MED 443
King Saud University

Anatomy 443

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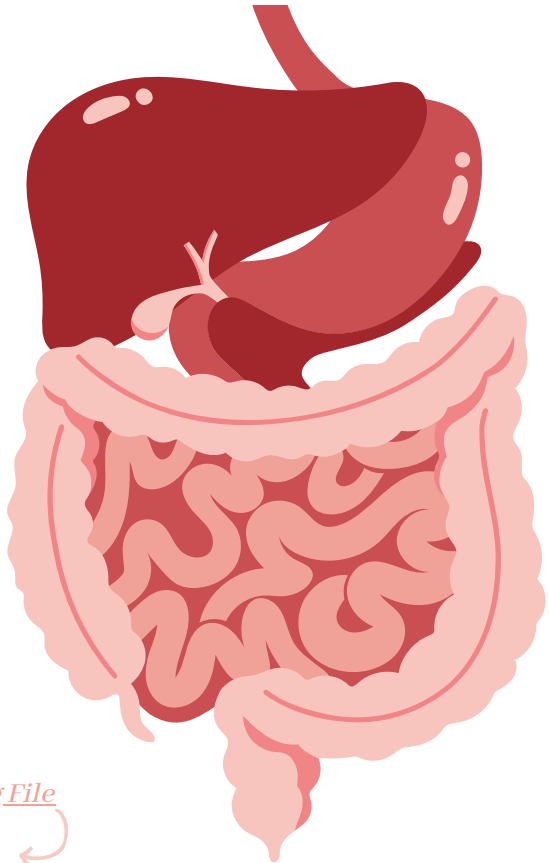
Introduction to Pluripotent Stem cells

GNT Block



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The Editing File

Objectives

- Stem Cell : Definition & main function within the body.
- Where can we find Stem Cells (location).
- Classifications of stem cells:
  1- Embryonic Stem Cell
  2- Adult stem cells (Tissue Specific Stem Cell)
  3- Induced Pluripotent Stem Cell (iPS) cells
- Different approaches for isolation of pluripotent stem cells.
- The Promise of Stem Cell Technology.

This lecture was presented by :

Dr. Amer Mahmoud Dr. Mona Al Safadi

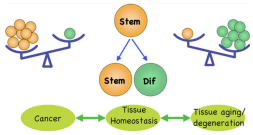
We recommend you watch these 2 videos: PART 1 & PART 2

You can find Atlas by Clicking HERE!

Stem cells

MCQ

Introduction of stem cells

Definition	A cell that has the ability to: ‣ **Continuously divide** and give rise to new copy of itself (**self-renew**). ‣ **Differentiate** into various kinds of cells/tissues, such as: - Endoderm (ex. hepatocytes) - Mesoderm (ex. cardiac myotubes) - Ectoderm (ex. neurons) 
Unique characteristics	(1) **Unlimited self renew** (Regeneration). (2) **Differentiation**(ex. beating cells of the heart muscles), triggered by: - Internal signals (specific genes) - External signals (Growth factors, cytokines like: TGF-B & FGF)
Function	Continuous Repair of defective cell types and regeneration of tissues.

Classification of stem cells

Based on potency

(1) Totipotent:

‣ The zygote at 1-3 days, differentiate into embryonic and extraembryonic cell types.

‣ Extraembryonic like: Placenta and umbilical cord

‣ Forms **embryo** and **placenta**.

(2) Pluripotent:

‣ Descendants of **Totipotent Cells** and differentiate into cells of the **3 germ layers**.

‣ Can just form the **embryo.**

(3) Multipotent:

‣ Produce cells of closely related cells (e.g. hematopoietic).

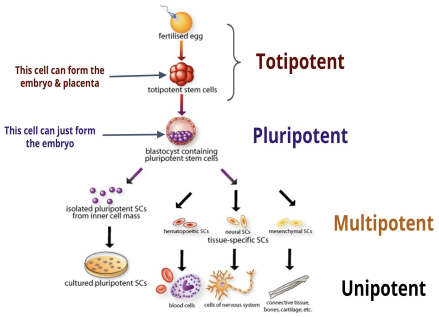
‣ **family stem cells.**

(4) Oligopotent:

‣ Differentiate into only a **few** cells, such as: **lymphoid** or **myeloid** stem cells.

(5) Unipotent:

‣ Produce only **one** cell type (e.g. **muscle** stem cells).



