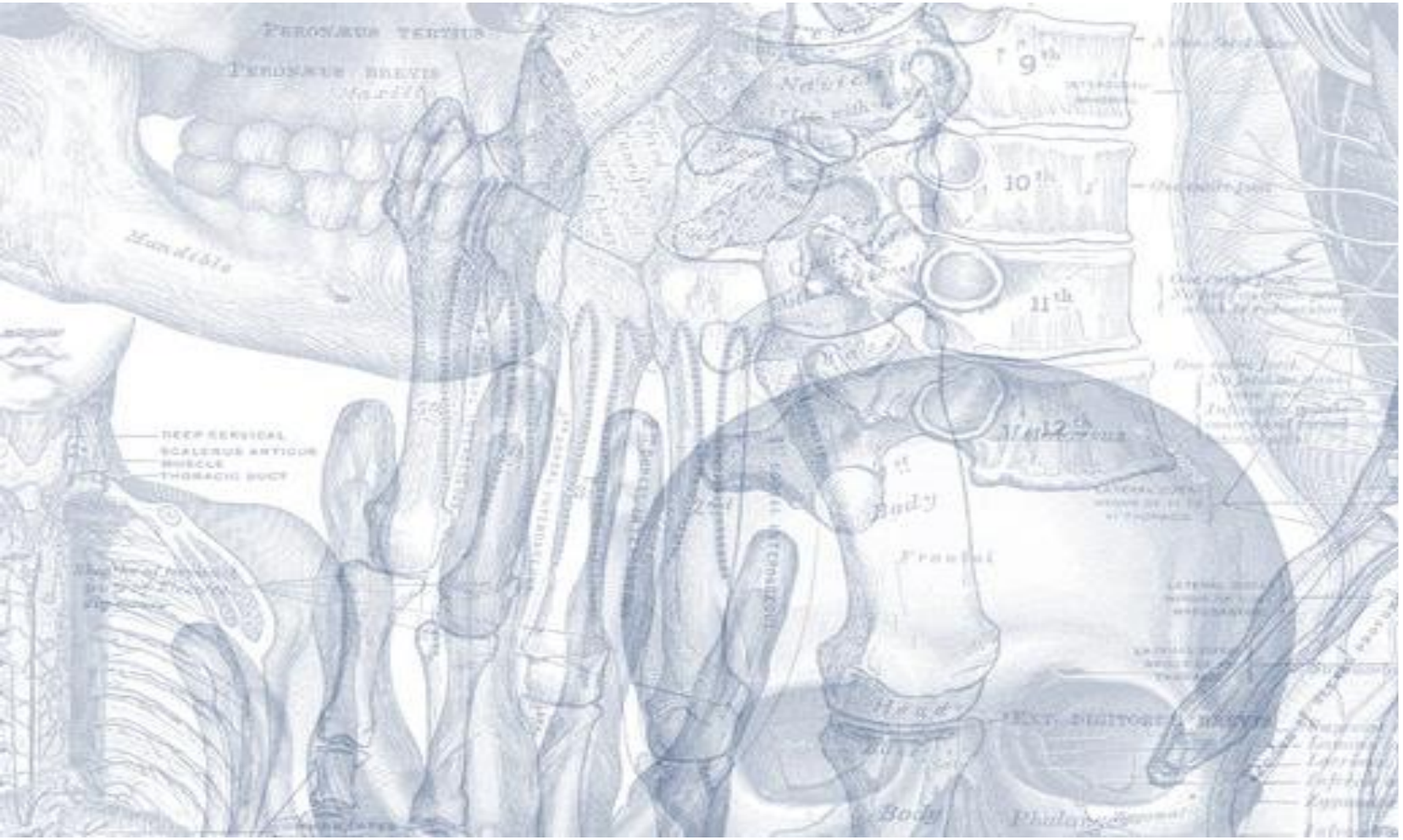
بسم الله الرحمن الرحيم



ANATOMY
TEAM 436

436
MEDICINE
KING SAUD UNIVERSITY

تمت المراجعة
شوق الأحمري & عبدالعزيز العنقري

Introduction to Pluripotent Stem Cells

Color Code

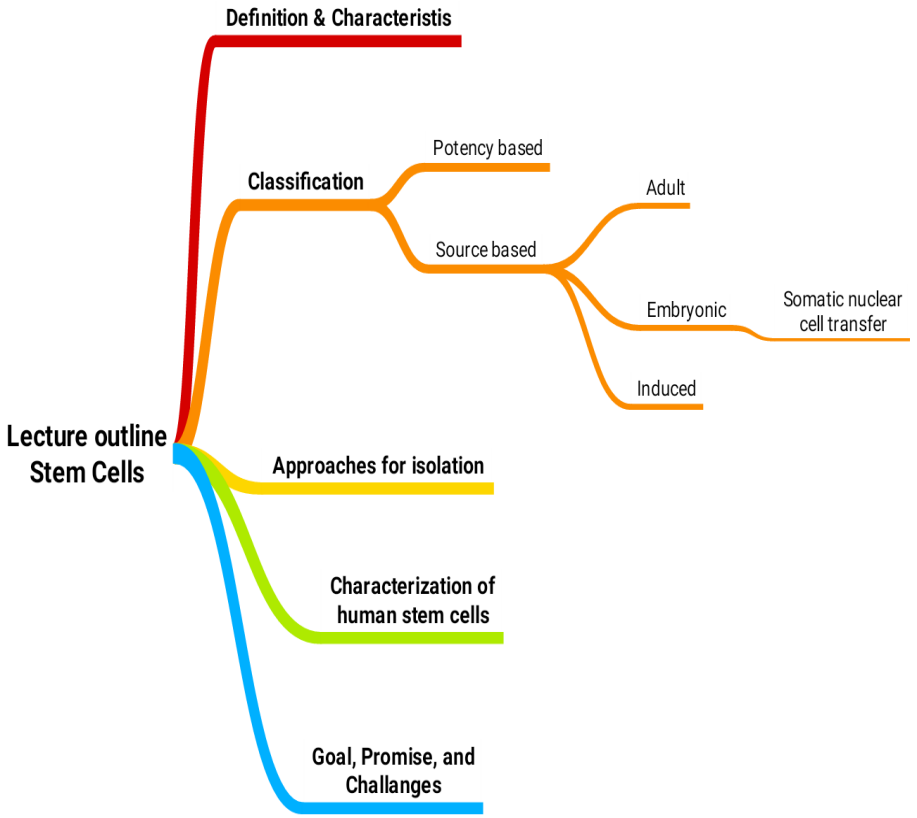
- **Important**
- **Doctors Notes**
- **Notes/Extra explanation**

Please view our Editing File before studying this lecture to check for any changes.

Objectives

By the end of the lecture you should be able to:

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



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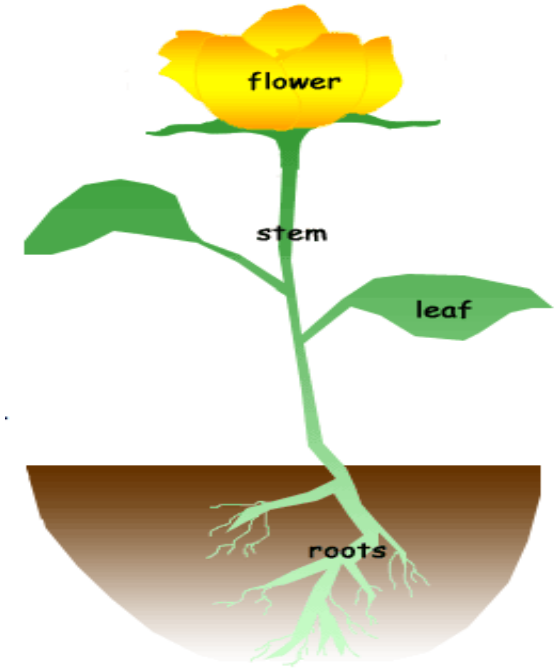
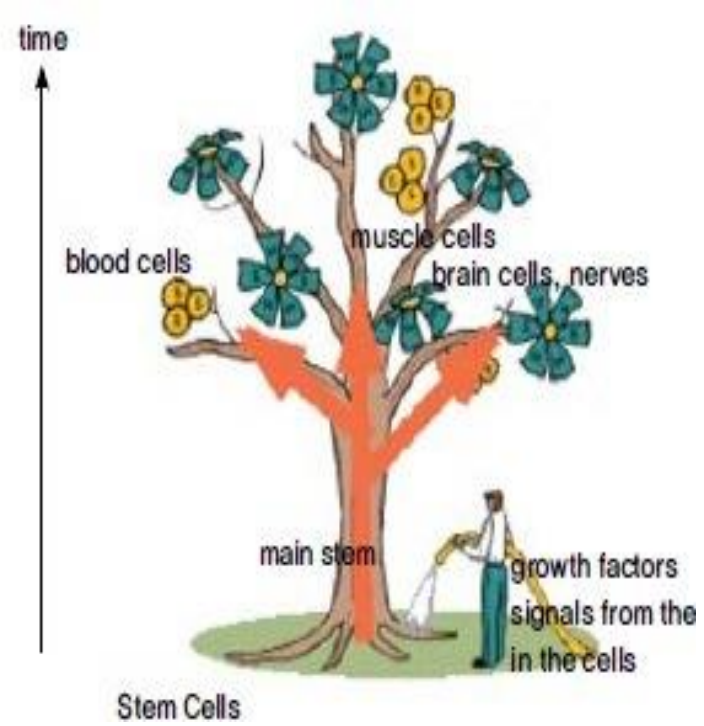
Stem Cells: Introduction

When we hear stem cells all these words come to mind but in fact the **main goal** of stem cell is to provide a cure and regenerate cells which couldn't regenerate





Here we can compare the stem cells to the roots of the plants. The root gives the stem which can give leaves and flowers, and in the same way stem cells can differentiate depending on the micro-environment factors into more than 200 types of cells, example muscle, nerve, blood, etc.

