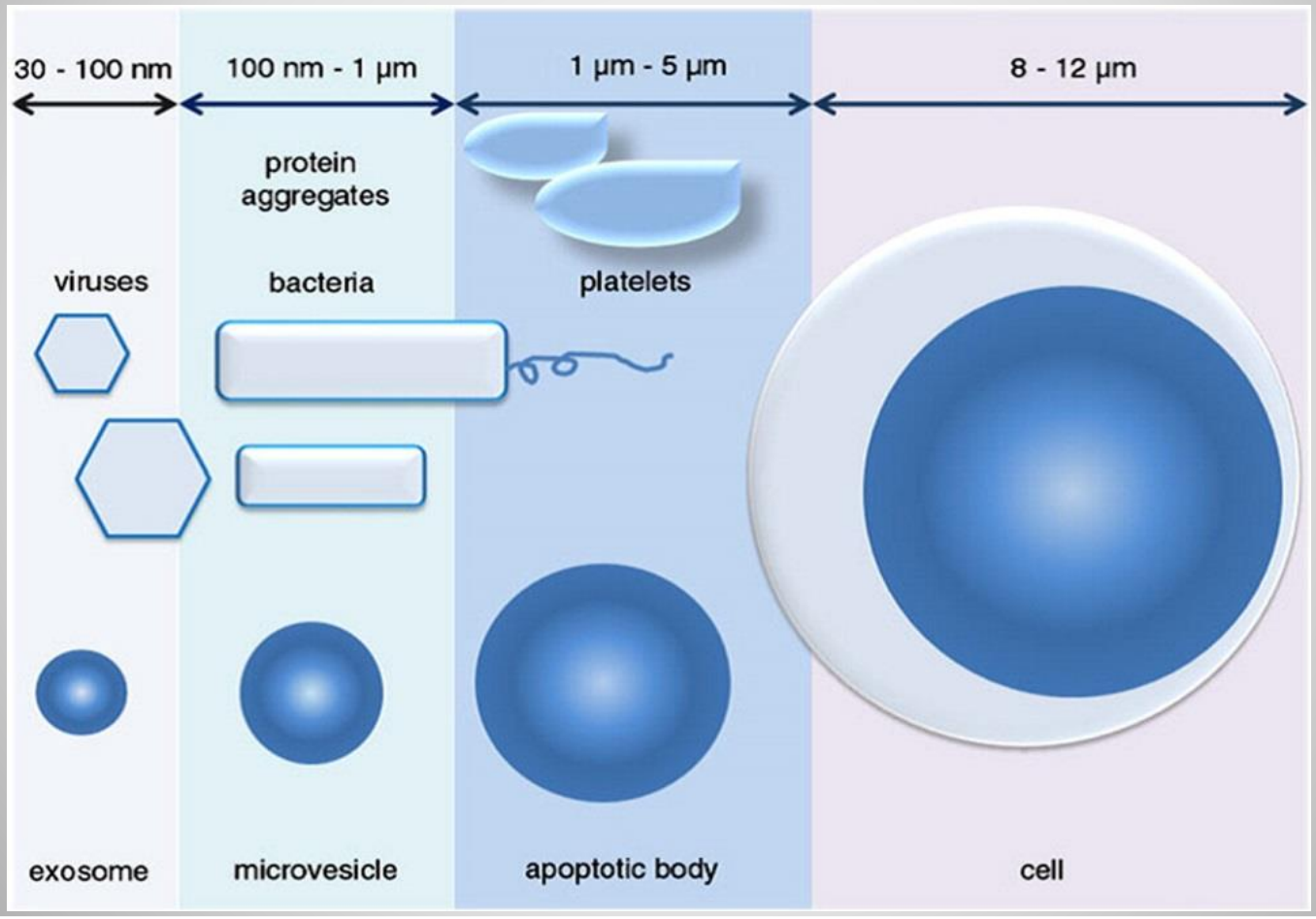



Dai, *et al. JMCC* 2008;44:607-17

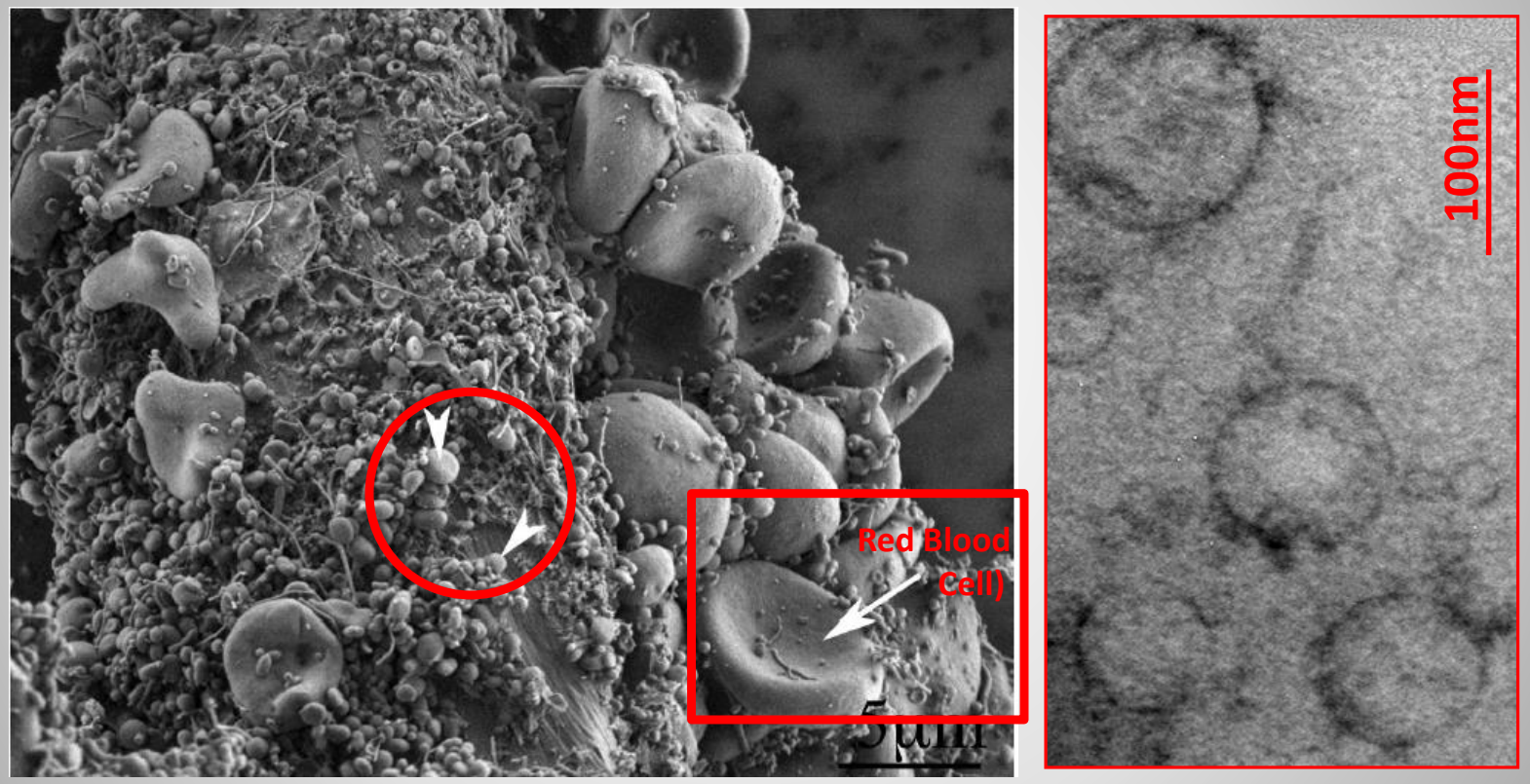
# Release of Extracellular Vesicles (EVs)

- A growing body of evidence demonstrates that the production of EVs is a universal feature of cellular life.
- The vesicles released by stem cells have a lipid bilayer and contain a cell-specific cargo of proteins, lipids, and genetic material.
- These vesicles mediate molecule transfer to neighbouring cells to alert them or to change their behaviour.
- Vesicles can influence the cells which they encounter via different mechanisms to alter their function and behaviour.

