STEM CELLS FOR TISSUE ENGINEERING

What are stem cells?

- **remarkable potential to develop into many different cell types during early life and growth**

- **serve as a sort of internal repair system, dividing essentially without limit to replenish other cells in life**

  When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

What are stem cells?

- All stem cells - regardless of their source (tissue or organ) — have 2 general properties that distinguish them from other cell types:

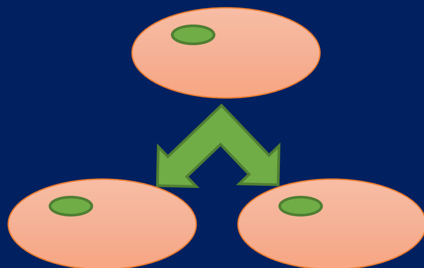
- unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity - self renewal

- under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions and give rise to specialized cell types - differentiation.

In some organs, such as the bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In others, however, such as the pancreas and the heart, stem cells only divide under special conditions.

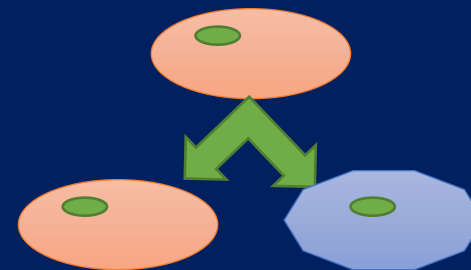
Stem cells stemness

Self-renewal



ability to make identical copies of themselves;
ability to go through numerous cycles of cell division mantaining the undifferentiated state

Differentiation



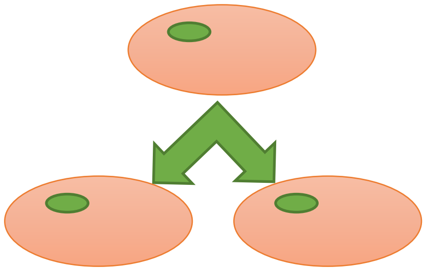
ability to form other cell types of the body - multilineage differentiaton

Interest for cell based therapies including *Tissue Engineering strategies*

Bilateral Cooperation with Industrialised Countries
BEAM - Master Joint Mobility Project
an EU Australian cooperation in Biomedical Engineering
Grant Agreement, 2014-1843/001 001-CPT EU-ICI-ECP
ICVS/3B's Associate Laboratory

Self-Renewal

Self-renewal



ability to make identical copies of themselves;
ability to go through numerous cycles of cell division mantaining the undifferentiated state

- why can embryonic stem cells proliferate long times in the lab without differentiating, but most non-embryonic stem cells cannot ?

- **what are the factors in living organisms that regulate stem cell proliferation and self-renewal**

?

Self-Renewal

- Understand **cell proliferation regulation during**

- **- normal embryonic development**
- **- the abnormal cell division that leads to cancer.**

- grow embryonic and non-embryonic stem cells more efficiently in the laboratory.

- Maintain stem cells unspecialized with specific factors and conditions.
- It has taken scientists many years of trial and error to learn to derive and maintain stem cells in the lab without spontaneously differentiation into specific cell types.

- why can embryonic stem cells proliferate long times in the lab without differentiating, but most non-embryonic stem cells cannot ?

- **what are the factors in living organisms that regulate stem cell proliferation and self-renewal**

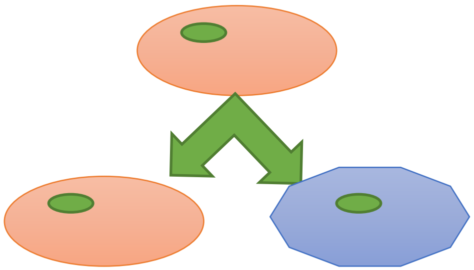
?

Stem cells differentiation

While differentiating, the cell usually goes through several stages, becoming more specialized at each step.

Scientists are just beginning to understand the signals inside and outside cells that trigger each stem of the

Differentiation



ability to form other cell types of the body - multilineage differentiaton

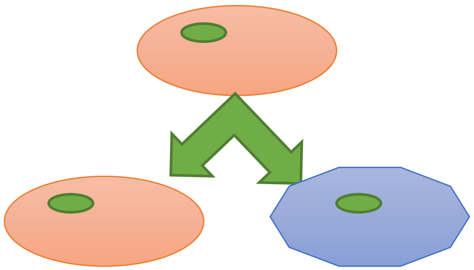
The internal signals are controlled by a cell's genes and carry coded instructions for all cellular structures and functions.

The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the microenvironment. The interaction of signals during differentiation causes the cell DNA to acquire epigenetic marks that restrict DNA expression in the cell and can be passed on through cell division

Stem cells differentiation

- Are the internal and external signals for cell differentiation similar for all kinds of stem cells?
- Can specific sets of signals be identified that promote differentiation into specific cell types?

