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Introduction to Pluripotent Stem Cells

What are Stem Cells?

A cell that has the ability:

- to continuously divide and give rise to new copy of itself (self-renewal)
- and other specialized (differentiated) cells/tissues (differentiation)

Endoderm: hepatocytes

Mesoderm:cardiac myotubes

Ectoderm:Neurons

Stem cells divide to new cell that has the potential to either remain a stem cell or become another type of cell with a more specialized function as cells of the blood, heart, bones, skin, muscles, brain etc, serving as a sort of repair system for the body.

characteristics of stem cells

- Self renewal
  regeneration
- Differentiation
  sort of repair system for the body
  - External signals (GF, cytokines)
  - Internal (specific genes) signals

If self renewal excess the differentiation > cancer
If differentiation excess the self renewal > aging/degeneration

Potential of stem cells:		
Totipetent (Total)	Pluripotent (ESC) (Plural)	multipotent (ASC) (Multiple)
fertilized egg (zygote)	from 4-5 day old embryo Blastocyst (inner cells mass)	Adult stem cells
From embryonic and extraembryonic* cells	From embryo only	Differentiate into related cells
Differentiate into any adult cell type	Potential to form all differentiated cell types except placenta	Form only multiple adult cell types + can't retain back or switch there limit

Hematopoietic SCs	Neural SCs	Mesenchymal SCs
Differentiate into all types of blood cells	Neurons and glial cells	Differentiate into Osteoblasts, Chondrocytes and Adipocytes. give: cartilage, bones & connective tissue

Fully mature

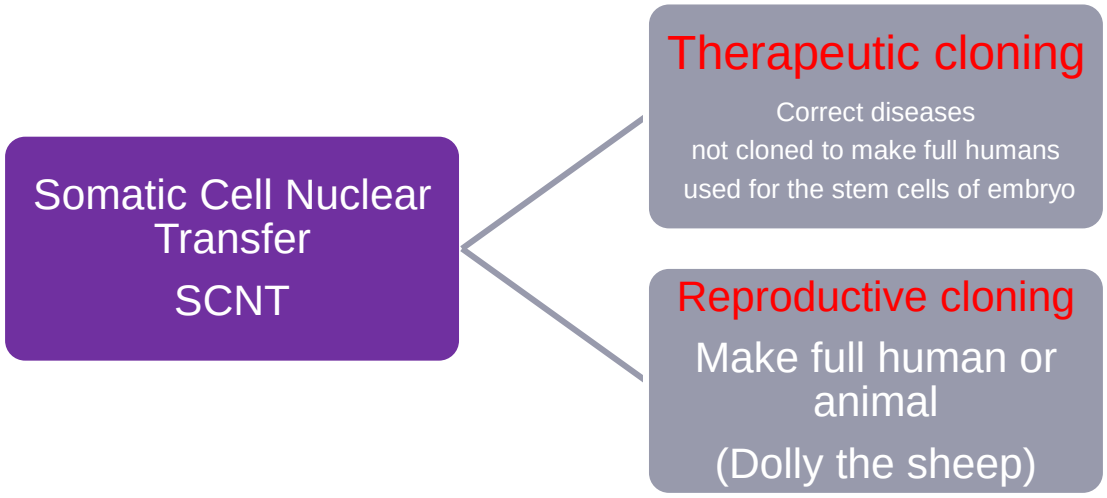
*Extra embryonic cell: placenta & umbilical cord

Generation of embryonic stem cells:

- Isolate and transfer of ICS into culture dish in culture media
- Culture at 37c and 5% CO2
- **Inner surface of culture dish is coated with inactivated *MEFs as a feeder layer:**
  - provides sticky surface for attachment
  - release nutrients
- Cells divide and spread over the dish
- ESCs are removed gently and plated into several different culture plates.

Why we inactivate the MEFs?

- To not facilitate the differentiation of the stem cells
- reduce the possibility of contamination



*MEFs: mouse embryonic fibroblast.

