Mesenchymal stem cells

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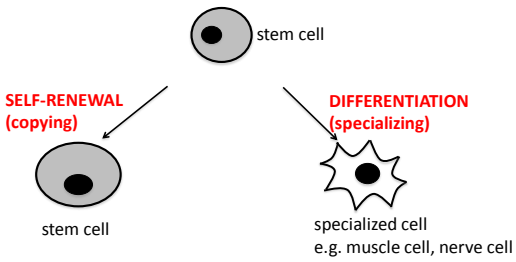
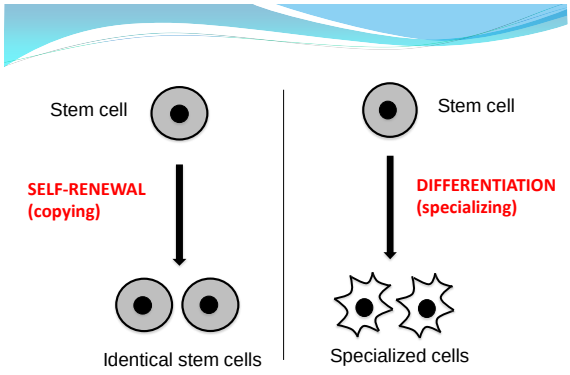
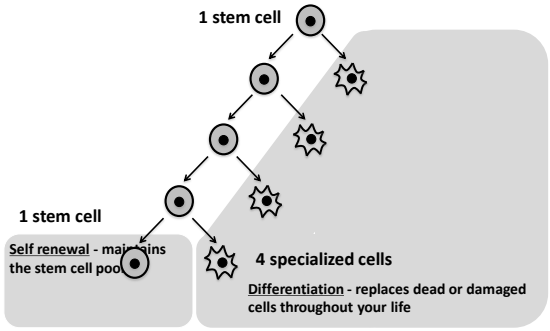
A Life Story

What is a stem cell?



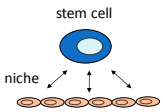


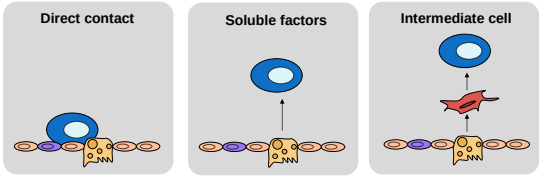
Why Stem Cells self-renew and differentiate?



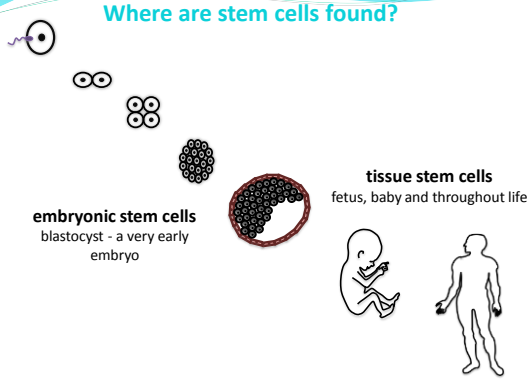
Stem cell niches

Niche
Microenvironment around stem cells that provides support and signals regulating self-renewal and differentiation





Where are stem cells found?

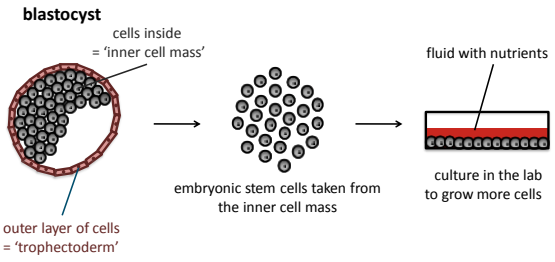


embryonic stem cells
blastocyst - a very early embryo

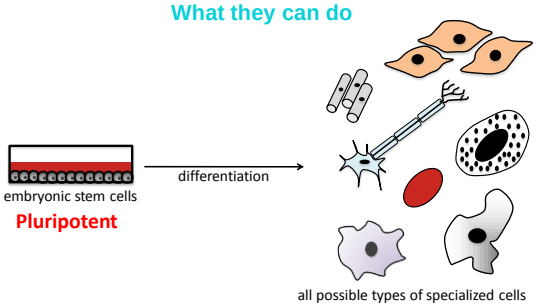
tissue stem cells
fetus, baby and throughout life

Embryonic Stem Cells

Embryonic stem (ES) cells



Embryonic stem (ES) cells: What they can do



Adult stem cells

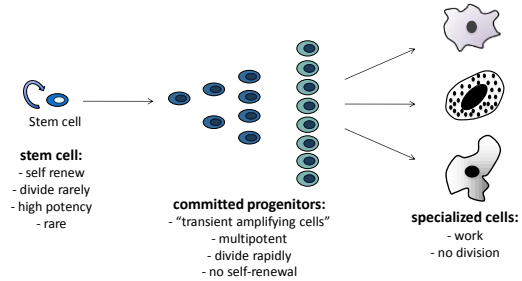
What are Adult Stem Cells?