Stem Cells

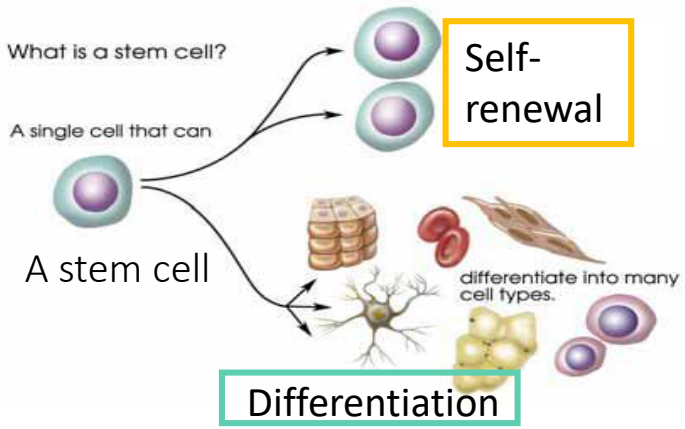
Definition

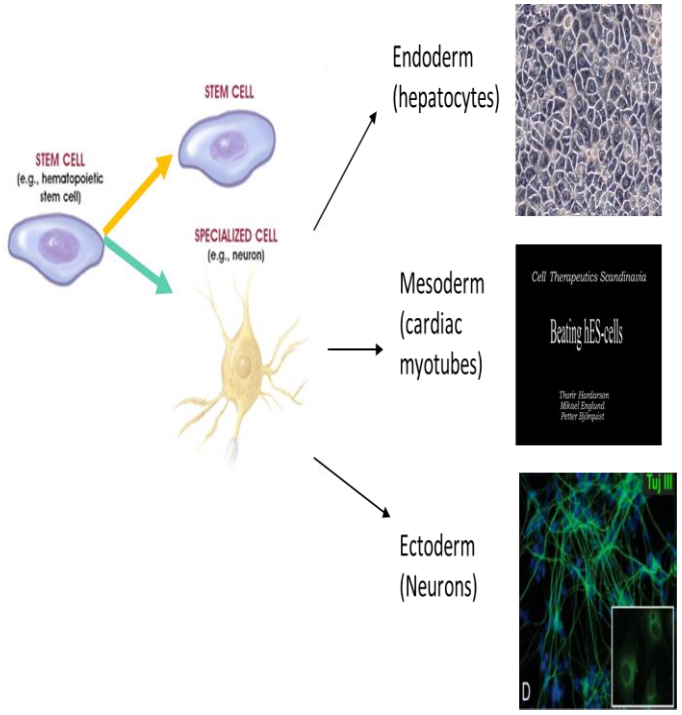
- A cell that has the ability:
  - to continuously divide and give rise to new copy of itself (self-renew)
  - and other specialized (differentiated) cells/tissues.
- Stem cells divide to new cell that has the potential to either remain a stem cell or become another type of cell with a more specialized function as cells of the **blood**, **heart**, **bones**, **skin**, **muscles**, **brain** etc, serving as a sort of repair system for the body.
- Main function within the body:
  - Continuous Repair of defective cell types and regeneration of tissues.

Example: diabetes treatment were the cells differentiate into islets of langerhan (which produce insulin) then we transplant it into the human.

We classify stem cells in 2 ways:

1. According to potency: totipotent, pluripotent multipotent, unipotent, etc.
2. According to source: embryonic, adult





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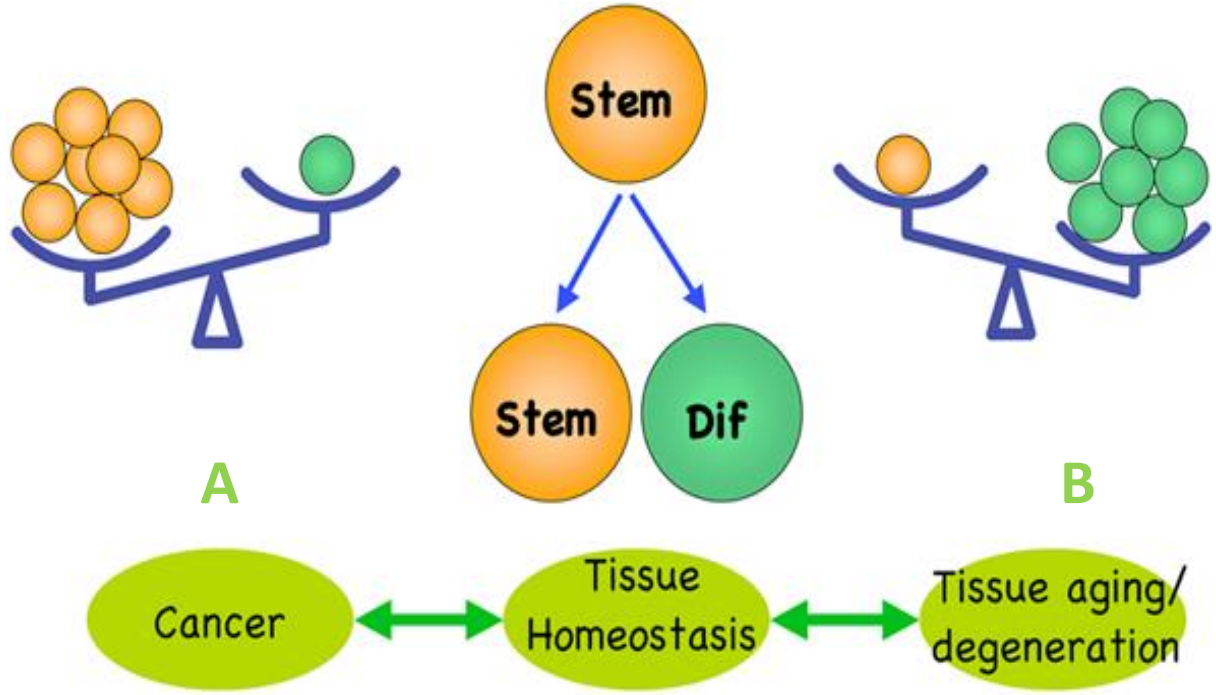
Stem Cells

Unique Characteristics of Stem Cells

- Unlimited self renewal (Regeneration) it divides to give copies of itself and this is the main idea behind treating degenerative disease.
- Differentiation (eg. beating cells of the heart muscles) which depends on:
  - Internal signals (specific genes)
  - External signals (GF, cytokines) → they amplify the microenvironment around cells

For tissue homeostasis or health to be maintained there has to be a **balance** between these two characteristics. If there is imbalance:

A. Too much regeneration and unlimited dividing will result in cancer
B. Too much differentiation without enough regeneration will lead to aging and degeneration