SOURCES OF STEM CELLS

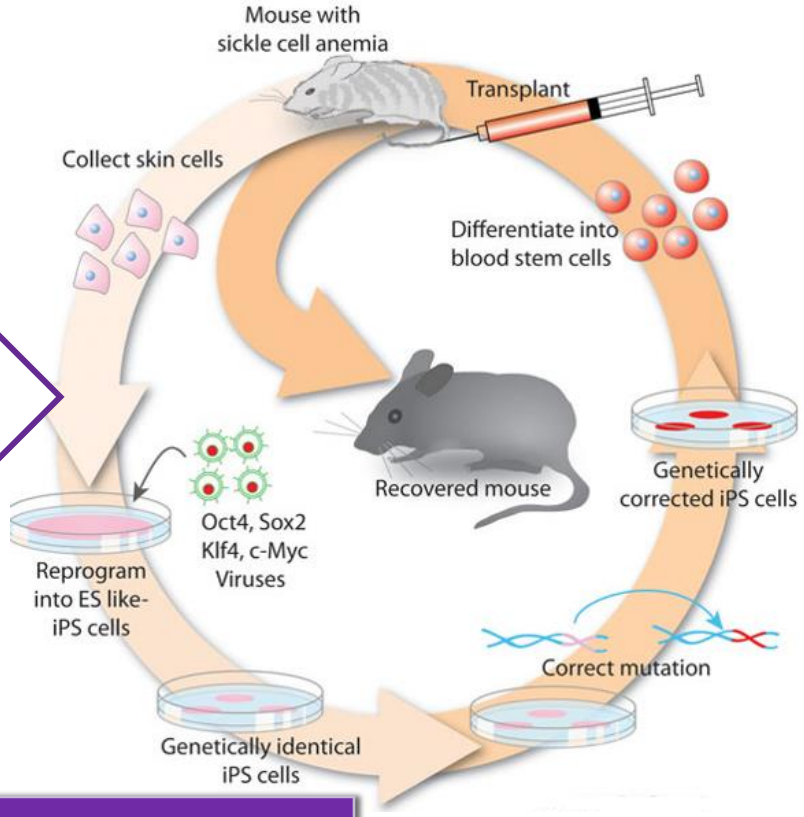
	Embryonic stem cells		Adult stem cells
	In Vitro Fertilization	Nuclear Transfer	Adult Stem Cells
advantages	• Produce all cell types • Abundant in IVF clinics • Egg can be tricked of being fertilized by an electric shock	• Produce all cell types • abundant in IVF clinics • Genetic matching to specific patients	• Genetic matching to patients • Shows promise in therapies.
disadvantages	• Limited cell lines. • Could produce teratomas	• Not available for Humans • Could produce teratomas	• Limited cell types. Difficult to isolate. • Not found in all tissues.
Ethical issues	• Requires consent. • Destroys Blastocyst	• Requires consent. • Destroys Blastocyst	-

Embryonic Stem Cells (ESC)	Adult Stem Cells (ASC):
• IVF embryos • Aborted embryos • cloned embryos	• Bone Marrow • Placental Cord • Mesenchymal Stem cells
Pluripotent	Multipotent
large number can be harvested	Limited numbers and more difficult to isolate
• immune rejection • Ethical concerns	• No immune rejection • No Ethical concerns

IPSCs (induced pluripotent stem cells):

- somatic cells that have been reprogrammed to a pluripotent state (embryonic stem cell like state).
- Return the differentaited cells (skin fibroblast, bone..) to their embryonic state

The first IPSCs was done by:
Takahashi and Yamanaka



Process:

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

The cells were differentiated into hematopoietic cells

The produced cells were transfused back into the sick mouse

Factors used in inducing PSCs:

Yamanaka factors	Thomson factors
OCT4, SOX2, Klf4,(Myc)	OCT4, SOX2, NANOG, Lin28

How does IPSC happen?

These genes code for specific transcription factors and are already found in our genome, but are added to increase or induce their transcription. These gene(**Oct8/Sox2/c-Myc/Klf4)**
then work on inducing and inhibiting other genes that cause the cell to reverse back in its lineage to its embryologic pluripotent stem cells (Dedifferentiation). These cells are then used for research mainly.

Challenges with Embryonic Stem Cells

- Developing chromosomal abnormalities in lab
- Extraction
- Cells needs to be properly differentiated
- SCs need to be monitored regularly after injection for therapy
- Host rejection
- Mouse feeder cells and xenotransplantation reactions

Goal of Stem Cell Therapies:

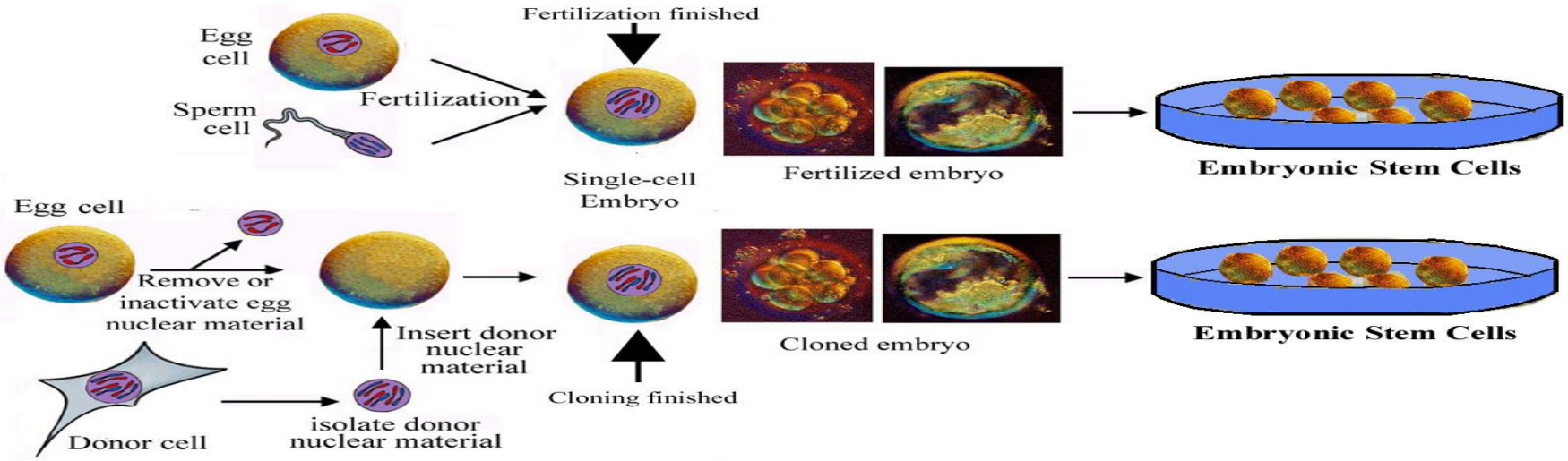
to promote cell replacement in organs that are damaged and do not have the ability for self repair