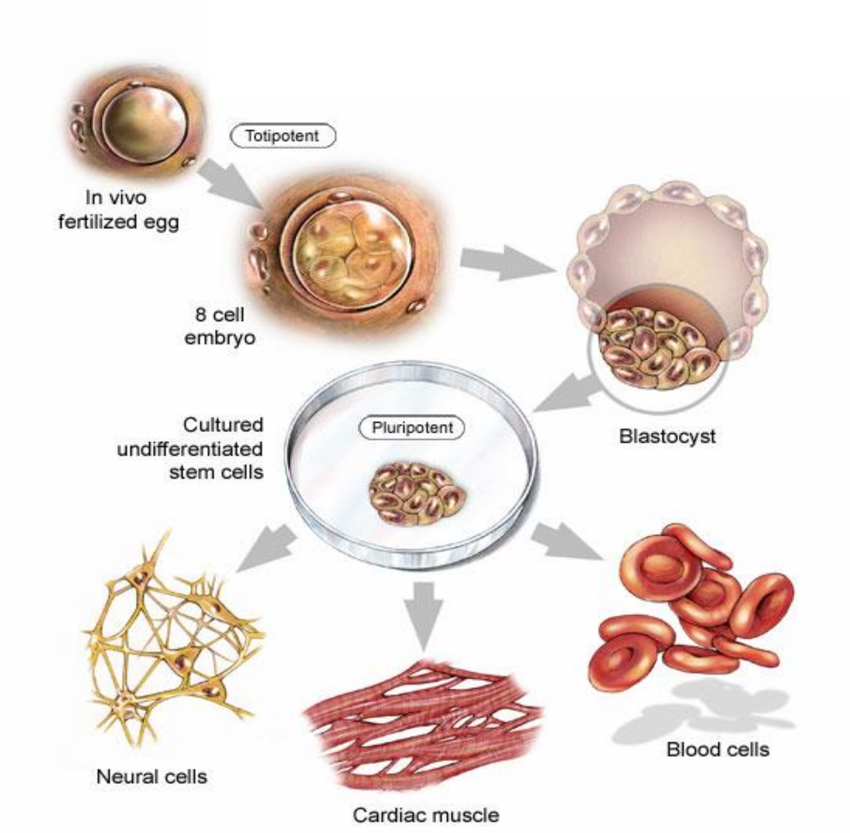
Differentiation



ability to form other cell types of the body

Addressing these questions may lead scientists to find new ways to control stem cell differentiation in the laboratory, thereby growing cells or tissues that can be used for specific purposes such as cell-based therapies, disease models or drug screening.

Stem cells can become everything?- Stem cells "types"



ECTODERM	MESODERM	ENDODERM
• Epidermis of skin and its derivatives (including sweat glands, hair follicles) • Epithelial lining of mouth and rectum • Sense receptors in epidermis • Cornea and lens of eye • Nervous system • Adrenal medulla • Tooth enamel • Epithelium or pineal and pituitary glands	• Notochord • Skeletal system • Muscular system • Muscular layer of stomach, intestine, etc. • Excretory system • Circulatory and lymphatic systems • Reproductive system (except germ cells) • Dermis of skin • Lining of body cavity • Adrenal cortex	• Epithelial lining of digestive tract • Epithelial lining of respiratory system • Lining of urethra, urinary bladder, and reproductive system • Liver • Pancreas • Thymus • Thyroid and parathyroid glands

Totipotent : ability to form an entire organism - fertilized oocyte and the cells after the first cleavage division

Pluripotent: ability to form all three germ layers, including germ cells, but not extra embryonic tissue as placenta and umbilical cord

– ***cell of the inner mass of the blastocyte***; when brought into culture they are called embryonic stem cells

Multipotent: ability to form multiple cell types – for example, mesenchymal stem cells can differentiate into bone, cartilage and fat cells

What are embryonic stem cells?

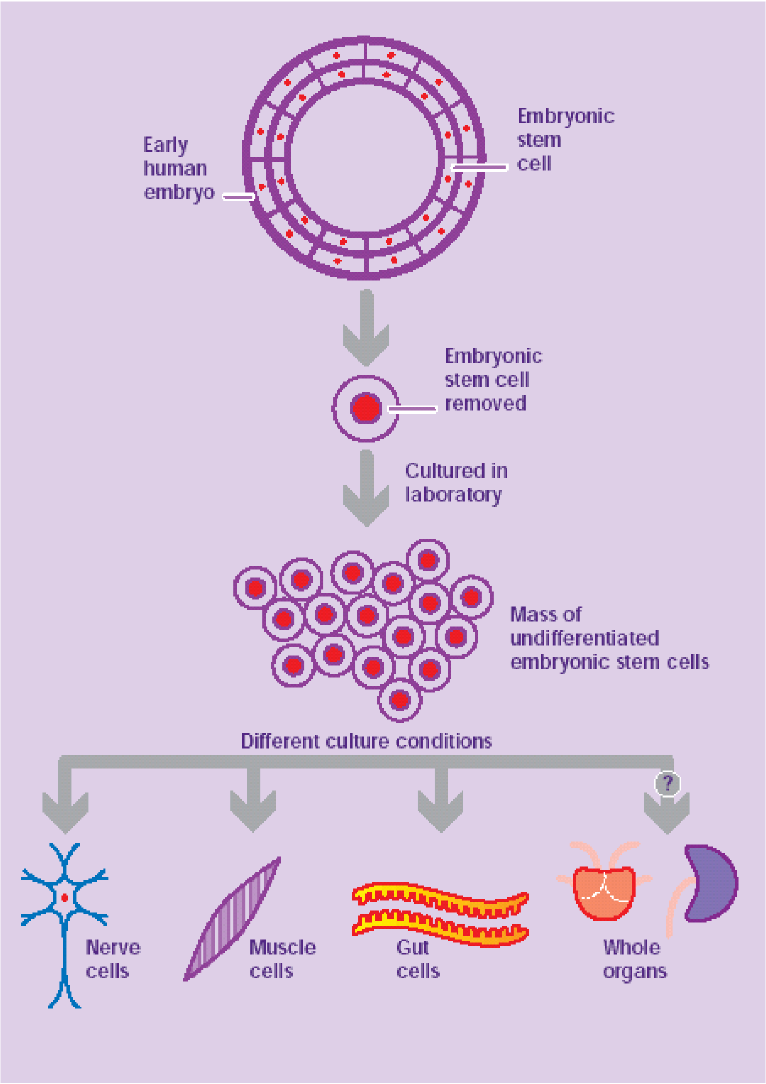
Embryonic stem cells

- are derived from embryos
- Most are derived from embryos that develop from eggs that have been fertilized *in vitro* and then donated for research purposes with informed consent of the donors. They are *not* derived from eggs fertilized in a woman's body.

- Scientists discovered ways to derive embryonic stem cells from early mouse embryos, in 1981.

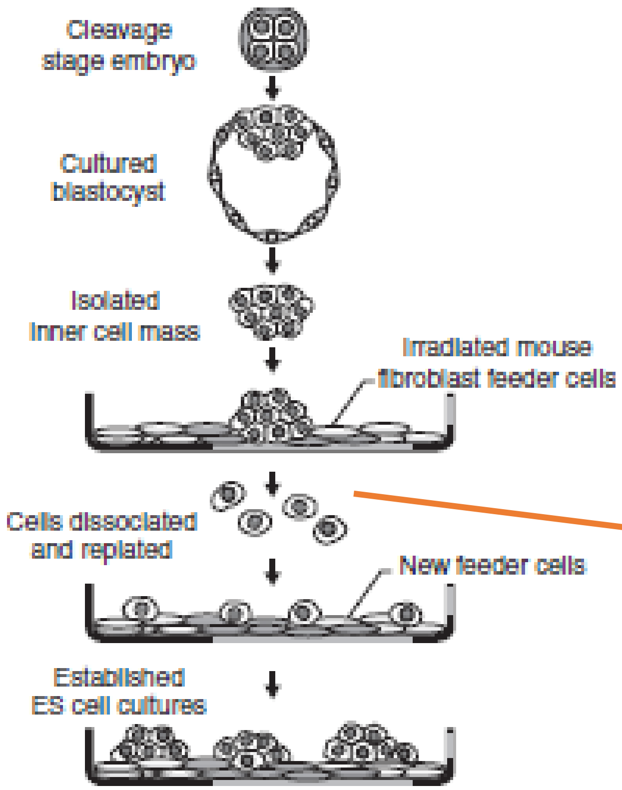
- The detailed study of the biology of mouse stem cells led to the discovery, in 1998, of a method to derive stem cells from human embryos and grow the cells in the laboratory.
  These cells are called human embryonic stem cells.