Based on the source

(1) Adult stem cells (tissue specific) :

‣ They are **Multipotent**.

‣ **Limited** numbers and more difficult to isolate.

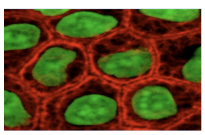
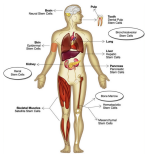
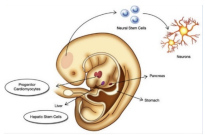
‣ **No** immune rejection & no ethical concerns.
(Note: it will be explained later on)

‣ Found in specific mature body tissues as well as the umbilical cord and placenta after birth.

‣ They also can be isolated of developing embryos different tissues.

‣ **Sources:**

1- Bone marrow. 2- Placenta cord. 3- Mesenchymal SC.

(2) Embryonic stem cells:

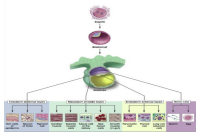
‣ They are **Pluripotent**.

‣ **Large** number can be harvested..

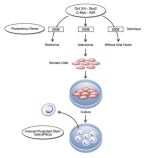
‣ May cause **immune rejection** & ethical concerns.
(Note: it will be explained later on)

‣ **Sources:**

1- IVF embryos. 2- **Aborted embryos**. 3- Cloned embryos.



(3) Induced



The history of medical sensation

1981: Marin Evans at the university of cambridge is first to identify embryonic stem cells (In mice).

1997: Ian Wilmut and colleagues at the Roslin Institute, Edinburgh. Dolly the sheep, the first artificial animal clone.

1998: James Thomson (University of Wisconsin) and John Gearhart (Johns Hopkins) isolated human embryonic stem cells and grew then in lab.

2001: Bush controversy.

2006: Shinya Yamanaka of kyoto university reprograms ordinary adult cells to form "induced stem cells".

2009: Obama-power.

2010: Medical treatment of Spinal injury using **hESCs**.

2012: Medical treatment of Blindness using **hESCs**.

2014: Human trials using iPSCs.

What are stem cell technologies?

Male slides

- Cloning technologies
  - is human cloning a technology?
  - What is different about cloning embryonic stem cells?
- Induced pluripotent stem cells
  - New ways to potentially avoid the use of embryos
  - Disease-specific stem cell lines created
  - The promise and potential pitfalls of this approach

WHEN does research actually become technology?

Somatic cell nuclear transfer (SCNT)

Cloning

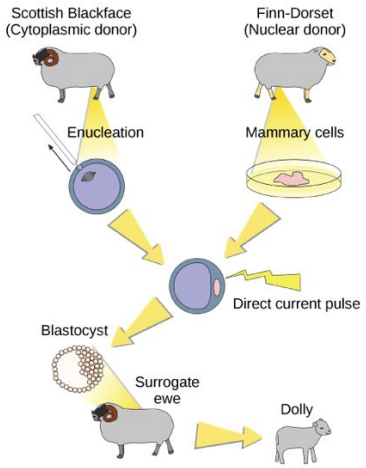
Definition

Cloning describes the processes used to create an exact genetic replica of another cell, tissue or organism. The copied material, which has the same genetic makeup as the original, is referred to as a clone.

Types of cloning

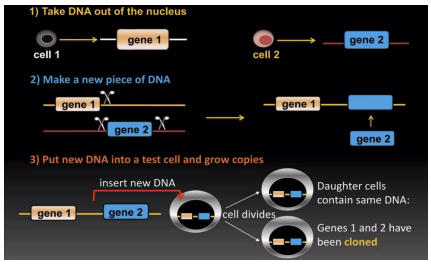
Reproductive cloning

- Use to make two identical individuals.
- Very difficult to do
- Illegal to do on humans
- Dolly the sheep, the first mammal cloned from an adult somatic cell, was created through somatic cell nuclear transfer (SCNT).
- The cloning of Dolly involved taking a somatic cell (a cell not involved in reproduction, in this case, a mammary cell) from the white face sheep and transferring its nucleus (containing genetic material) into an egg cell that had its nucleus removed from the black sheep. The resulting cell, with the genetic material from the white sheep cell, was then stimulated to divide and develop into an embryo. This embryo was implanted into a surrogate sheep, leading to the birth of Dolly.



