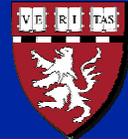




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TISSUE ENGINEERING

IV. Stem Cells

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CELLS FOR TISSUE ENGINEERING/ REGENERATIVE MEDICINE

- **Autologous**
 - Differentiated parenchymal cells of same or other tissue type
 - Undifferentiated or differentiated stem cells (adult)
- **Allogeneic**
 - Same as above
 - Fetal stem cells
 - Embryonic stem cells

NEED FOR STEM CELLS IN TISSUE ENGINEERING/REGENERATIVE MEDICINE

Problems in Using Differentiated Cells

- Limited availability of differentiated autologous cells.
- Morbidity of a harvest procedure and donor site.
- Limited proliferative capacity and biosynthetic activity.

WHAT ARE STEM CELLS?

- Cells with the capacity for unlimited or prolonged self-renewal.
- Cells that can produce at least one type of highly differentiated descendant.

<http://www.nih.gov/news/stemcell/primer.htm>

NATIONAL INSTITUTES OF HEALTH
May 2000

Stem Cells: A Primer

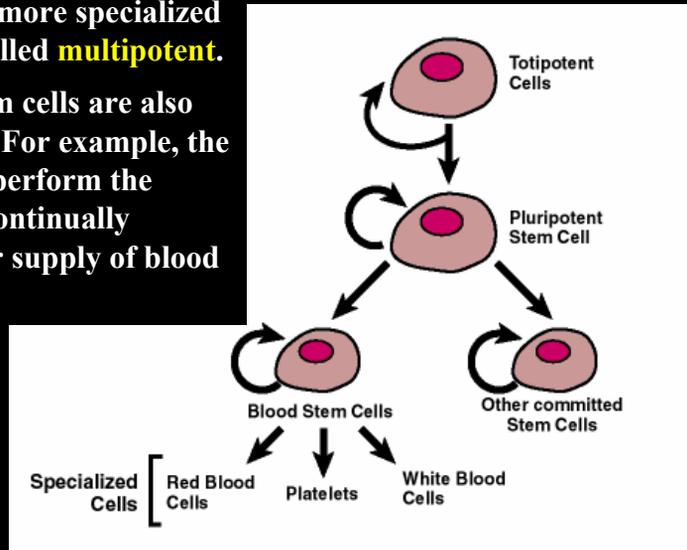
Definitions

Stem cells - cells that have the ability to divide for indefinite periods in culture and to give rise to specialized cells.

- **Multipotent** -giving rise to many cell types.
- **Pluripotent** -capable of giving rise to most tissues of an organism.
- **Totipotent** - having unlimited capability. Totipotent cells have the capacity to specialize into extraembryonic membranes and tissues, the embryo, and all postembryonic tissues and organs.

- **Pluripotent** stem cells undergo further specialization into stem cells that are committed to give rise to cells that have a particular function. These more specialized stem cells are called **multipotent**.
- Multipotent stem cells are also found in adults. For example, the blood stem cell perform the critical role of continually replenishing our supply of blood cells .

<http://www.nih.gov/news/stemcell/primer.htm>



How are pluripotent stem cells derived?

- Pluripotent stem cells can be isolated directly from the inner cell mass of human embryos at the blastocyst stage and cultured to produce a pluripotent stem cell line.
- Pluripotent stem cells can be isolated from fetal tissue obtained from terminated pregnancies. Cells can be taken from the region of the fetus that is destined to develop into the testes or the ovaries.

<http://www.nih.gov/news/stemcell/primer.htm>

Potential Applications of Pluripotent Stem Cells

- Help to understand the events that occur during development.
 - identification of the factors involved in the cell specialization; "decision-making" genes .
- Change the way drugs are developed and tested for safety.
 - new medications could be initially tested using human cell lines.
- Generation of cells and tissue that could be used for so-called "cell therapies."
 - donated organs and tissues are often used to replace ailing or destroyed tissue.
 - the number of people suffering from these disorders far outstrips the number of organs available for transplantation.