Derivation of hES cells lines

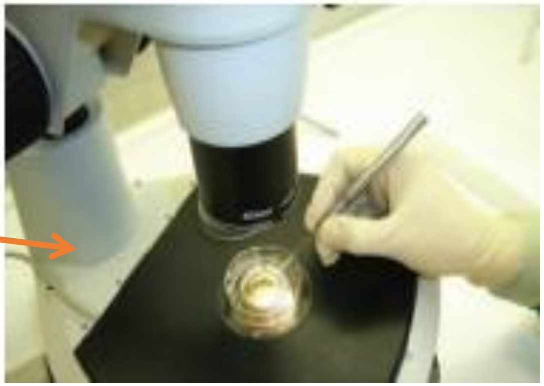


- The zygote, the fertilized egg, is grown *in vitro* until it reaches the blastocyst stage.
- Instead of being transferred to the uterus, these 5-day old blastocysts are used to isolate human embryonic stem cells. The blastocysts contain approximately 200–250 cells, of which 30–34 cells form the inner cell mass.
- Human ES cells have to be cultured on a feeder layer, cells used in co-culture to maintain pluripotent stem cells.

Derivation of hES cells lines (cont.)



Several human ES cell lines cannot be dissociated with trypsin. Therefore, these colonies are mechanically dissected by cutting them in pieces with a knife made of a glass capillary:



The colony pieces are then transferred to a new dish with feeder cells.

Embryonic stem cells are very sensitive to temperature and pH change, and when colonies overgrow, they also tend to differentiate. Therefore, ES cells have to be cared for every day, also in the weekend and during holidays.

Characterization of embryonic stem cells

Scientists who study human embryonic stem cells have not yet agreed on a standard battery of tests that measure the cells' fundamental properties. However, laboratories that grow human embryonic stem cell lines use several kinds of tests, including:

1. **Growing and subculturing the stem cells for many months.**
   This ensures that the cells are capable of long-term growth and self-renewal. Scientists inspect the cultures to see that the cells look healthy and remain undifferentiated.

2. **Determine the presence of transcription factors (TF) that are typically produced by undifferentiated cells. Two of the most important TF are Nanog and Oct4.**
   Transcription factors help turn genes on and off at the right time, which is an important part of the processes of cell differentiation and embryonic development. In this case, both Oct 4 and Nanog are associated with maintaining the stem cells in an undifferentiated state, capable of self-renewal.

3. **Determine the presence of particular cell surface markers that are typically produced by undifferentiated cells.**

Characterization of embryonic stem cells (Cont.)

4. **Examining the chromosomes**

   Assess whether the chromosomes are damaged or if the number of chromosomes has changed. It does not detect genetic mutations in the cells.

4. **Determining whether the cells can be re-grown, or subcultured, after freezing, thawing, and re-plating**.

5. **Testing whether the human embryonic stem cells are pluripotent** by

   1) allowing the cells to differentiate spontaneously in cell culture;

   2) manipulating the cells so they will differentiate to form cells characteristic of the three germ layers; or

   3) injecting the cells into a mouse with a suppressed immune system to test for the formation of a benign tumor called a teratoma.

Differentiation of embryonic stem cells

- **Under appropriate conditions, embryonic stem cells can remain undifferentiated (unspecialized)**

but

- **if cells are allowed to clump together to form embryoid bodies, they differentiate spontaneously.**

  Although spontaneous differentiation is a good indication that a culture of embryonic stem cells is healthy, it is not an efficient way to produce cultures of specific cell types.

- **So, to generate cultures of specific types of differentiated cells: e.g. heart muscle cells, blood cells, or nerve cells it is import to control the differentiation of embryonic stem cells.**

- **chemical composition of the culture medium,**
- **the surface of the culture dish,**
- **modify the cells by inserting specific genes**

⟺

Some basic protocols or "recipes" for the directed differentiation of embryonic stem cells into some specific cell types have been

Differentiation of embryonic stem cells

Direct the differentiation of ES cells into specific cell types

⇩

Generate differentiated cells to treat certain diseases in the future

Parkinson´s
Diabetes
traumatic spinal cord injury
Duchenne's muscular dystrophy
heart diseases
vision and hearing loss
….

Currently 28 clinical studies ongoing/finished with ES cells (www.clinicaltrials.gov)

Video on how stem cells work and develop

http://www.youtube.com/watch?v=mUcE1Y_bOQE&feature=related

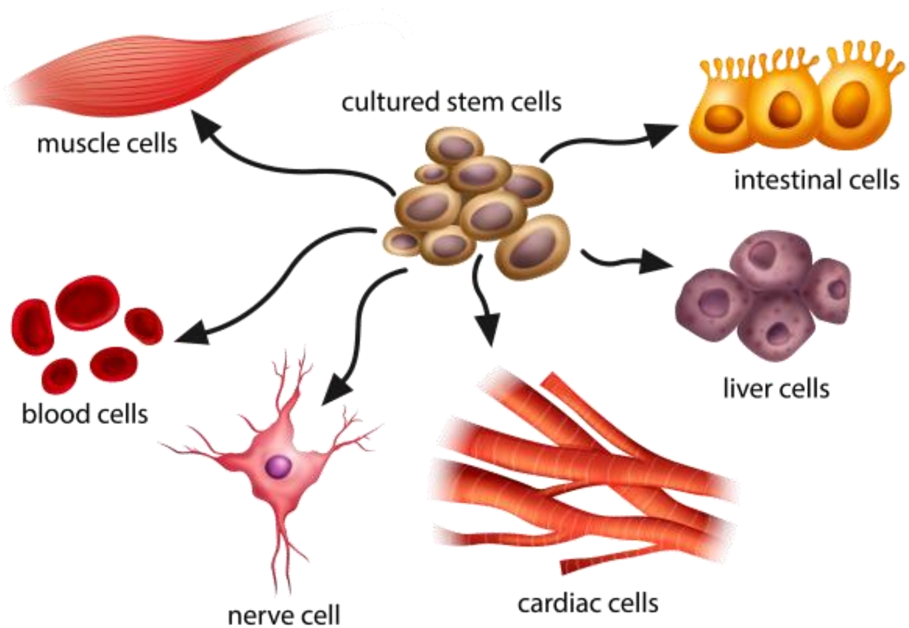
Human Adult stem cells

What are adult stem cells?