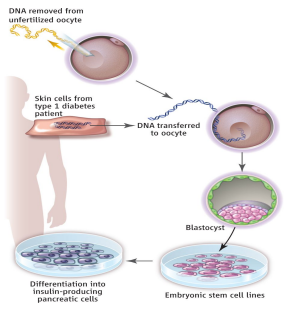
Molecular cloning

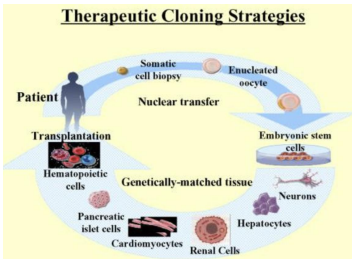
- Use to study what a gene does.
- Routine in the biology labs.
- Cut and paste.



Therapeutic cloning

- Therapeutic cloning uses stem cells to correct diseases and other health problems that someone may encounter.
- Therapeutic cloning does not clone to make full humans but rather is used for the stem cells of embryo.
- The process is similar to reproductive cloning, where a somatic cell is fused with an egg cell, but in therapeutic cloning, the purpose is to generate embryonic stem cells with the same genetic material as the patient. These stem cells can then be coaxed to differentiate into various cell types, offering the potential for personalized regenerative medicine.





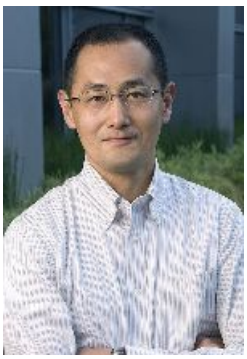
The first IPSCs

MCQ

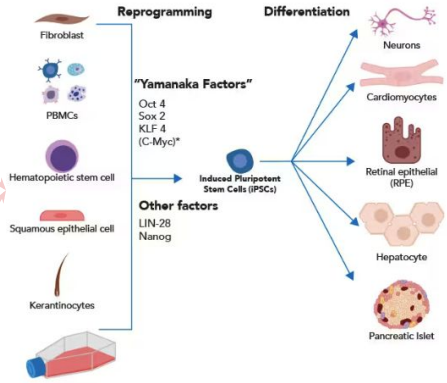
General info

In late 2006 the group of **Takahashi and Yamanaka** reported the stimulation of cells of adult and embryonic origin to pluripotent stem cells called **induced pluripotent stem** (iPS) cells.

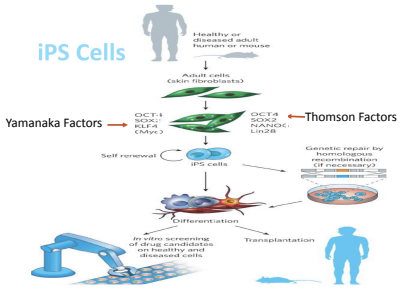
- The method was described by Yamanaka in which the skin cells of laboratory mice were genetically manipulated and returned back to their embryonic state.
- Induced pluripotent stem cells are somatic cells that have been reprogrammed to a pluripotent state (embryonic stem cell like state).



- Somatic cells (Such as the **fibroblasts**) can be reprogrammed by introduction of four transcription factors **Oct4, Sox2, Klf4 and c-Myc**, often referred to as "**Yamanaka Factors.**", Thomson factors : OCT4, SOX2,NANOG and Lin28. **LIN-28 and Nanog,** has been shown to increase reprogramming efficiency.
- After reprogramming to a "de-differentiated" state, iPSCs can generate virtually any cell type.



- Several difficulties are to be overcome before iPSCs can be considered as a potential patient-specific cell therapy.
- It will be crucial to characterize the development potential of human iPS cell line in the future.

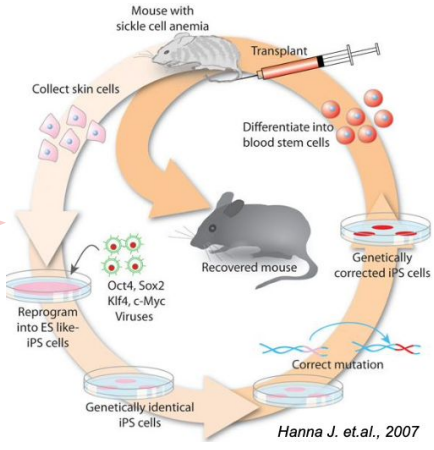


- **Experimental application of IPSCs was done on a mouse:**

1- Skin cells were taken from the tail tip of a sickle-cell model mouse.

2- The cells were differentiated into pluripotent stem cells then to hematopoietic cells.