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

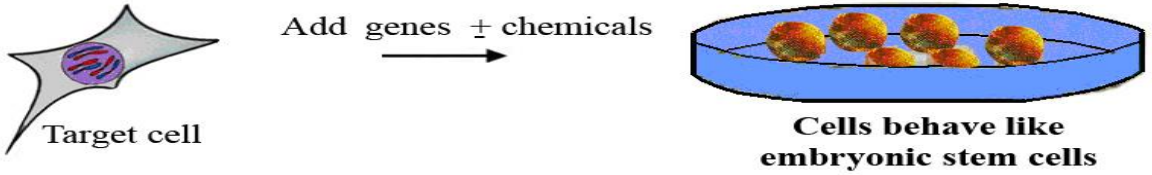
Embryonic Stem Cells

from Embryos created by Fertilization or by Cloning (Somatic Cell Nuclear Transfer)



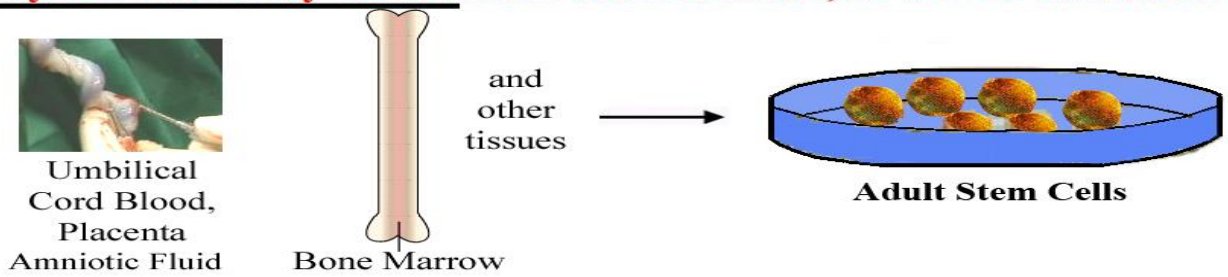
Induced Pluripotent Stem Cells (iPS cells)

from Normal Cells that are Reprogrammed to behave like Embryonic Stem Cells



Adult Stem Cells

Stem Cells normally found in body tissues from birth onward, as well as umbilical cord, etc.



MCQ's

1. Which of the following is a source of adult stem cells?

A. Morula
B. Placenta
C. Bone Marrow
D. Nuclear Transfer

2. Adult stem cells are :

A- totipotent .
B- Multipotent .
C- unipotent .
D- tripotent .

3. Mesenchymal can differentiate into:

A- glial cells
B- adipocyte
C- blood cells
D- neurones

4. OCT4, SOX2,Klf4, Myc are:

A- Viruses
B- protiens
C- gene correctors
D- vitamins

5- in (In Vitro Fertilization) the egg can be fertilizes by:

A- another egg
B- sperm
C- electric shock
D- unneucletaed cell

6-If differentiation excess the self renewal that will cause:

A- cancer
B- degeneration
C- normal amount of stem cells
D- none of above

7. Which of the following are pluripotent stem cells?

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 cell 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

8. Important limitation of using cloned ESCs (SCNT-ESCs) clinically:

a. Immune rejection
b. Produce limited number of cell types
c. Destruction of human embryos
d. Difficult to grow and culture in the laboratory

9.What are Yamanaka factors?

a. OCT3/4, SOX2, KLF4, c-Myc
b. Growth factors
c. Cytokines
d. OCT3/4, SOX2, Nanog

10. Mesenchymal stem cells are examples of:

a. Pluripotent stem cells
b. Multipotent stem cells
c. Totipotent stem cells
d. Induced pluripotent stem cells (iPS cells)

GOOD LUCK DOCTORS

Answers:
1. C
2. B
3. B
4. A
5. C
6. B
7. D
8. C
9. A
10. B

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