- An adult stem cell is an undifferentiated cell, found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ.

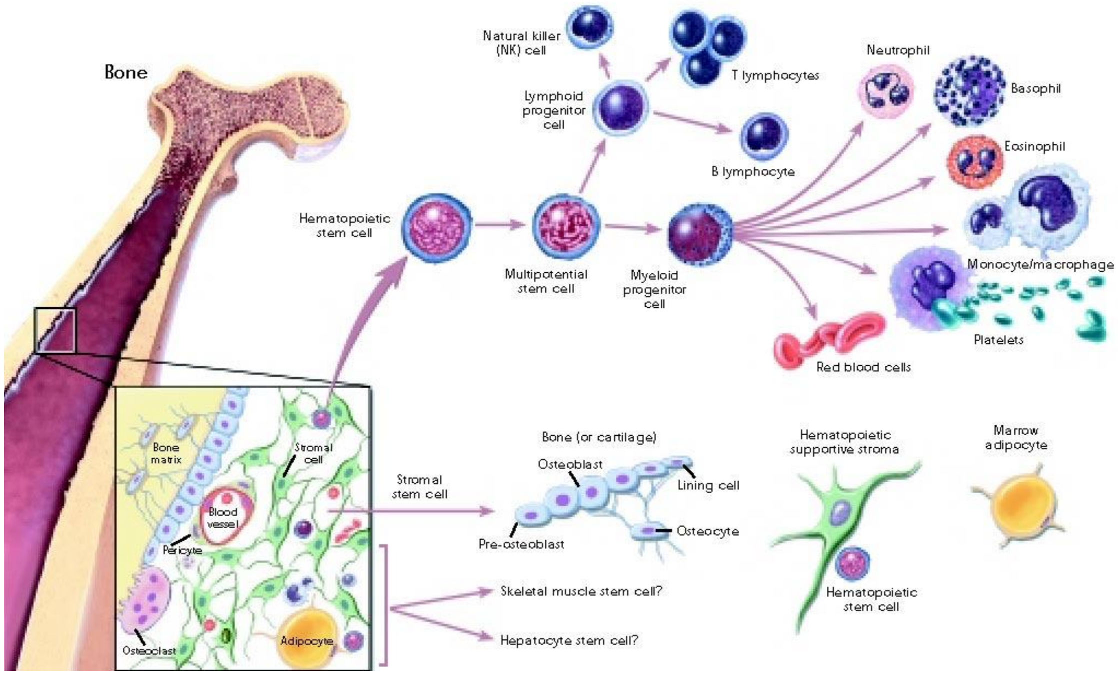
- The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found.

- Adult stem cells are present in practically all tissues, which make adult stem cells appealing for cell based therapies including transplants.



http://biostemtechnologies.com/stemcells/

Hematopoietic and Mesenchymal stem cells



history of adult stem cells began in the 1950s, when researchers discovered that the bone marrow contains at least 2 kinds of stem cells:

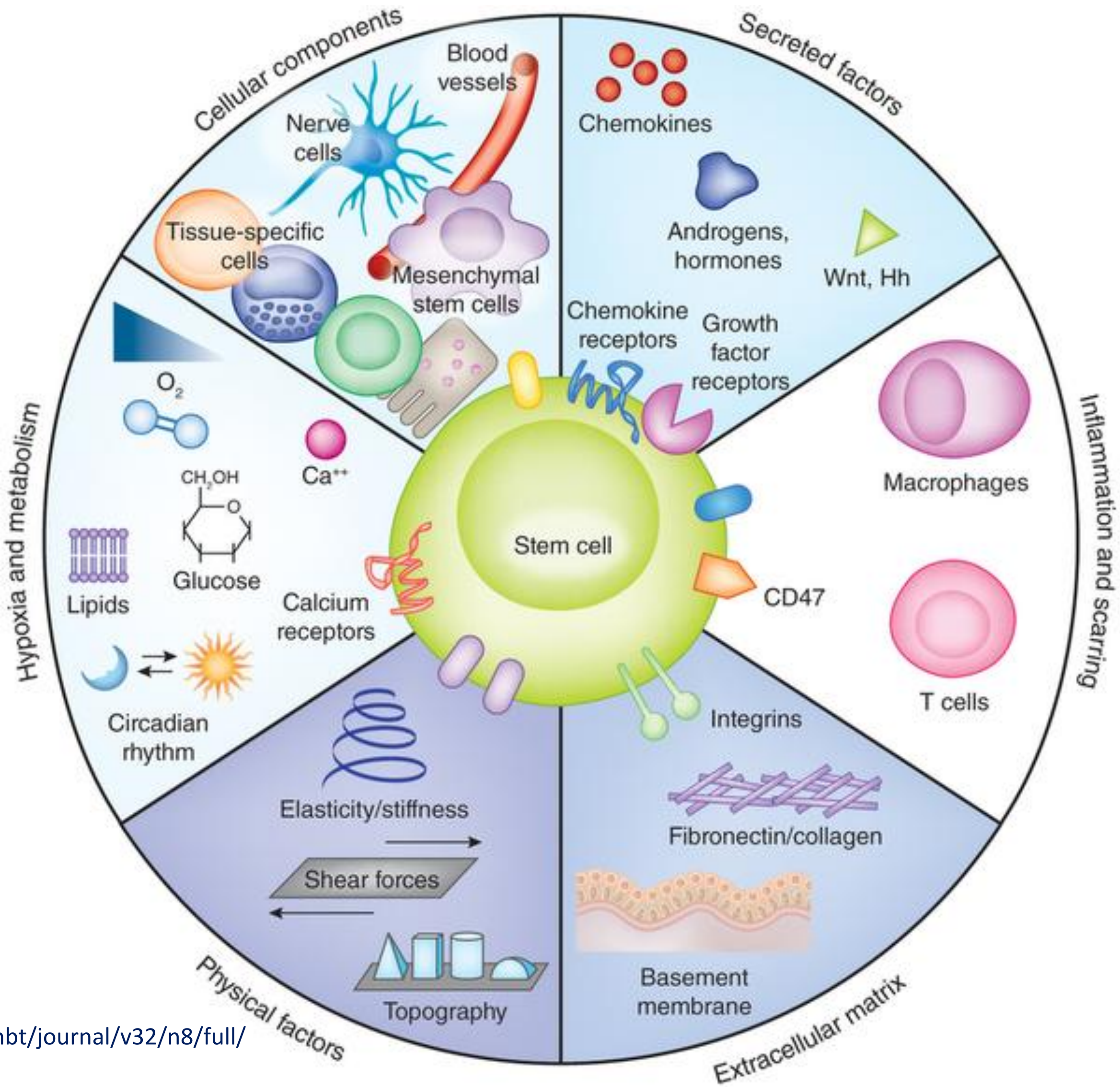
- **hematopoietic stem cells**
- **marrow stromal stem cells**