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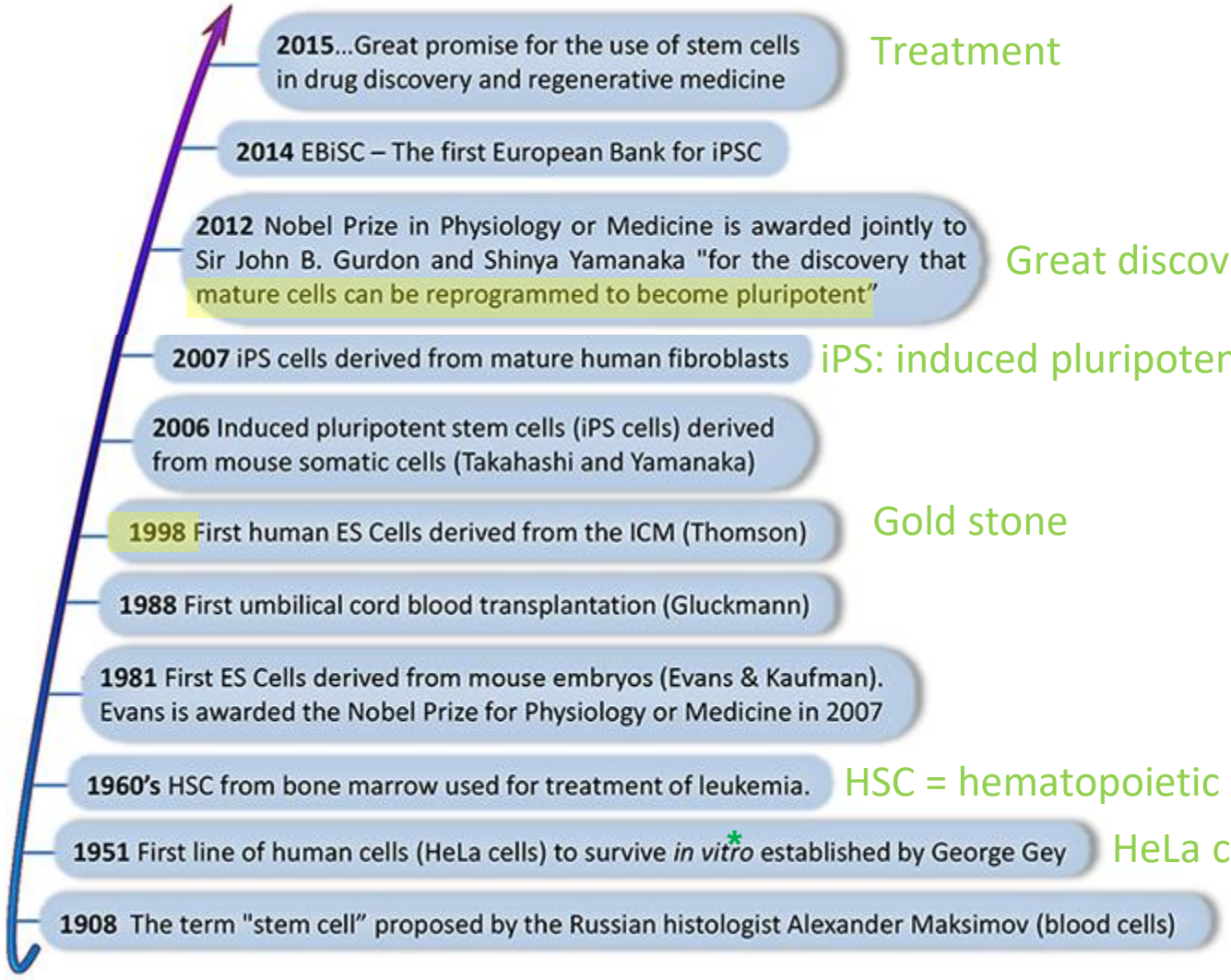
The History of Stem Cells *for your information*



Treatment

Great discovery

iPS: induced pluripotent stem cell

Gold stone

HSC = hematopoietic stem cell transplanted to leukaemia patient

HeLa cells can divide unlimitedly

* In vitro= Outside the body

Classification of stem cells (**potency** based)

Important

Potency = قدرة الخلية

(morula) **Totipotent (Total)**

1-3 days, differentiate into (intra)embryonic and extraembryonic cell types.

(blastocyst) **Pluripotent (plural)** Most important one that we use

Descendants of totipotent cells and differentiate into cells of **3 germ layers**

Without placenta and extraembryonic cell types

Multipotent (multiple)

Produce cells of **closely related** of cells (e.g. hematopoietic from bone marrow) family stem cells

Oligopotent محدود

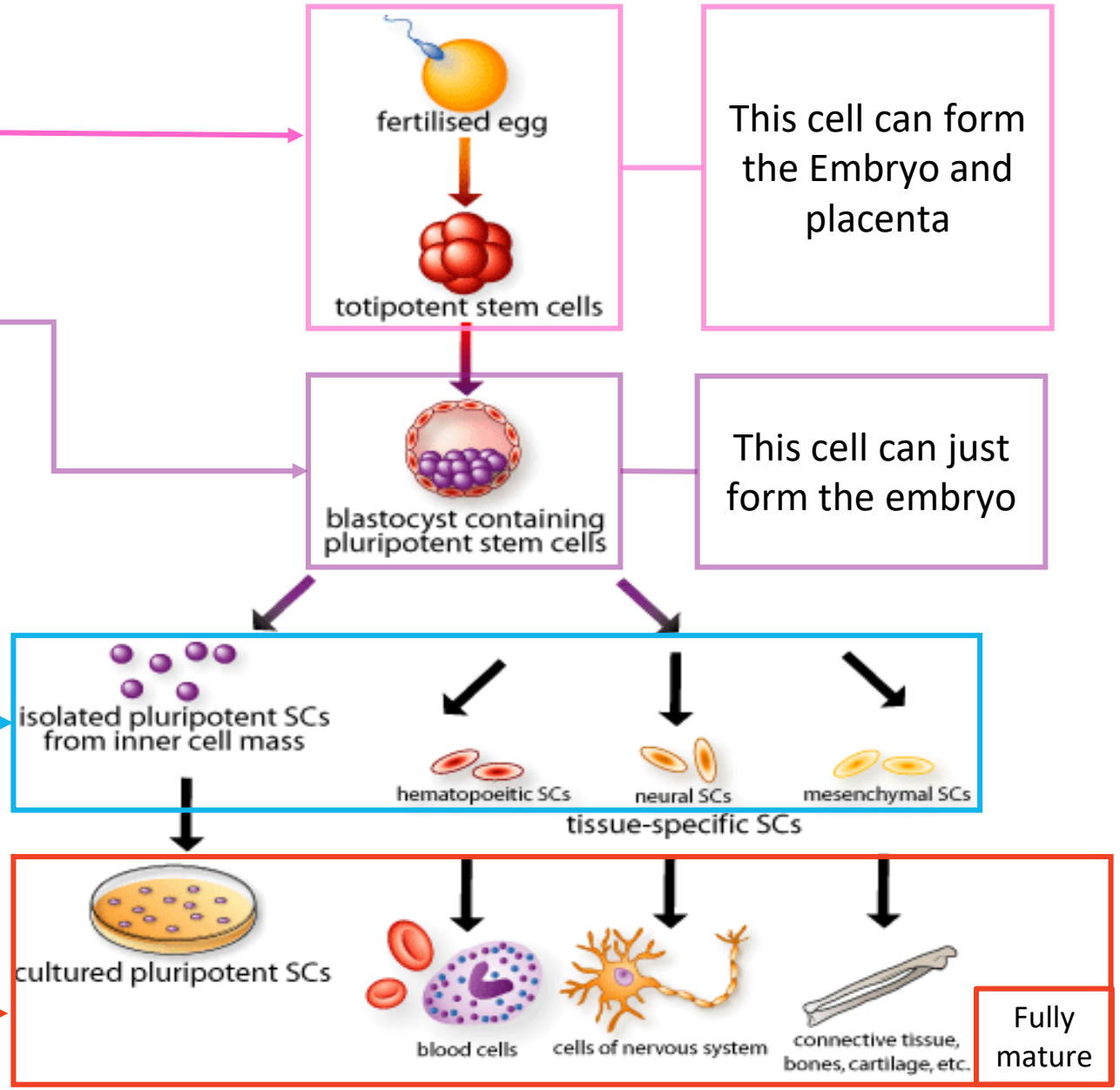
Differentiate into ONLY a **few cells**, such as lymphoid or myeloid stem cells

Unipotent

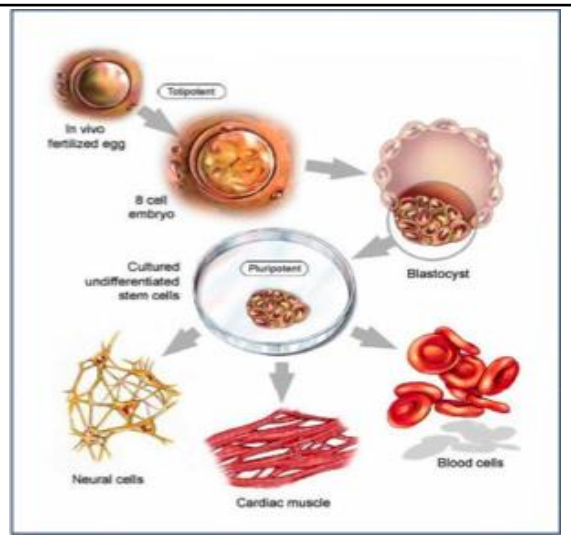
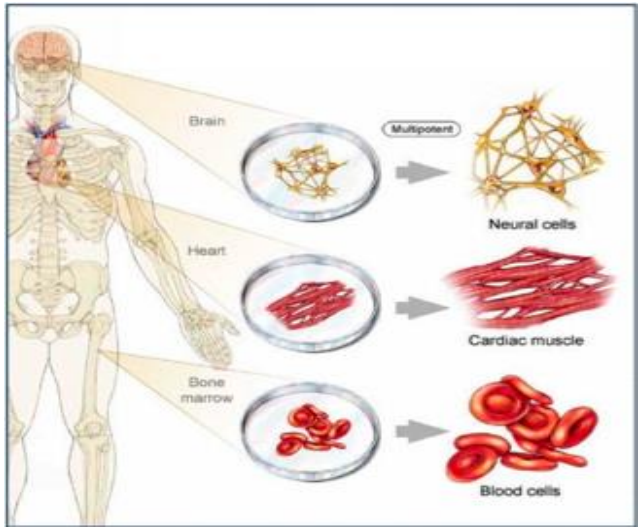
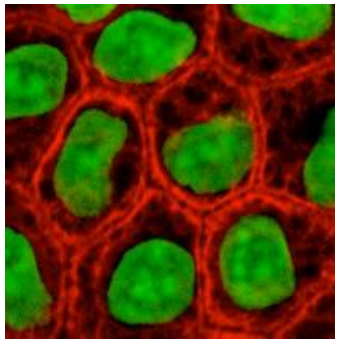
Produce ONLY **one** cell type (e.g. muscle stem cells & testes)