Adult Stem Cells

- Multipotent stem cells can be found in some types of adult tissue. In fact, stem cells are needed to replenish the supply cells in our body that normally wear out (*e.g.*, the blood stem cell).
- Multipotent stem cells have not been found for all types of adult tissue, but discoveries in this area of research are increasing.
 - neuronal stem cells have been isolated from the rat and mouse nervous systems. The experience in humans is more limited.
 - a kind of cell that may be a neuronal stem cell has been isolated from adult brain tissue.

Do adult stem cells have the same potential as pluripotent stem cells?

- Until recently, little evidence that multipotent cells such as blood stem cells could change course and produce cells other than a blood stem cell or a specific type of blood cell.
 - In animals, it has been shown that some adult stem cells are able to develop into other types of specialized cells.
 - In mice neural stem cells placed into the bone marrow appeared to produce a variety of blood cell types.
 - In rats, stem cells found in the bone marrow were able to produce liver cells.
- Even after a stem cell has begun to specialize, the adult stem cell may, under certain conditions, be more flexible than first thought.

<http://www.nih.gov/news/stemcell/primer.htm>

Why not just pursue research with adult stem cells?

- Multipotent cells have great potential for use in cell therapies with the following advantages.
 - Transplantation of *ex vivo*-manipulated autologous cells would not likely result in rejection.
 - Reduce or even avoid the practice of using stem cells derived from human embryos or human fetal tissue, sources that trouble many people on ethical grounds.

<http://www.nih.gov/news/stemcell/primer.htm>

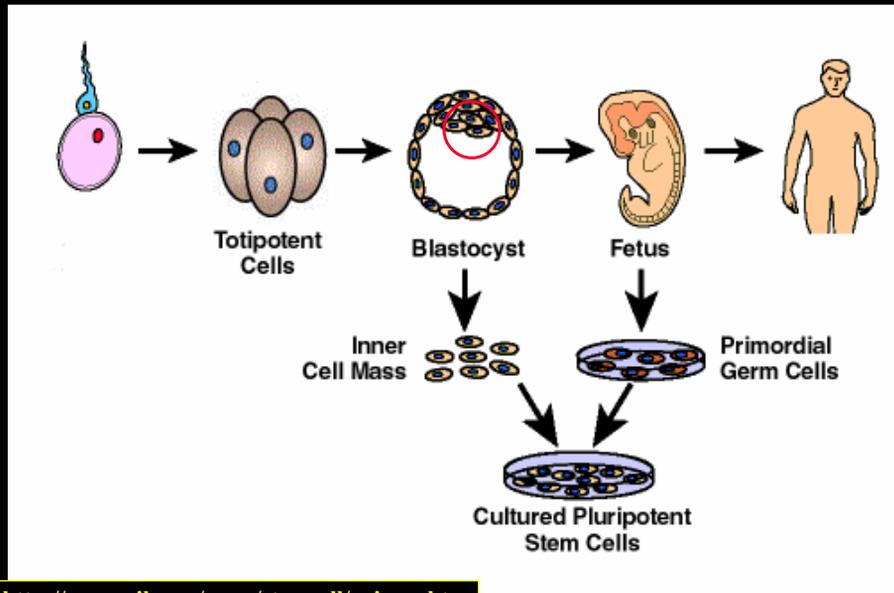
STEM CELL HISTORICAL PERSPECTIVE

- The finding that the adherent cell population derived from adult whole marrow includes mesenchymal (multipotent) stem cells dates back almost 3 decades.
 - AJ Friedenstein, *et al.*, *Exp. Hematol.* 2:83-92, 1974
- Early finding of the requirement for MSCs as “support cells” for hematopoietic stem cell cultures.
 - TM Dexter, *et al.*, *J. Cell. Physiol.* 91:335-344, 1977
 - AJ Friedenstein, *et al.*, *Transplantation* 17:331-40., 1974
- Only a few years since pluripotent stem cells isolated from embryos and fetuses.
 - JA Thomson, *et al.*, *Science* 282:1145 (1998)
 - MJ Shamlott, *et al.*, *PNAS* 95:13726 (1998)

EMBRYONIC AND FETAL STEM CELLS HISTORICAL PERSPECTIVE

- In November, 1998, James Thomson of the University of Wisconsin isolated embryonic stem cells from “surplus” embryos.
- A few days later, John Gearhart of the Johns Hopkins University published word that he cultured a line of stem cells from germ cells from aborted fetuses.