# Types of EVs

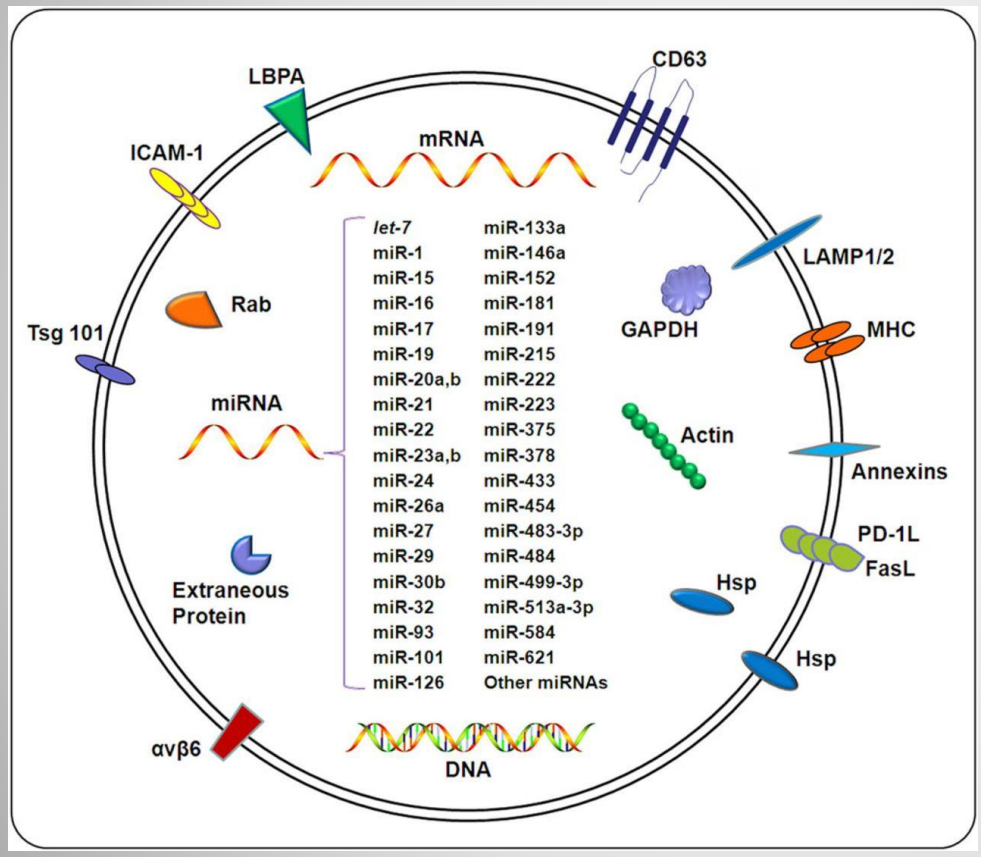

30 - 100 nm
100 nm - 1 µm
1 µm - 5 µm
8 - 12 µm
protein aggregates
viruses
bacteria
platelets
exosome
microvesicle
apoptotic body
cell