Nullpotent

The terminal cells

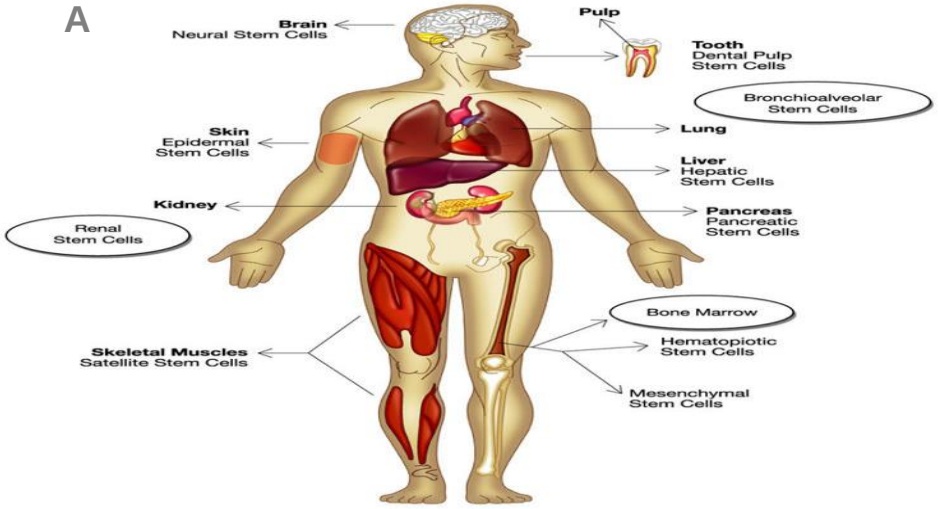


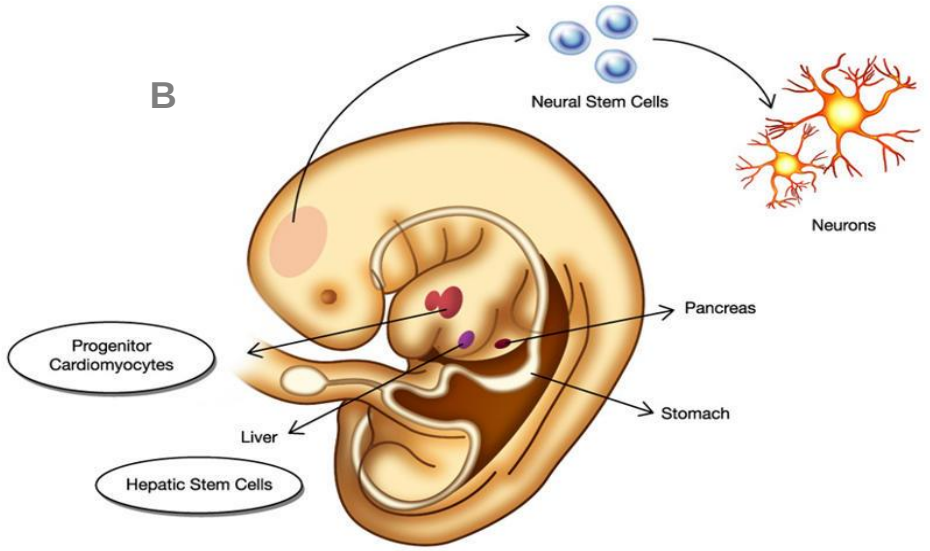
Classification of stem cells (**source** based)

	Embryonic Stem Cells (ESC)	**Adult Stem Cells (ASC)**
Source	✿ IVF embryos ✿ Aborted embryos ✿ cloned embryos	✿ Bone Marrow ✿ Placental Cord ✿ Mesenchymal Stem cells
Potency	✿ Pluripotent ✿ large number can be harvested advantage	✿ Multipotent ✿ Limited numbers and more difficult to isolate disadvantage
Note	✿ May cause immune rejection ✿ Ethical concerns disadvantage	✿ No immune rejection (Because it comes form the patient to the same patient) ✿ No Ethical concerns advantage
Picture	 	 

Now we will discuss each type in more detail

1- Adult* stem cells (Tissue Specific Stem Cell)





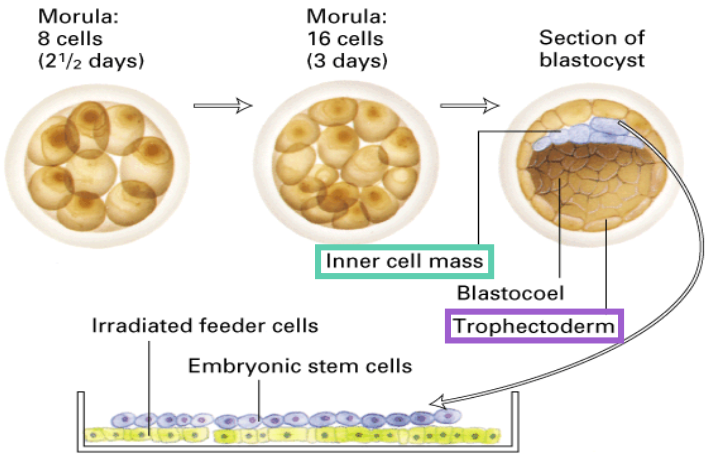
* Adult means any cell after day 14 when it becomes multipotent. note that there is a difference between embryonic and fetus stem cells. Fetus stem cells are considered adult stem cells

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

B. They also can be isolated of developing embryos' different tissues (after Day 14)

- Can be derived from:
  - Bone marrow
  - Placental cord
  - Mesenchymal stem cells.
  - Menstrual blood may also contain stem cells
  - Mothers milk can also contain adult stem cells (which reminds us of القرابة بالرضاعةsince some stem cells actually transfer from the mothers milk to the baby)

2- Embryonic Stem Cell (ESC)



- Embryonic human stem cells were first isolated in 1995 by Dr. James Thomson.
- Derived from 4-5 day old embryo (Blastocyst) (IVF*, SCNT**) which consists of:
  - Trophoblast
  - Blastocoel
  - **Inner Cell Mass** (ICS) this is the part we care about

*IVF: In Vitro Fertilization is a process of fertalization by extracting eggs and a sperm, manually combining them in a laboratory dish then transferring them to a uterus
**SCNT: Somatic Cell Nuclear Transfer (will be discussed later)

- **Generation of embryonic stem cells:**

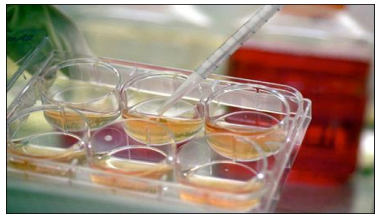
Isolate and transfer of ICS into culture dish in culture media

1

Culture at 37c and 5% CO2

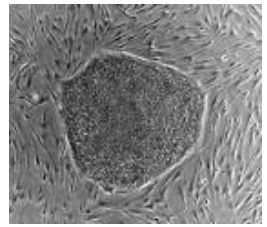
2

Inner surface of culture dish is coated with inactivated MEF*s as a feeder layer: provides sticky surface for attachment and release nutrients



3

Cells divide and spread over the dish



4

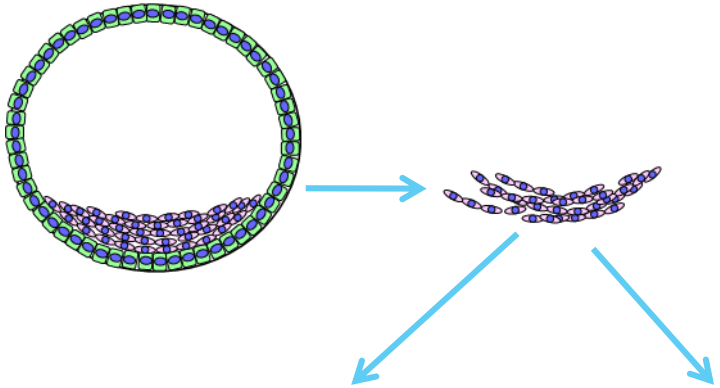
ESCs are removed gently and plated into several different culture plates.