- **hematopoietic stem cells or blood-forming, stem cells from bone marrow have been used in transplants for 40 years.**
- **bone marrow stromal stem cells (also called mesenchymal stem cells), or skeletal stem cells by some), discovered a few years later, make up a small proportion of the stromal cell population that can generate bone, cartilage, fat, support the formation of blood cells, and fibrous connective tissue.**

Where are adult stem cells found?

- Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis.

- Stem cell niche: an area of a tissue that provides a specific microenvironment, in which stem cells are present in an undifferentiated and self-renewable state. Cells of the stem-cell niche interact with the stem cells to maintain them or promote their differentiation.



http://www.nature.com/nbt/journal/v32/n8/full/nbt.2978.html

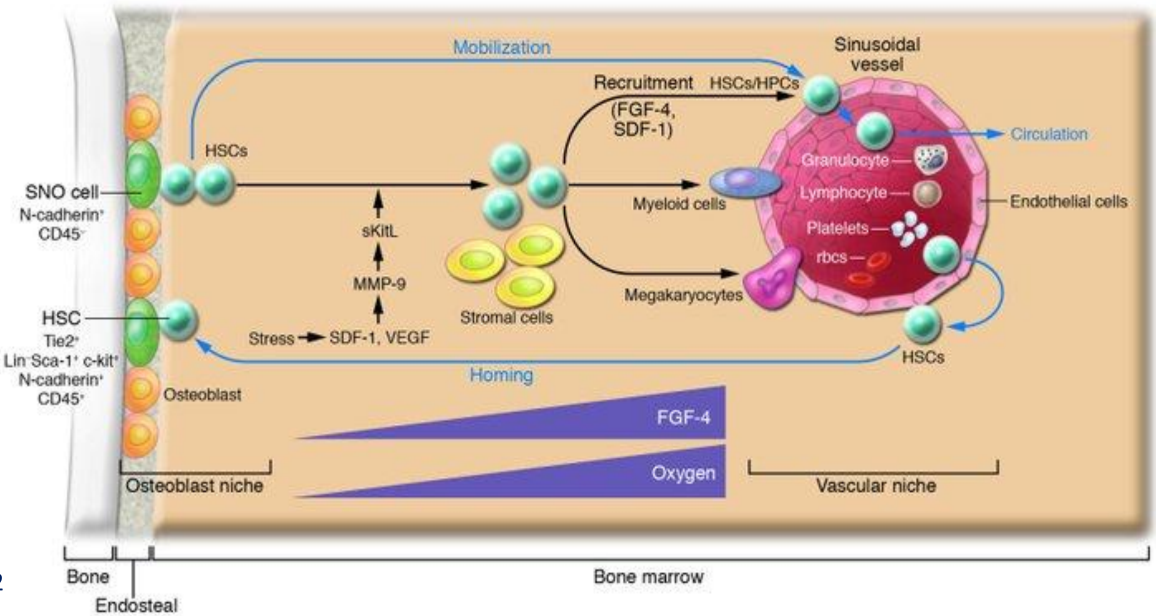
Stem cells niches- examples:

- Stem cells may remain quiescent (non-dividing) for long periods until they are required to mature cell types to maintain tissues or stimulated by disease or tissue injury.

- In many tissues, current evidence suggests that some stem cells are pericytes, cells that compose the outermost layer of small blood vessels, due to the shared markers and clonally multipotent.

- Stem cells exist in very few numbers, and growing large quantities of adult stem cells in cell culture labs and manipulate them to generate specific cell types has been a challenging approach to treat injury or disease.

The osteoblastic and vascular niches in bone



http://www.jci.org/articles/view/28568/figure/2

Differentiation pathways of adult stem cells

Hematopoietic stem cells give rise to all the types of blood cells:
red blood cells, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, and macrophages.

Mesenchymal stem cells give rise to a variety of cell types: bone cells (osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes), and other kinds of connective tissue cells such as those in tendons.

Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles

Follicular stem cells can give rise to both the hair follicle and to the epidermis.

Neural stem cells in the brain give rise to its three major cell types: nerve cells (neurons) and two categories of non-neuronal cells—astrocytes and oligodendrocytes.

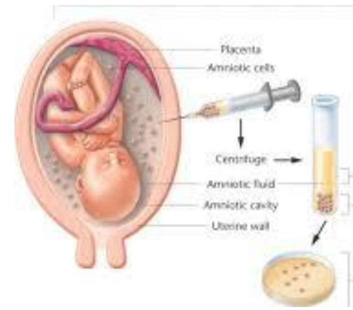
Epithelial stem cells in the lining of the digestive tract occur in deep crypts and give rise to several cell types: absorptive cells, goblet cells, paneth cells, and enteroendocrine

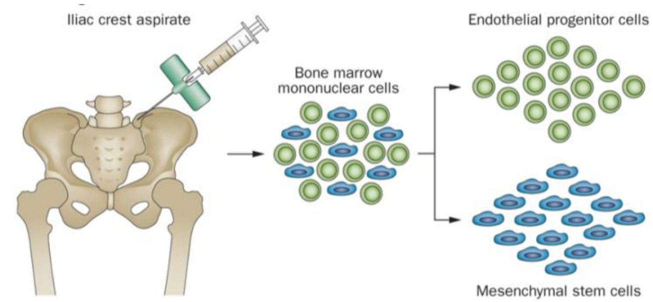
Epidermal stem cells give rise to keratinocytes, which migrate to the surface of the skin and form a protective layer.