# Morphology of Exosomes




Exosomes are the best characterized class of vesicles

# Exosomes as the Carriers



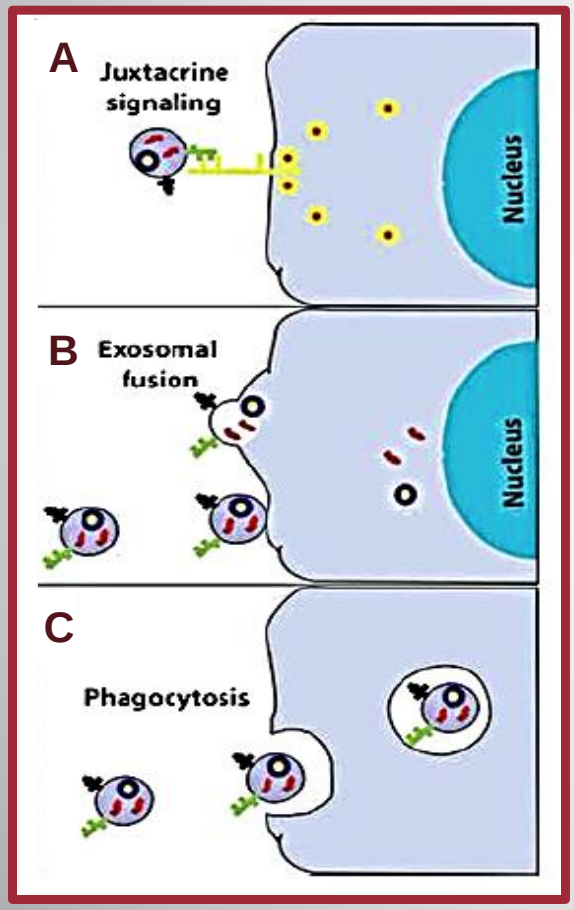

- **mRNA**
- **DNA**
- **microRNA**
- **Protein**

  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

# Exosomes-target Cells Interaction



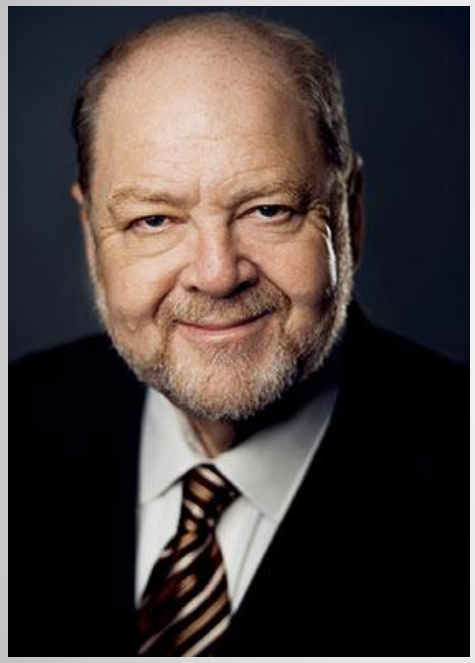

**A** Activate cell surface receptors via protein and bioactive lipid ligand

**B** Fuse with the cellular membrane of the target cells

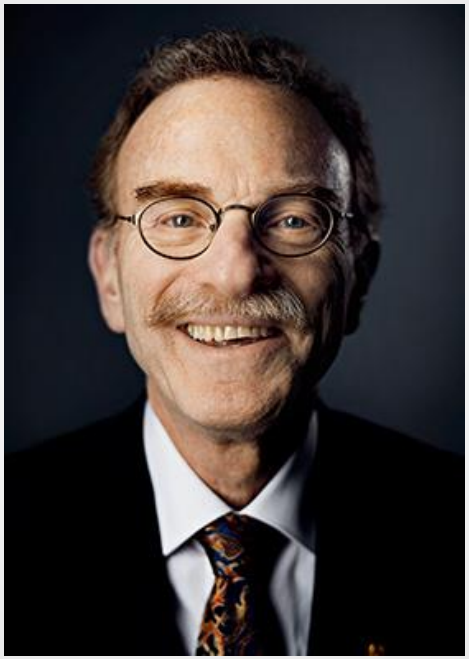
**C** Directly phagocytosis by recipient cells

*J Mol Med* (2013) 91:431–437

# Nobel Prize in Physiology or Medicine 2013



**James E. Rothman**



**Randy W. Schekman**

**Thomas C. Südhof**

***for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells.***

# Therapeutic Potential of Exosomes

1. Exosomes recapitulate regenerating effects of parent stem cells in various animal models and in preclinical studies.
2. Exosomes eliminate many issues associated with the use of whole cells, including the risk of occlusion in microvasculature when systematic administration, recipient immune response, differentiation into inappropriate cell types, and pro-arrhythmic side effects.
3. Exosomes therapeutic potential can be significantly enhanced by pre-conditioning or genetic manipulation of the parent stem cells.
4. In addition, exosomes have a unique rigid lipid membrane that makes them insensitive to freeze-thaw cycles and resistant to bursting in a hypotonic environment. They may be produced in industrial quantities.

***Thank you very much!***