How are **pluripotent** stem cells derived?



Sci. Express, Feb. 12, 2004

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See Hwang, Woo Suk, et al. "Evidence of a Pluripotent Human Embryonic Stem Cell Line Derived from a Cloned Blastocyst." *Science Express* (February 12, 2004).

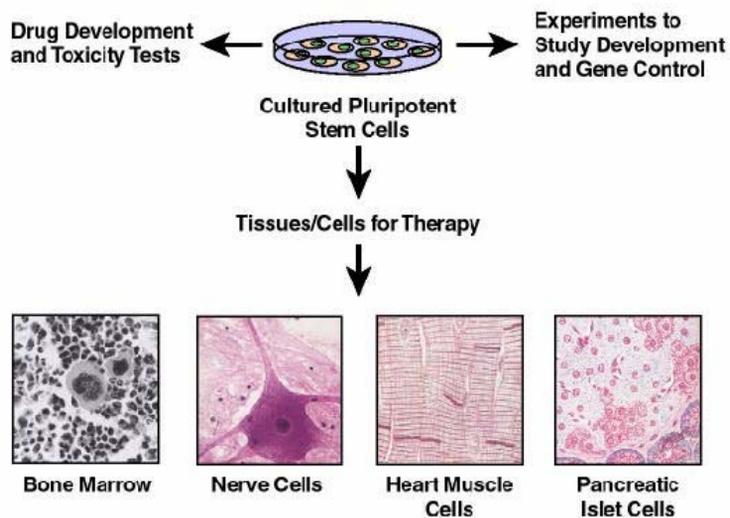
Commercial Interest in Stem Cells is High

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Table summarizing companies working with stem cells, from *Science* 287 (2000): 1420.

Science 287:1420 (2000)

The Promise of Stem Cell Research



<http://www.nih.gov/news/stemcell/primer.htm>

CELLS BEING REFERRED TO AS “STEM CELLS”

- Can divide in culture for only a limited number of passages and still be induced to differentiate into selected cell types (*i.e.*, cannot divide indefinitely).
- Can only be induced to differentiate into only a few specialized cell types.
- Most tissues appear to contain such cells.

NEED FOR STEM CELLS IN TISSUE ENGINEERING/REGENERATIVE MEDICINE

Problems in Using Differentiated Cells

- Limited availability of differentiated autologous cells.
- Morbidity of a harvest procedure and donor site.
- Limited proliferative capacity and biosynthetic activity.

Can Adult Stem Cells Suffice?

Science 292: 1820 (2001)

- Stem cells can be found in many tissues of the body and developing embryos and fetuses
 - ES cells are **pluripotent**; with the correct cues they can give rise to any kind of cell in the body
 - Adult stem cells are **multipotent**; they can produce many, but not all, cell types
- Adult bone marrow cells have been in use for more than a decade, whereas embryonic stem (ES) cells were isolated for the first time 3 years ago
- Surprising flexibility of adult stem cells found in many tissues
- ES cells multiply more readily and seem far more proficient in producing certain specialized cell types