3- The produced cells were transfused back into the sick mouse and it recovered (Note that rejection by the immune system has a very low chance to occur because the genetic material was originally from the mouse itself and not from a different one).



Generation of embryonic SCs

(1) Embryonic human stem cells were first isolated in 1995 by Dr. James Thomson.
Derived from 4-5 day old embryo (blastocyst):

1-Trophoblast. 2-Blastocoel. 3-Inner Cell Mass (ICS).

(2) Isolate and transfer of ICS into culture dish in culture media at 37 C and 5% CO2.

(3) Inner surface of culture dish is **coated** with inactivated **MEFs** (Mouse embryonic Fibroblasts) as a feeder layer:

- It Provides sticky surface for **attachment** - It Releases **nutrients**.

(4) Cells divide and spread over the dish.

(5) ESCs are removed gently and plated into several different culture plates.

Challenges with Embryonic stem cells

Male slides MCQ

- Abnormalities in chromosome number and structure were found in some human ESC lines.
- Stem cells need to be differentiated to the appropriate cell types before they can be used clinically.
- Stem cell development or proliferation must be controlled once placed into patients (risk of teratoma formation).
  - **Teratoma** is a tumor composed of mixed tissue pattern such as: skin with keratin, brain tissue, striated and smooth muscle, lymphoid tissue.
- The use of mouse "feeder" cells to grow ESC could result in problems due to xenotransplantation (The surgical transfer of cells or whole organs from an organism of one species to an organism of a different species).
- Possibility of rejection of stem cell transplants as foreign tissues is very high.

Stem cell therapies

The goal

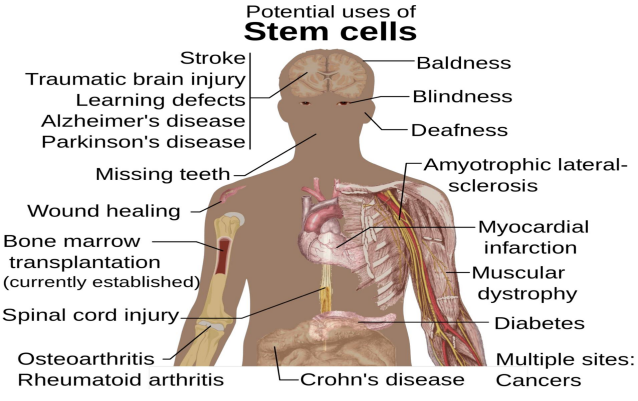
The goal of stem cell therapies is to promote cell replacement in organs that are damaged and do not have the ability for self repair.

The promise of stem cell technology

- Replacement of tissues/organs
- Repair of defective cell types
- Study cell differentiation
- Toxicity testing.
- Understanding prevention and treatment of birth defects.
- Study of development and gene control.
- Study of drugs therapeutic potential.

The Obstacles of stem cell research

- How to find the right type of stem cells?
- How to completely differentiate Stem Cells to desired cell type?
- How to put the stem cells into the right place?
- Will the stem cells perform the desired function in the body?
- Differentiation protocols for many cell types have not been developed.



MCQs

Q1. Which of the following are pluripotent stem cells? (from slides)			
A. Cells has the potential to differentiate into any adult cell type forming an entire organism	**B.** Cells that has limited potential to form only multiple adult types	**C.** Cells that don't have the ability for self renewal	**D.** Cells has the potential to form all differentiated cell types except placenta
Q2. Important limitation of using cloned ESCs (SCNT-ESCs)? (from slides)			
A. Immune rejection	**B.** Produced limited number of cell types	**C.** Destruction of human embryos	**D.** Difficult to grow and culture in the laboratory
Q3. Mesenchymal stem cells are examples of: (from slides)			
A. Totipotent SC	**B.** Pluripotent SC	**C.** Multipotent SC	**D.** Induced pluripotent SC
Q4. Which of the following is a type of Multipotent stem cells?			
A. Muscle SC	**B.** myeloid SC	**C.** Hematopoietic SC	**D.** lymphoid SC
Q5. What are Yamanaka factors? (From slides)			
A. Oct4, Sox2, Klf4, c-Myc	**B.** Growth factors	**C.** Cytokines	**D.** Oct3/4, SOX2, NANOG
Q6. Which of the following can cause teratoma?			
A. Unipotent SC	**B.** Embryonated SC	**C.** Oligopotent SC	**D.** Nullipotent SC

A1. D A2. C A3. C A4. C A5. A A6. B

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