Harvesting, isolation and culture of stem cells

1. Harvesting
- Invasive
- Less invasive procedures

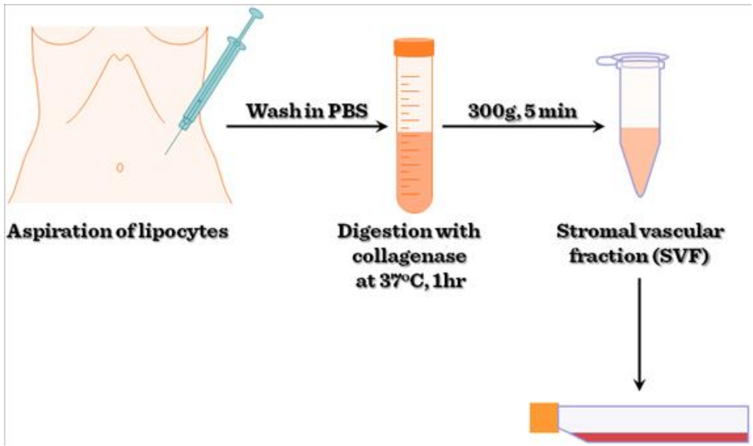
http://bio3400.nicerweb.com/
Locked/media/ch22/amn





http://www.nature.com/nrcardio/journal/v11/n4/fig_tab/nrcardio.2014.9_F2.html

2. Isolation
- Digestion - enzymes
- Selection – adhesion, gradient, centrifugation, antibody driven separation (FACS/magnetic beads)



http://www.tankonyvtar.hu/hu/tartalom/tamop425/0011_1A_3D_en_book/ch01s02.html

3. Culture
- Expansion
- Diferentiation
- Biochemical supplements
- Static/dynamic

International Society for Cellular Therapy ISCT

Cytotherapy (2006) Vol. 8, No. 4, 315–317

Taylor & Francis
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POSITION PAPER

Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement

M Dominici[1], K Le Blanc[2], I Mueller[3], I Slaper-Cortenbach[4], FC Marini[5], DS Krause[6], RJ Deans[7], A Keating[8], DJ Prockop[9] and EM Horwitz[10]

Table 1. Summary of criteria to identify MSC

1	Adherence to plastic in standard culture conditions		
2	Phenotype	Positive ($\geq 95\%+$)	Negative ($\leq 2\%+$)
		CD105	CD45
		CD73	CD34
		CD90	CD14 or CD11b
			CD79α or CD19
			HLA-DR
3	*In vitro* differentiation: osteoblasts, adipocytes, chondroblasts (demonstrated by staining of *in vitro* cell culture)		

International Society for Cellular Therapy
ISCT

Cytotherapy (2005) Vol. 7, No. 5, 393–395

Taylor & Francis
Taylor & Francis

POSITION PAPER

Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement

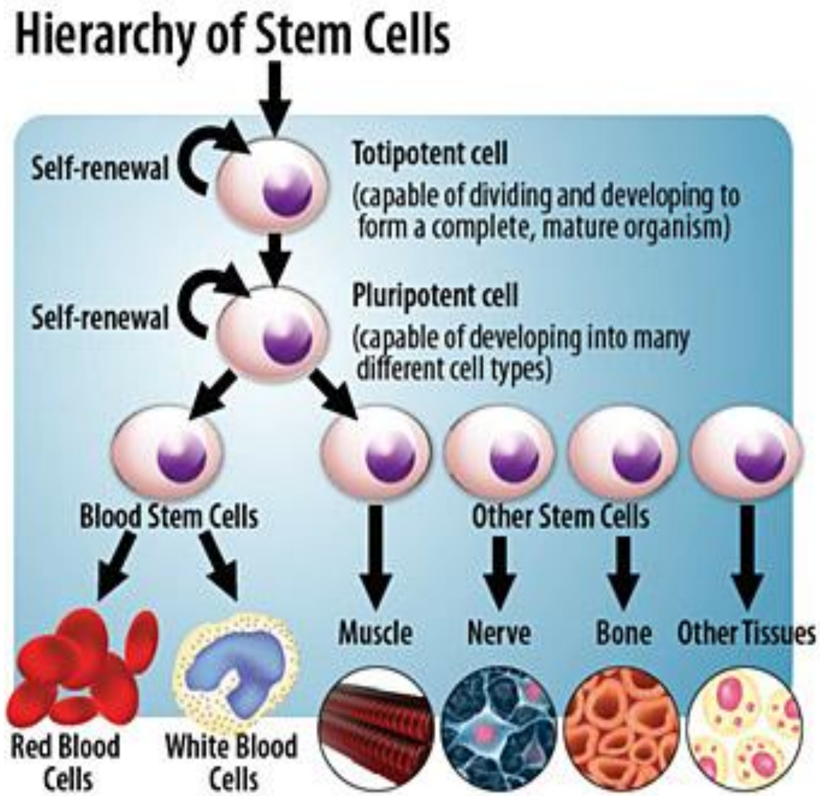
EM Horwitz[1], K Le Blanc[2], M Dominici[3], I Mueller[4], I Slaper-Cortenbach[5], FC Marini[6], RJ Deans[7], DS Krause[8] and A Keating[9]

The plastic-adherent cells isolated from BM and other sources have come to be widely known as mesenchymal stem cells (MSC). However, the recognized biologic properties of the unfractionated population of cells do not seem to meet generally accepted criteria for stem cell activity, rendering the name scientifically inaccurate and potentially misleading to the lay public. Nonetheless, a bona fide MSC most certainly exists. To address this inconsistency between nomenclature and biologic properties, and to clarify the terminology, we suggest that the fibroblast-like plastic-adherent cells, regardless of the tissue from which they are isolated, be termed multipotent mesenchymal stromal cells, while the term mesenchymal stem cells is used only for cells that meet specified stem cell criteria. The widely recognized acronym, MSC, may be used for both cell populations, as is the current practice; thus, investigators must clearly define the more scientifically correct designation in their reports. The International Society for Cellular Therapy (ISCT) encourages the scientific community to adopt this uniform nomenclature in all written and oral communications.

Keywords

Mesenchymal stem cell, MSC, stromal cell.