- An adult stem cell is an **undifferentiated** (or partially-differentiated) cell found in tissues and organs.
- They can **self-renew** and **differentiate** to become most or all of the **specialized** cell types within their specific tissue lineage.
- Adult stem cells
  - Maintain cell populations
  - Help you heal
  - Play a role in aging

How Regeneration Works in adult stem cells

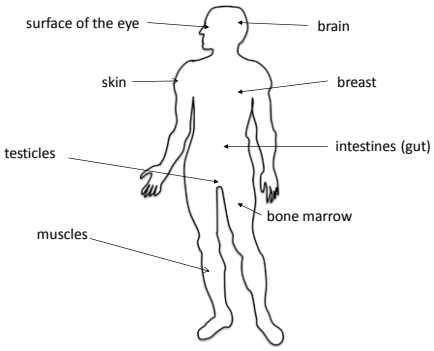
- Adult stem cells normally remain quiescent (non-dividing) for relatively long periods of time until they are activated by signals to maintain tissues
- When activated they divide through a process called asymmetric cell division.
- Through this process they are able to maintain their populations and differentiate into the desired cell types by the creation of a progenitor cell.
- A progenitor cell, in contrast to stem cells, is already far more specific: they are pushed to **differentiate** into their "target" cell.

Principles of renewing tissues



Location of Adult Stem Cells

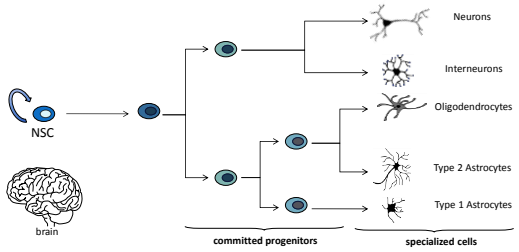
- Adult stem cells and progenitor cells reside through out your body
- These stem cells reside in a specific area of each tissue called the "stem cell niche"
- This niche is a particular microenvironment that fosters the growth of resident stem cells
- Mutations in cells, signals they receive, and changes in the microenvironment can activate a stem cell



Types of Adult Stem Cells

- Neural stem cells: neurons, glial cells
- Epithelial stem cells: skin, linings
- Hematopoietic stem cells: blood and immune system
- Mesenchymal stem cells: bone, cartilage, fat, muscle, tendon/ligament

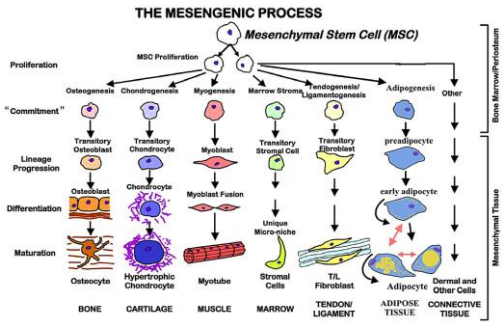
Neural stem cells (NSCs)



Mesenchymal stem cells

- Mesenchymal stem cells (MSCs) are of stromal origin and have been isolated from placenta, adipose tissue, lungs, bone marrow, bone, blood, umbilical cord, amniotic fluid and teeth.
- Since the first discovery that adult bone marrow contains rare, yet powerful multipotent progenitor cells (Friedenstein et al., 1968), often referred to as mesenchymal stem cells (MSCs), multipotent adult progenitor cells, marrow stromal cells, or mesenchymal progenitors (Salem et al., 2010), it is now apparent that many adult tissues harbor cells that have similar characteristics (da Silva Meirelles et al., 2006).
- These cells exhibit multiple functions and are involved in tissue maintenance and repair, as well as in the regulation of hematopoiesis and immune responses (Chamberlain et al., 2007).

Adult MSCs can be defined as undifferentiated cells that can renew themselves and have the ability, once cultured under specific growth conditions, to differentiate into multiple lineages of mesodermal tissues, such as skeletal muscle, bone, tendons, cartilage, and fat, or nonmesenchymal cell-lineages, such as neuron-like cells, hepatocytes and pancreatic-like cells (Prockop et al., 1997).