The Mesengenic Process

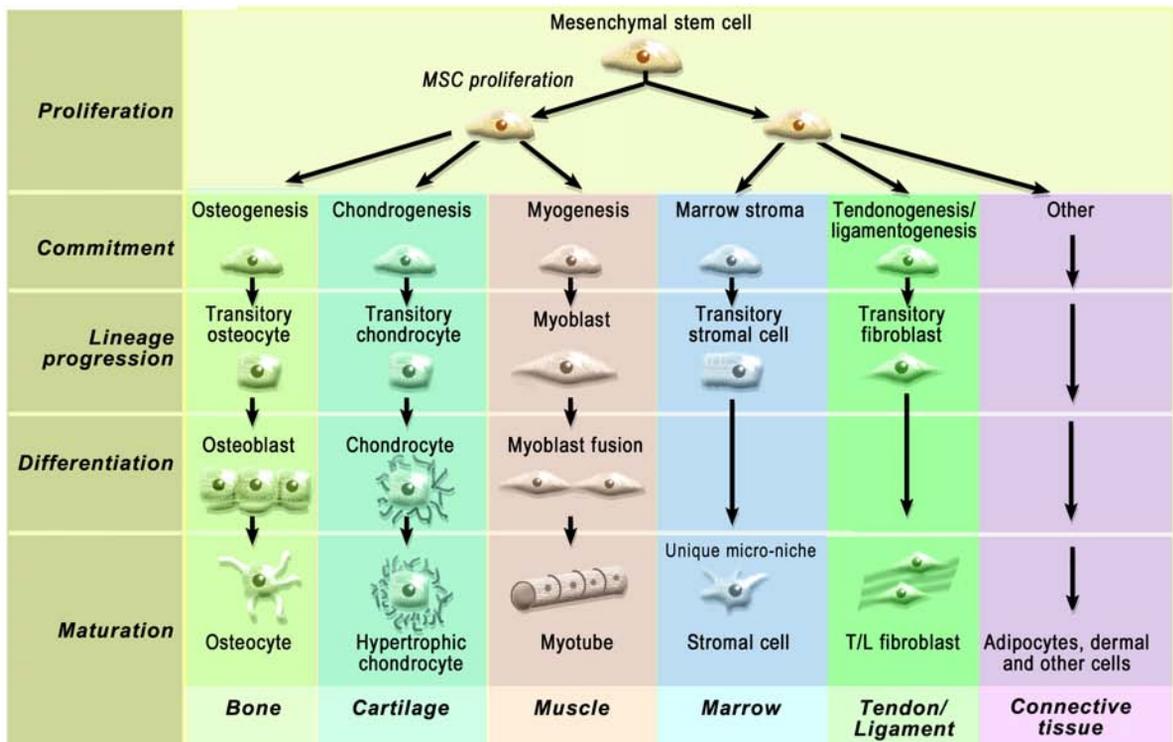


Figure by MIT OCW.

Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies

- Determined if a population of stem cells could be isolated from human adipose tissue.
- Human adipose tissue, obtained by suction-assisted lipectomy (*i.e.*, liposuction), was processed to obtain a fibroblast-like population of cells or a processed lipoaspirate (PLA).
- PLA cells can be maintained *in vitro* for extended periods with stable population doubling and low levels of senescence.
- Immunofluorescence and flow cytometry show that the majority of PLA cells are of mesodermal or mesenchymal origin with low levels of contaminating pericytes, endothelial cells, and smooth muscle cells.

PA Zuk, *et al.* Tiss. Engr. 7:211(2001)

Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies

- PLA cells differentiate *in vitro* into adipogenic, chondrogenic, myogenic, and osteogenic cells in the presence of lineage-specific induction factors.
- The data support the hypothesis that a human lipoaspirate contains multipotent cells and may represent an alternative stem cell source to bone marrow-derived MSCs.

PA Zuk, *et al.* Tiss. Engr. 7:211(2001)

STEM CELLS FROM MARROW

Rationale for Clinical Value

Historical Perspective

- 1869** Autologous marrow induces bone at heterotopic sites (E. Goujon)
- 1919** Marrow has osteogenic activity (A Keith)
- 1961** Osteogenic properties of marrow (RG Burwell)
- 1986** Intra-op centrifugation of marrow and percutaneous injection for treating non-unions (J Connolly, et al., Neb. Med. J. 71:105)
- 1995** Marrow infiltrating into defects in articular cartilage provide stem cells for chondrogenesis