Summary: Stem cells "types"



- Embryonic / fetal stem cells: responsible for embryonic and fetal development and growth

- Embryonic Germ cells
  (derived from a specific part of the embryo called the gonad ridge, and are isolated from foetuses older than 8 weeks of development and can only survive about 70 to 80 cell divisions.)

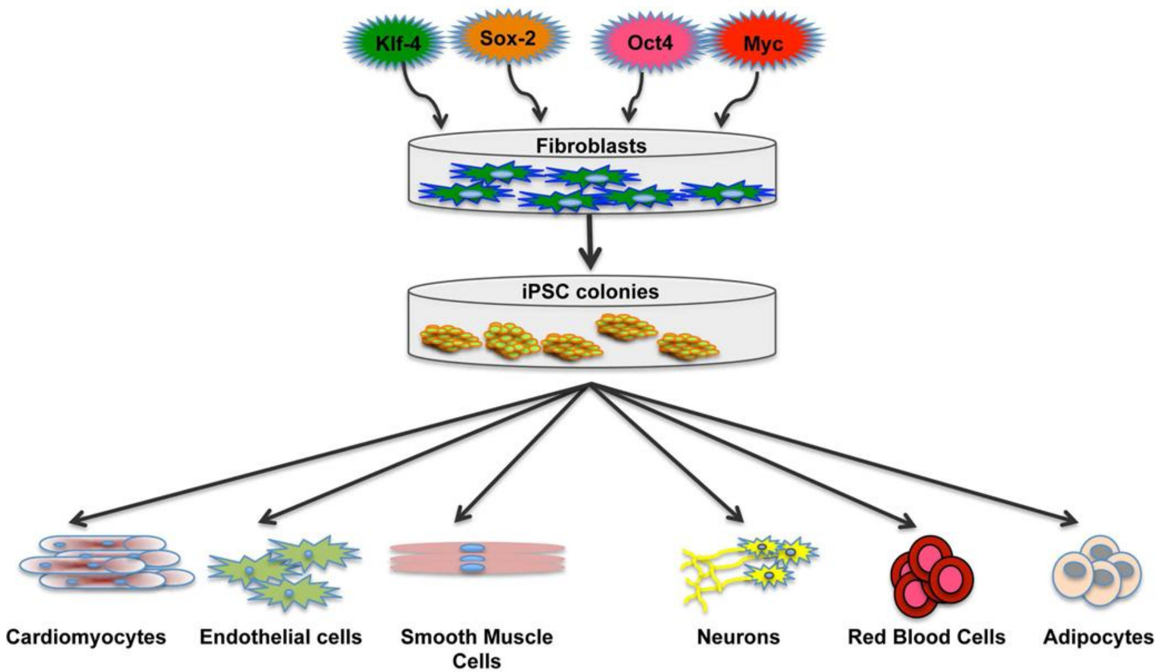
- Adult Stem Cells (somatic cells): responsible for growth, tissue maintenance, regeneration and repair of diseased or damaged tissue

- Induced pluripotent stem cells (iPS cells)

induced pluripotent stem cells (iPSCs)

The exogenous expression of pluripotency genes or transcription factors were identified to "reprogramme" genetically specialized (mouse) adult cells to assume a stem cell-like state.

Mouse iPSCs were first reported in 2006, while human iPSCs were first reported in late 2007.



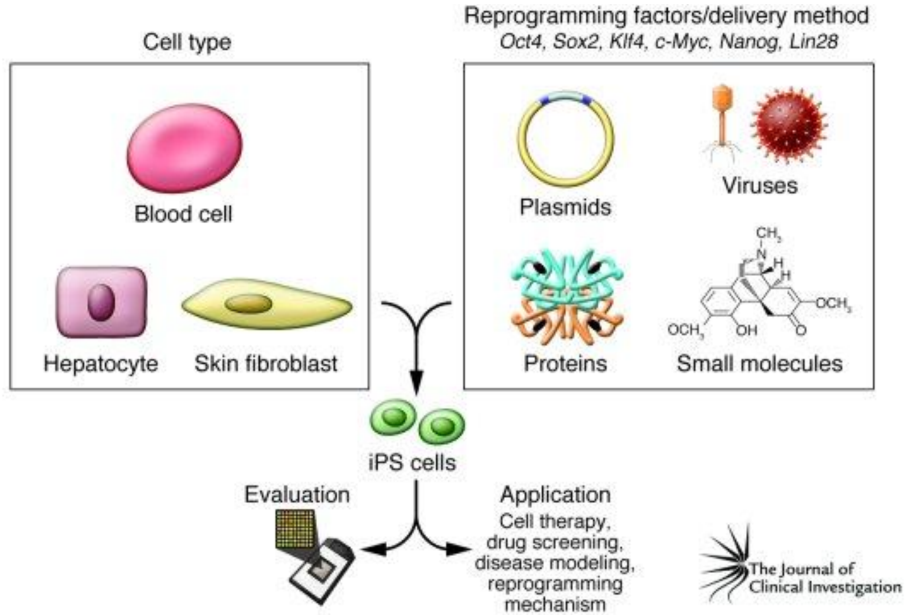
https://msakeblog.wordpress.com/category/technology-and-e-health/

Induced pluripotent stem cells (iPSCs) are a type of pluripotent stem cells that can be generated directly from adult cells and reprogrammed to an embryonic stem cell–like state by an exogenous express of genes or factors important for maintaining the defining properties of embryonic stem cells.

induced pluripotent stem cells (iPSCs)

- iPSCs are already promising tools for
  - drug development and screening
  - cell based therapies
  - disease modelling

due to the ability to develop into any cell type and recapitulate human disease with potential to repair or replace diseased, injured or aged cells within the body.



- Genetic manipulation and viruses are common delivery strategies to introduce the reprogramming factors (genomic integration) into adult cells.
- In animal studies, the virus used to introduce the stem cell factors may cause cancers, thus this process must be carefully controlled and tested as well as the production efficiency and application safety in human cell therapy.
- Moreover, hPSCs may retain epigenetic memory that in some case may add valuable biological information but may also be disadvantageous.

Transdifferentiation of adult stem cells

- The conversion of a cell type present in one tissue or organ into a cell type from another tissue or organ without going through a pluripotent cell state is called trans-differentiation.

- Transdifferentiation between some cell types can occur naturally in response to injury and can be induced experimentally.

- Transplanted stem cells may be secreting factors that encourage the recipient's own stem cells to begin the repair process.

- Even when transdifferentiation has been detected, only a very small percentage of cells undergo the process.