Characteristics

- In culture mesenchymal stem cells posses a fibroblast like phenotype and share similarities in immunophenotype and differentiation assays.
- These are following criteria's in order to indentify MSC:
  1) must have adherence to plastic
  2) formation of colonies
  3)Fibroblast like morphology
  4) expression of CD105, CD73 and CD90, and lack of expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules;
  5) must have differentiation potential
- Despite this phenotype similarity, gene profile among all these cells may be different.
- Human mesenchymal stem cells are to isolate by their ability to adhere to plastic plates compare to murine.

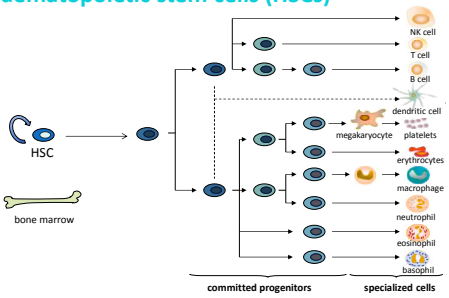
Hematopoietic stem cells

The stem cells that forms blood and immune cells are known as Hematopoietic stem cells.
Give rise to all the blood cell types:

- **Myeloid** (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells)
- **Lymphoid** (T-cells, B-cells, NK-cells)

Found in the bone marrow from very early on in development, as well as in umbilical cord blood and placental tissue

Haematopoietic stem cells (HSCs)



Stromal stem cells

- In the early 1970s many investigators confirmed that bone marrow also has cells with fibroblastic morphology that could differentiate into that could differentiate into bone, cartilage, fat and muscle (Prockop, 1997).
- The cells are marrow stromal cells.
- Primary human marrow stromal cells (MSCs) have been cultured from adult bone marrow (BM) mononuclear cells (MNCs).
- These cells have fibroblast-like morphology and express CD29, CD44, CD105 and CD166. They lack expression of CD14, CD34, and CD45.
- MSCs differentiates in vivo and in vitro into osteoblasts, chondrocytes, myocytes, adipocytes and beta-pancreatic islets cells. MSC also trans-differentiate into neuronal cells.

Dental Stem Cells

- Dental pulp is the soft living tissue inside a tooth. Stem cells are found inside the soft living tissue. Scientists have identified the mesenchymal type of stem cell inside dental pulp.
- Dental pulp stem cells can provide a unique alternative to embryonic stem cells.
- The DPSC are easily isolated compared to other postnatal stem cells types.

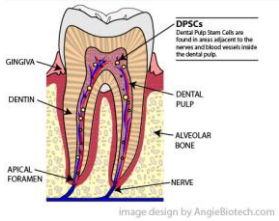
Human Dental Stem cells Sources

Since the discovery adult human dental pulp stem cells in 2000, several other types of DSCs have isolated and characterized from mature and immature dental pulps.

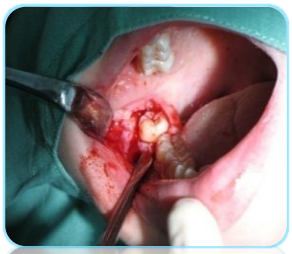
- Human Dental Pulp Stem Cells (DPSCs-Post-natal dental pulp stem cells)
  Gronthos et al, 2000
- Human Exfoliated Deciduous Teeth (SHED)
  Miura et al, 2003
- Periodontal Ligament Stem Cells(pdlscs)
  Seo et al, 2004
- Stem Cells From Apical Papilla (SCAP)
  Sonoyama et al, 2006, 2008
- Dental Follicle Progenitor Cells (DFPCs)
  Morsczeck et al, 2005

Dental Pulp Stem Cells Isolation

During development tooth morphognesis progress though mutually inductive signalling between interacting oral epithelial and mesenchymal cells of neural ectodermal origion. This results in the formation of hard external enamel layer of crown, a higly minralized a cellular matrix produced by specialized epithelial cells known as ameloblasts, which undergo apoptosis during tooth eruption and are absent in mature teeth.