5

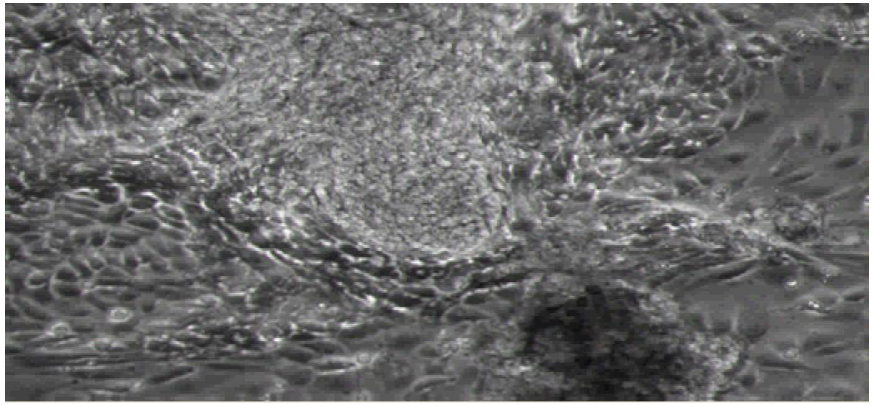
*MEF: mouse embryo fibroblast, they are used as a feeder layer to prevent differentiation of embryonic stem cells. They stop the division but the cells are still alive

2- Embryonic Stem Cell (ESC)

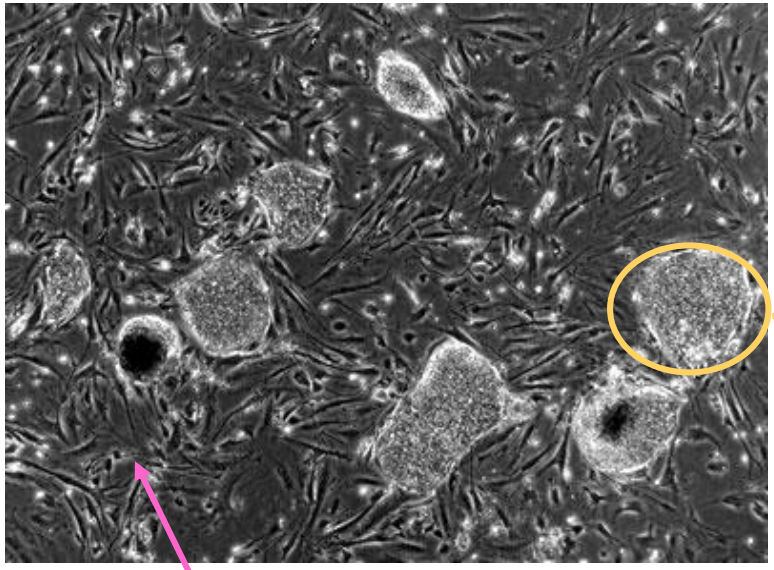
What do cultured ES cells look like?



Beating cardiomyocytes derived from hESCs

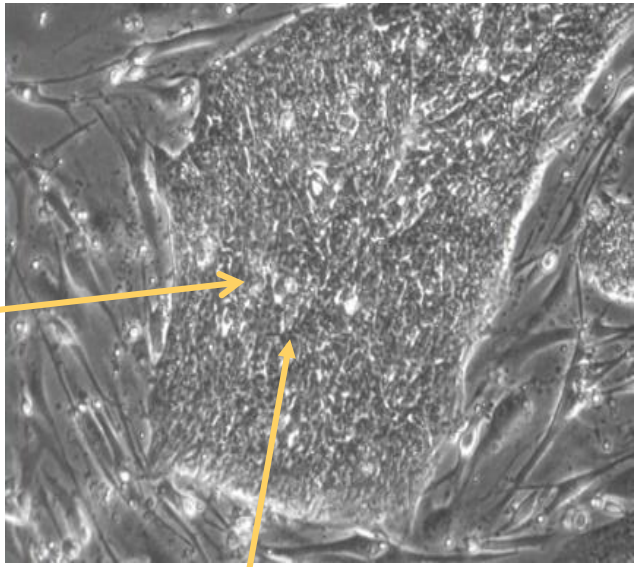


This is a video showing cardiac cells beating. (to view it download the ppt version)

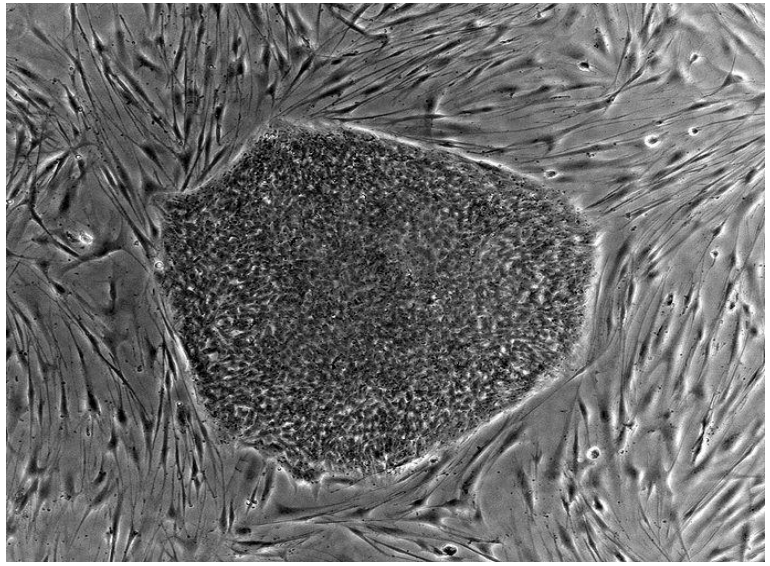


Mouse embryonic cells (feeder layer)

The lines around that surround ESC



Embryonic stem cell colony with distinct border



Embryonic stem cells in the dish

2- Embryonic Stem Cell (ESC)

Challenges with Embryonic Stem Cells

- Recently, **abnormalities in chromosome** number and structure were found in some human ESC lines. (long term and long time culture lead to abnormal chromosomes)
- Stem cell development or proliferation **must be controlled** once placed into patients. (to not cause cancer)
- Stem cells need to be **differentiated** to the appropriate cell types **before** they can be used clinically. (if they are inserted before they are differentiated they might multiply and form cancer)
- The use of mouse "feeder" cells to grow ESC could result in problems due to **xenotransplantation***. (the feeder layer is supposed to be removed before we use the ESC but all isolation methods don't guarantee not having feeder layer in the sample. Now there are medias to grow without feeder layer)
- Possibility of **rejection** of stem cell transplants as foreign tissues is very high.

*xenotransplantation: process of transplanting tissues between organisms.

Somatic Cell Nuclear Transfer (SCNT)

A new technique to prevent graft immuno-rejection

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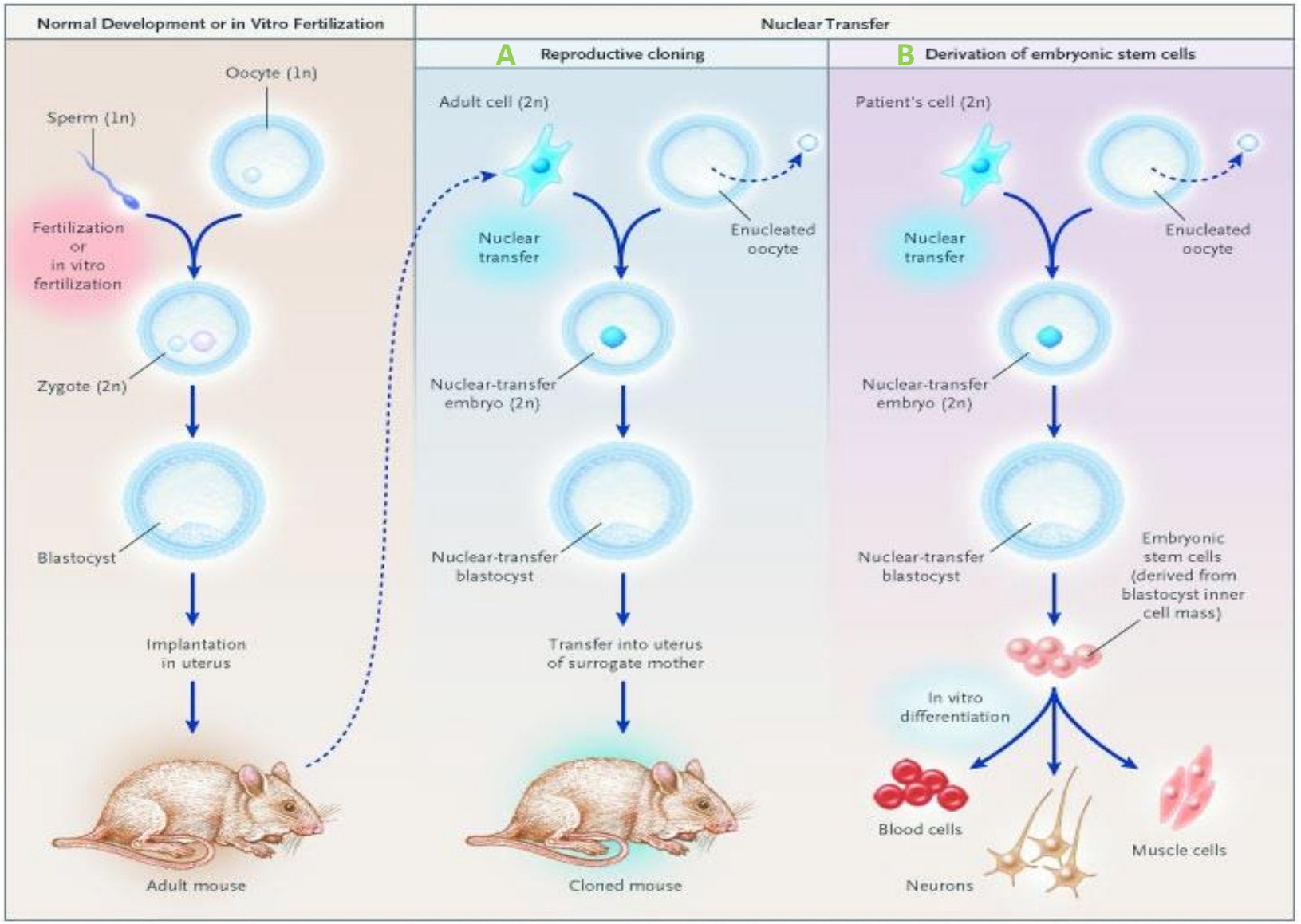
- Recall that all cells have 46 chromosomes except sex cells which have 23 chromosomes.
- In normal fertilization a sperm (23 chromosomes) and an oocyte (23 chromosomes) fuse to make a zygote (46 chromosomes) which becomes a blastocyst then continues to develop into a full organism.