Potential uses of human stem cells

- Bone marrow transplant is the most widely used stem-cell therapy, but some therapies derived from umbilical cord blood are also in use.

- Studies of human embryonic stem cells
  - information about the complex events that occur during human development.
  - Understanding of the genetic and molecular controls of division and differentiation may yield Insights on cancer and birth defects, how such diseases arise and suggest new strategies for therapy.

Potential uses of human stem cells

- <u>Human stem cells</u>
  - Screen and test the safety of new drugs on differentiated cells generated from human iPSCs in a wider range of cell types. The availability of iPSCs would allow drug testing. However, to screen drugs effectively, the conditions must be identical when comparing different drugs. Therefore, scientists will have to be able to precisely control the differentiation of stem cells into the specific cell type on which drugs will be tested is still in development
  - <u>Cancer cell lines</u>, e.g. are used to screen potential anti-tumor drugs.

Cell-based therapies - examples

The most important potential application of human stem cells is the generation of cells and tissues for cell-based therapies.

- **Today, donated organs and tissues are often used to replace damaged tissue, but the need for transplantable tissues and organs far outweighs the available supply.**
- **Stem cells, directed to differentiate into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases including Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis.**

Cell-based therapies - examples

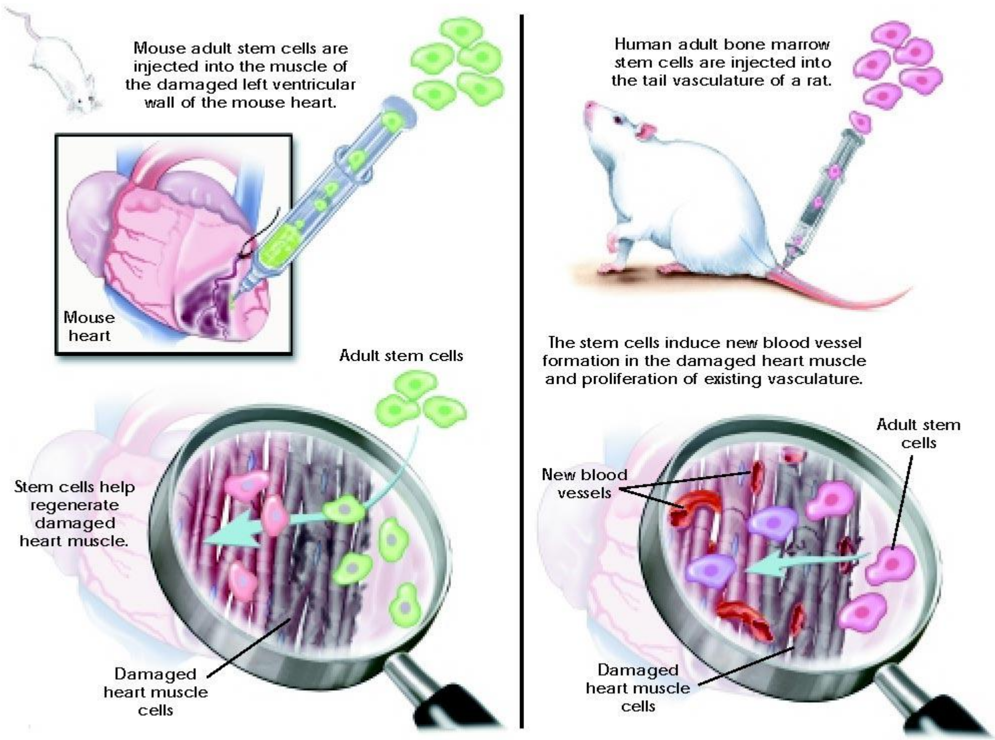
type 1 diabetes

The cells of the pancreas that normally produce insulin are destroyed by the patient's immune system.

It may be possible to direct the differentiation of human embryonic stem cells in cell culture to form insulin-producing cells that eventually could be used in transplantation therapy for patients with diabetes.

Cell-based therapies - examples

It may become possible to generate healthy heart muscle cells in the lab and then transplant those cells into patients with chronic heart disease.



The generation of heart muscle cells or stimulation the growth of new blood vessels to repopulate the heart tissue by these cells is actively under investigation.

Injected cells may accomplish repair by secreting GFs, rather than actually incorporating into the heart.

Cell-based therapies – Future perspectives

To be useful for transplant purposes, stem cells must be reproducibly made to:

I. Proliferate extensively and generate sufficient quantities of tissue.
II. Differentiate into the desired cell type(s).
III. Survive in the recipient after transplant.
IV. Integrate into the surrounding tissue after transplant.
V. Function appropriately for the duration of the recipient's life.
VI. Avoid harming the recipient in any way.
VII. Also, to avoid the problem of immune rejection, research strategies to generate tissues that will not be rejected.

To summarize, stem cells offer exciting promise for future therapies, but significant technical hurdles still remain to be overcome through years of intensive research.

Key questions that remain to be answered

- How many kinds of adult stem cells exist, and in which tissues do they exist?
- How do adult stem cells evolve during development and how are they maintained in the adult? Are they "leftover" embryonic stem cells, or do they arise in some other way?
- Why do stem cells remain in an undifferentiated state when all the cells around them have differentiated? What are the characteristics of their “niche” that controls their behavior?
- Do adult stem cells have the capacity to transdifferentiate, and is it possible to control this process to improve its reliability and efficiency?
- If the beneficial effect of adult stem cell transplantation is a trophic effect, what are the mechanisms? Is donor cell-recipient contact required, secretion of factors by the donor cell, or both?
- What are the factors that control adult stem cell proliferation and differentiation?
- What are the factors that stimulate stem cells to relocate to sites of injury or damage, and how can this process be enhanced for better healing?

FINAL CONCLUSIONS (STEM CELLS)

- **Given their unique regenerative abilities, stem cells offer new potentials for treating diseases such as diabetes, and heart disease. However, much work remains to be done in the lab and the clinic to understand how to apply these cells for cell-based therapies to treat disease and damaged tissues, which is also referred to as regenerative or reparative medicine.**

- **Stem cell studies enable scientists to learn about the cells' essential properties and what makes them different from specialized cell types. Scientists are already using stem cells expanded and manipulated in the lab to screen new drugs and to develop model systems to study normal growth and identify the causes of genetic defects.**

- **Research on stem cells continues to advance knowledge on the organism development from a single cell and how healthy cells replace damaged cells in adult organisms.**