1. The tooth is extracted under local anesthesia.

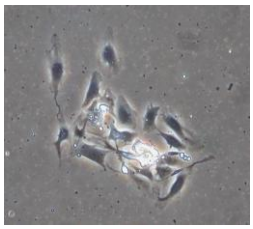
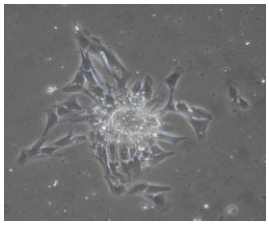


Thanks to Dr Soukup for providing pictures

2. The pulp tissue was separated from the pulp cavity.

Thanks to Dr Soukup for providing pictures

3. The pulp was kept in DMEM with 10% glucose. The pulp is trypsinised, and cultured, the cells are isolated from their ability to adhere with the culture plate.

Thanks to Dr Soukup for providing pictures

Dental Pulp Stem Cells Characteristics

- Research on the characteristics and differentiation of DPSC have become a hot topic in the field of dentistry.
- Like all other adult stem cells the DPSC are in a quiescent state in dental pulp and can perform continuous cell division during tissue injury/ regeneration.

- The DPSC have two types of growth pattern

1. They can divide into two identical daughter stem cells by the symmetric cells division

or

2. They can give birth to one altered daughter stem cells and one progenitor cell with self renewal capacity by the asymmetric cell division.

The dental pulp stem cells are undifferentiated mesenchymal stem cells in the dental pulp tissue and characterized by their ability to:

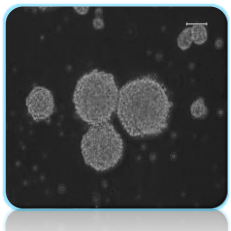
- Self renewal
- Colony formation capacity (an average of 40 dental pulp derived CFU-F were generated per 10000 cells plated)
- Mutlipotent differentiation
- Dental pulp stem cells are characterized as CD29+ CD44+ CD73+ CD90+ CD166+ CD184+ Bcrp1+ Musashi-1+ Nanog+ Sox-2+ cells.

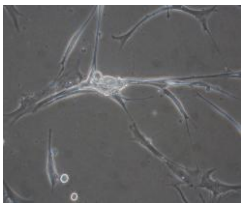
Dental Pulp Stem Cells Differentiation

- Through the addition of tissue-specific cytokines, differentiated cells were obtained in vitro fromDPSCs not only of mesenchymal linage but also of endo- and ecto-dermal linage.

- DPPSCs were able to form both embryoid bodies-like structures (EBs) in vitro and teratoma-like structures that contained tissues derived from all three embryonic germ layers when injected in nude mice.

Neurons





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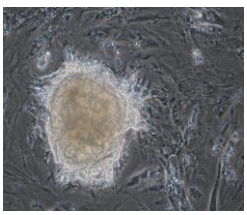
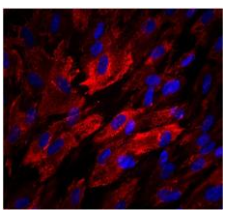
Osteocytes





procollagen

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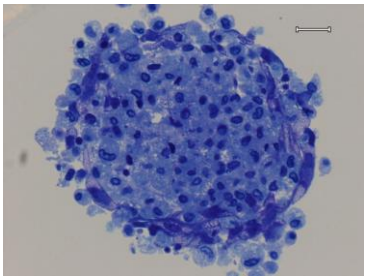
Chondrocytes



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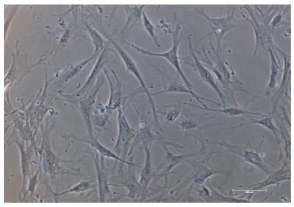
Umbilical Cord Mesenchymal stem cells

- The youngest most primitive MSCs can be isolated from umbilical cord.
- The umbilical cord contains two arteries and one vein, which are surrounded by mucoid connective tissue, and this is called the Wharton's jelly.
- The stem cells are isolated from Wharton's jelly and Umbilical cord blood.
- The MSCs are found in much higher concentration in Wharton's jelly then in umbilical blood.
- The umbilical cord is easily obtained from after the birth of new born.

Adipose tissue MSCs

- Adipose tissue is a type of connective tissue which is found under the skin (subcutaneous fat), around internal organs (visceral fat), in bone marrow (yellow bone marrow) and in breast tissue.
- Adipose tissue like bone marrow is derived from the embryonic mesoderm and contains the stroma that is easily isolated compared to bone marrow.
- The existence of stem cells within adipose tissue was reported for the first time in 2001.
- Adipose tissue is one of the richest source of MSCs.
- When compared to bone marrow, there is more than 500 times of more stem cells in 1gm of fat when compared to 1gm of aspirated bone marrow.
- Like other MSCs these cell also differentiates into adipocytes, myocytes, chondrocytes, endothelial and neuronal cells.



Morphology of human adipose stem cells in vitro

Thank You