- In nuclear transfer we take an oocyte and remove its nucleus then take a nucleus from a somatic cell (which already has 46 chromosomes) and insert it.
- Then it is exposed to electrical induction to divide and form blastocyst.
- Then if we want (A) reproductive cloning we transfer it to a surrogate mother or (B) we take the inner cell layer from the blastocyst and grow a specific type of tissue

Note: because the DNA is derived from the same person there is no immunological reaction

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Somatic Cell Nuclear Transfer (SCNT)

Nuclear transfer (cloning) can be used in 2 ways: reproductive (producing identical offspring) or therapeutic (which is the main goal)

Reproductive Cloning

Dolly is a sheep that was cloned from another sheep using the same method we discussed before. An oocyte was deprived of its nucleus and a different nucleus was inserted and the blastocyst was reinserted into a surrogate mother.

To learn more about dolly →

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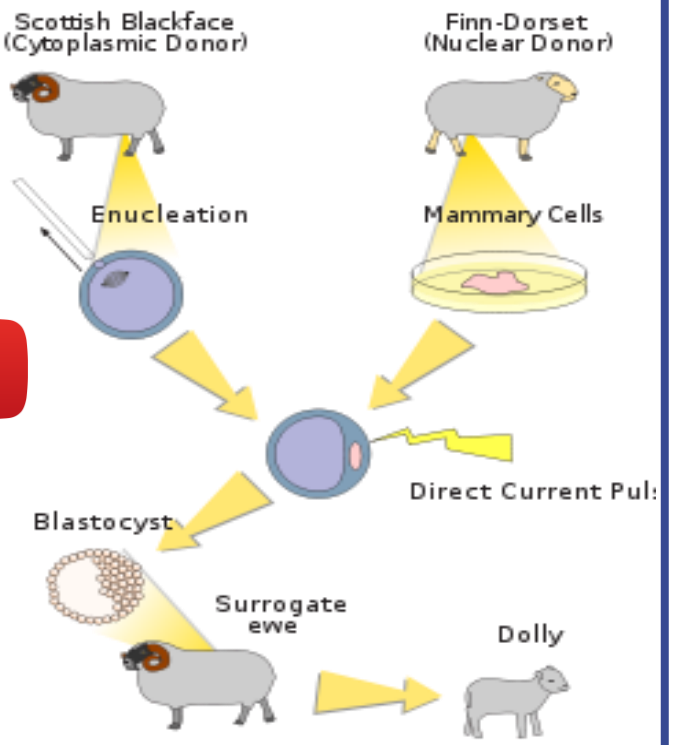
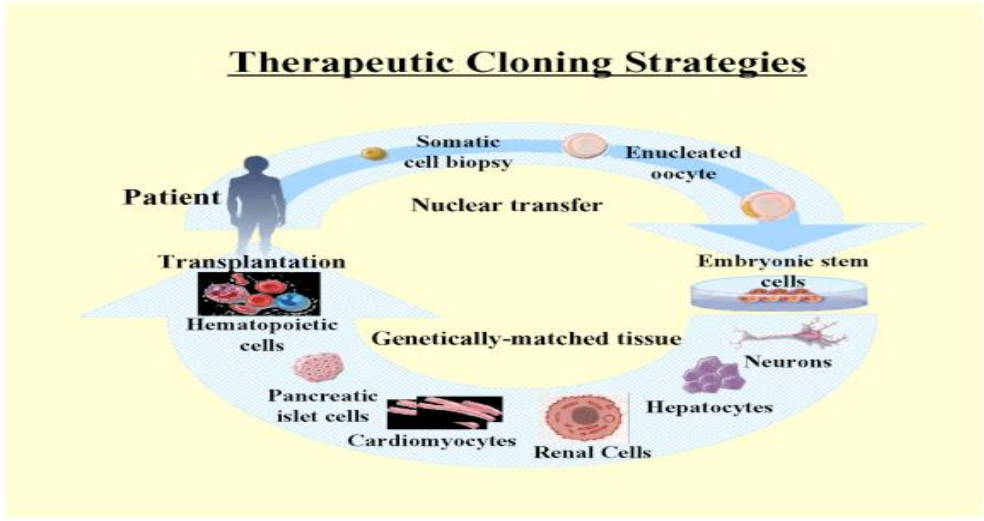
(July 1996 – February 2003)



Therapeutic Cloning

- Therapeutic cloning uses stem cells **to correct diseases** and other health problems that someone may encounter.
- Therapeutic cloning **does not cloned to make full humans** but rather is used for the stem cells of embryo



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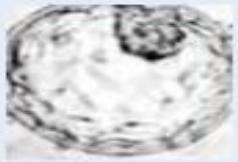
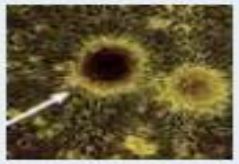
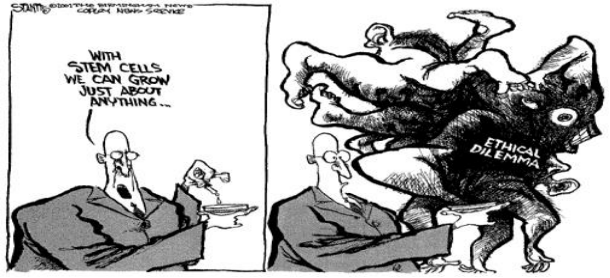
COMPARISON OF THE DIFFERENT SOURCES OF STEM CELLS

	Embryonic Stem Cells		Adult Stem Cells
	 **In Vitro Fertilization**	 **Nuclear Transfer**	 **Adult Tissues**
Attributes	• can produce all cell types • relatively easy to identify, isolate, maintain, and grow in the laboratory • large source of "excess" blastocysts from IVF clinics	• can produce all cell types • relatively easy to identify, isolate, maintain, and grow in the laboratory • stem cells may be genetically matched to patient	• demonstrated success in some treatments • stem cells may be genetically matched to patient
Limitations	• limited number of cell lines available for federally funded research • risk of creating teratomas (tumors) from implanting undifferentiated stem cells	some studies were successful • ~~not yet~~ achieved with human cells • risk of creating teratomas (tumors) from implanting undifferentiated stem cells	• produce limited number of cell types • not found in all tissues • difficult to identify, isolate, maintain, and grow in the laboratory
Ethical Concerns	• destruction of human blastocysts • donation of blastocysts requires informed consent	• destruction of human blastocysts • donation of eggs requires informed consent • concern about misapplication for reproductive cloning	• no major ethical concerns have been raised

A BIG problem stem cell researchers faced was the **ethical** concerns



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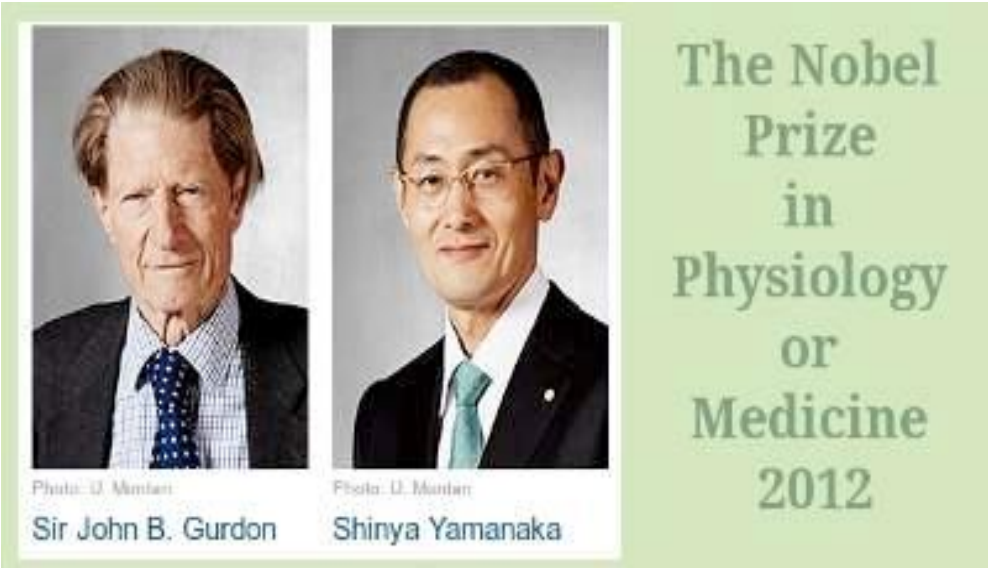
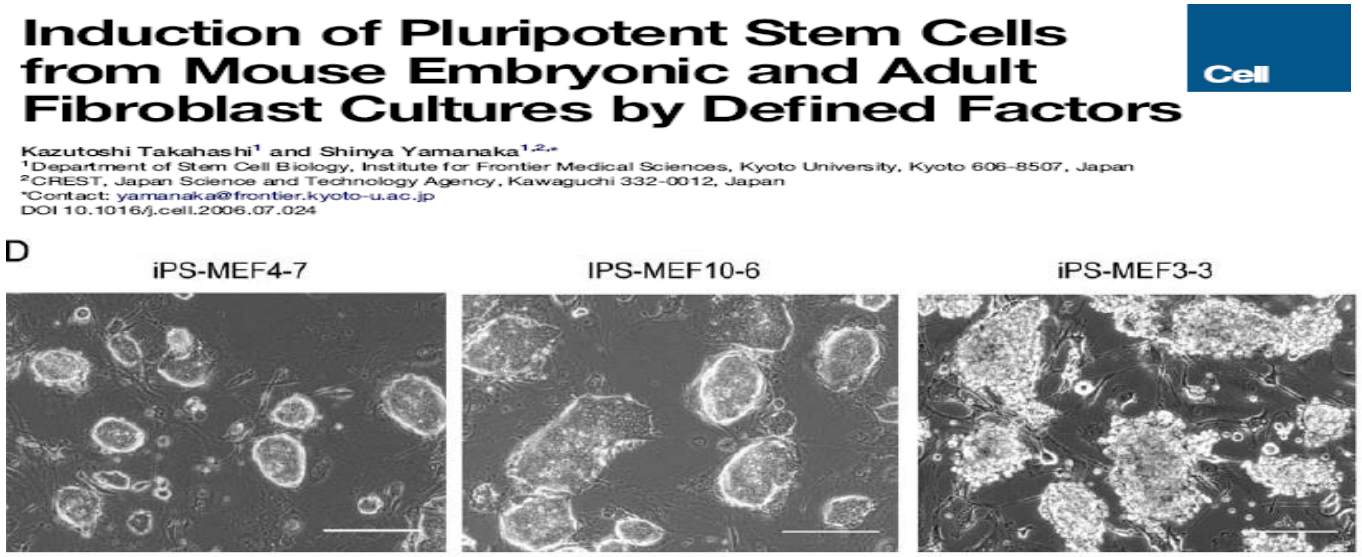
The First iPS

- 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.
- This was a milestone because they found a solution to the ethical dilemma and immunological reaction. They took somatic cells and redirected them to pluripotent stem cells. Because they did not use embryos (this eliminated the ethical problem) and they used cells from the same person (so no immunological reaction) This was a medical breakthrough for which they won Nobel prizes.





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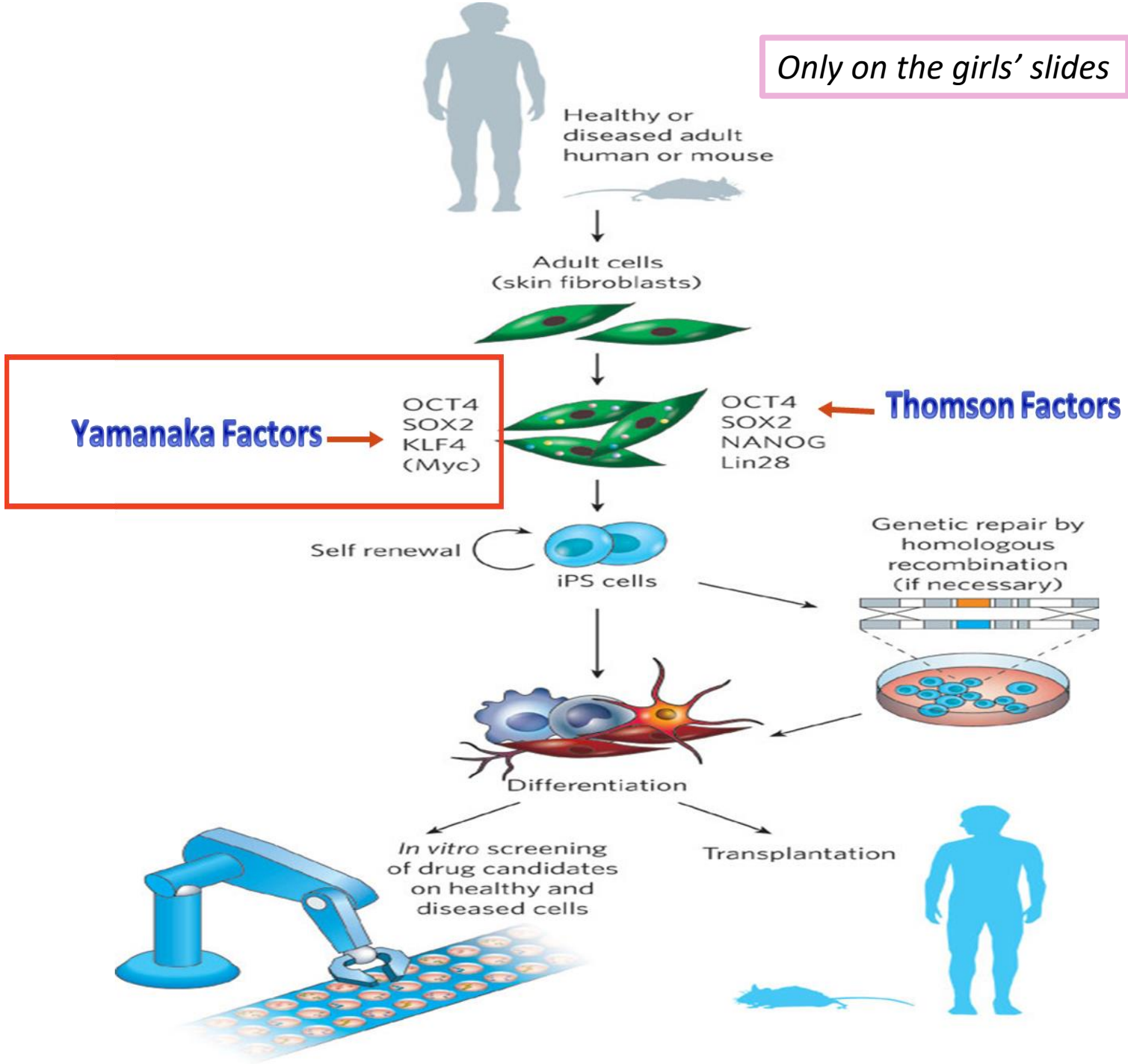
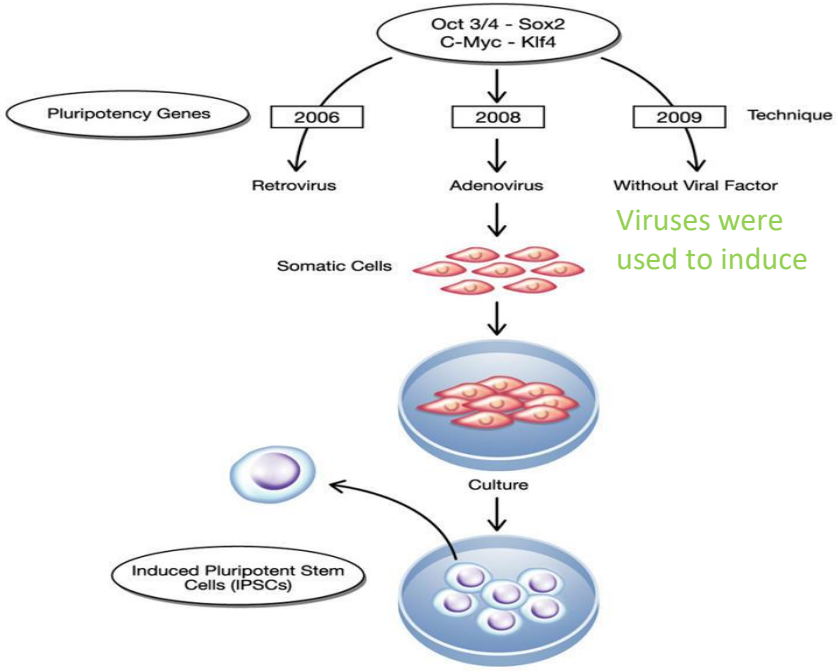
iPS Cells

- Yamanaka started with **24 pluripotent transcription factors** and did a lot of trials then found that **only four** are the ones that can be used (**now called Yamanaka factors OCT4, SOX2, KLF4 and Myc**).
- These factors reprogram somatic stem cells into pluripotent state. The transcription of the Yamanaka factors is done by viruses vectors (retrovirus and adenovirus and in 2009 they found it can be done without viruses).
- Another researcher called Thomson also found his pluripotent factors (OCT4, SOX2, NANOG and Lin28).
- After that the pluripotent stem cells can be differentiated in-vitro to the desired cell type.
- If there are any mutations causing a disease it can be fixed by homologous recombination before transplanting into the body.



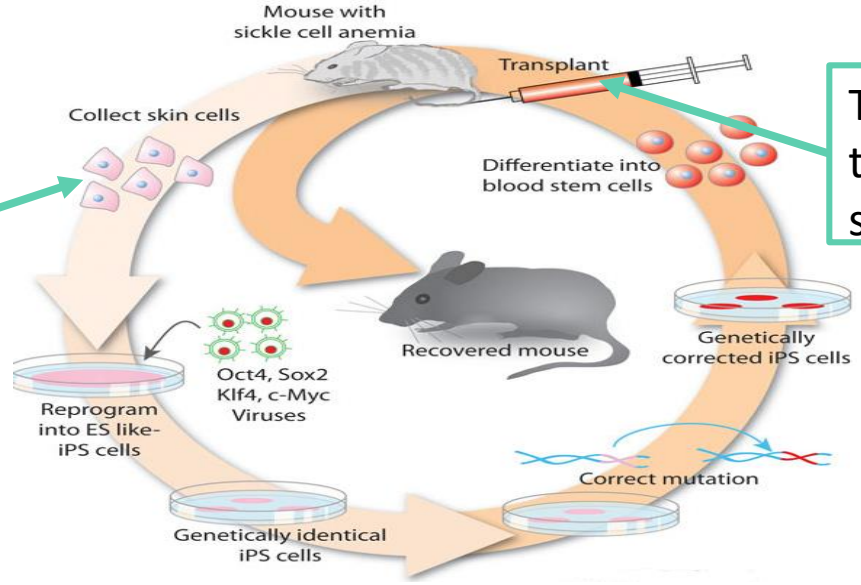
Induced Pluripotent Stem (iPS) Cells

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



Viruses were used to induce

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



The produced cells were transfused back into the sick mouse

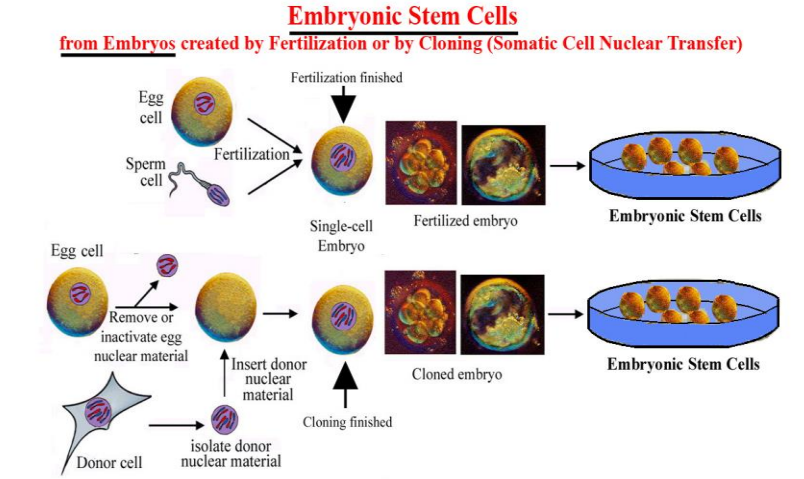
The cells were differentiated into hematopoietic cells.

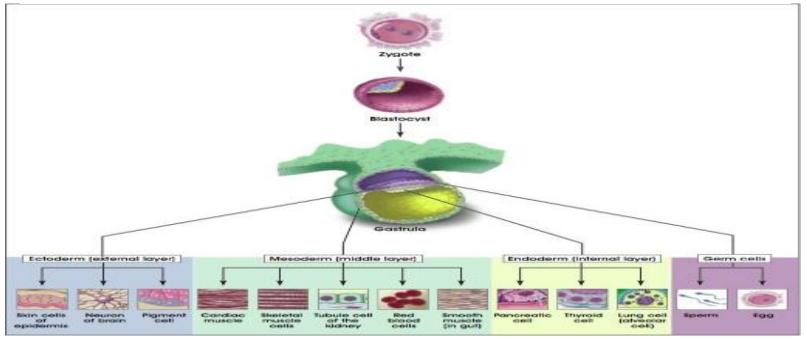
Classification of stem cells (**source** based)

After this breakthrough we now have 3 classes
(*this slide is a summary of everything we talked about before)*

1- Embryonic

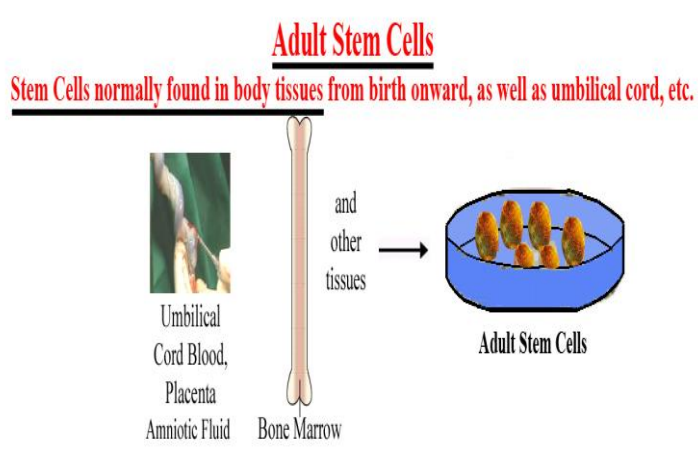
(pluripotent + totipotent)

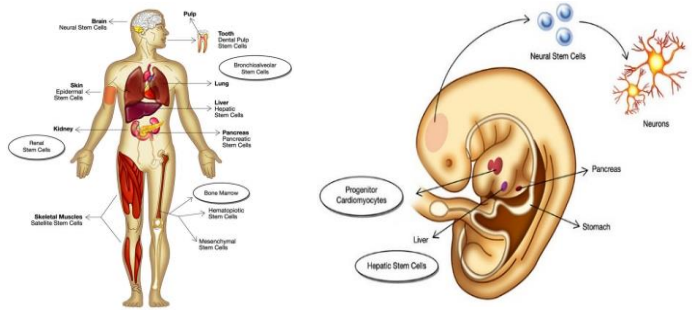




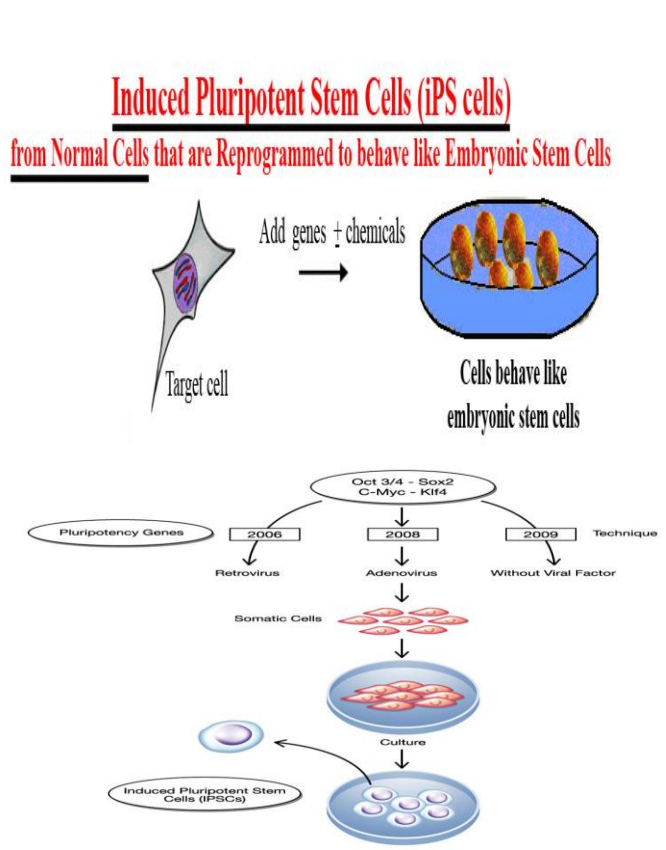
2- Adult (tissue specific)

(multipotent + oligopotent + unipotent)

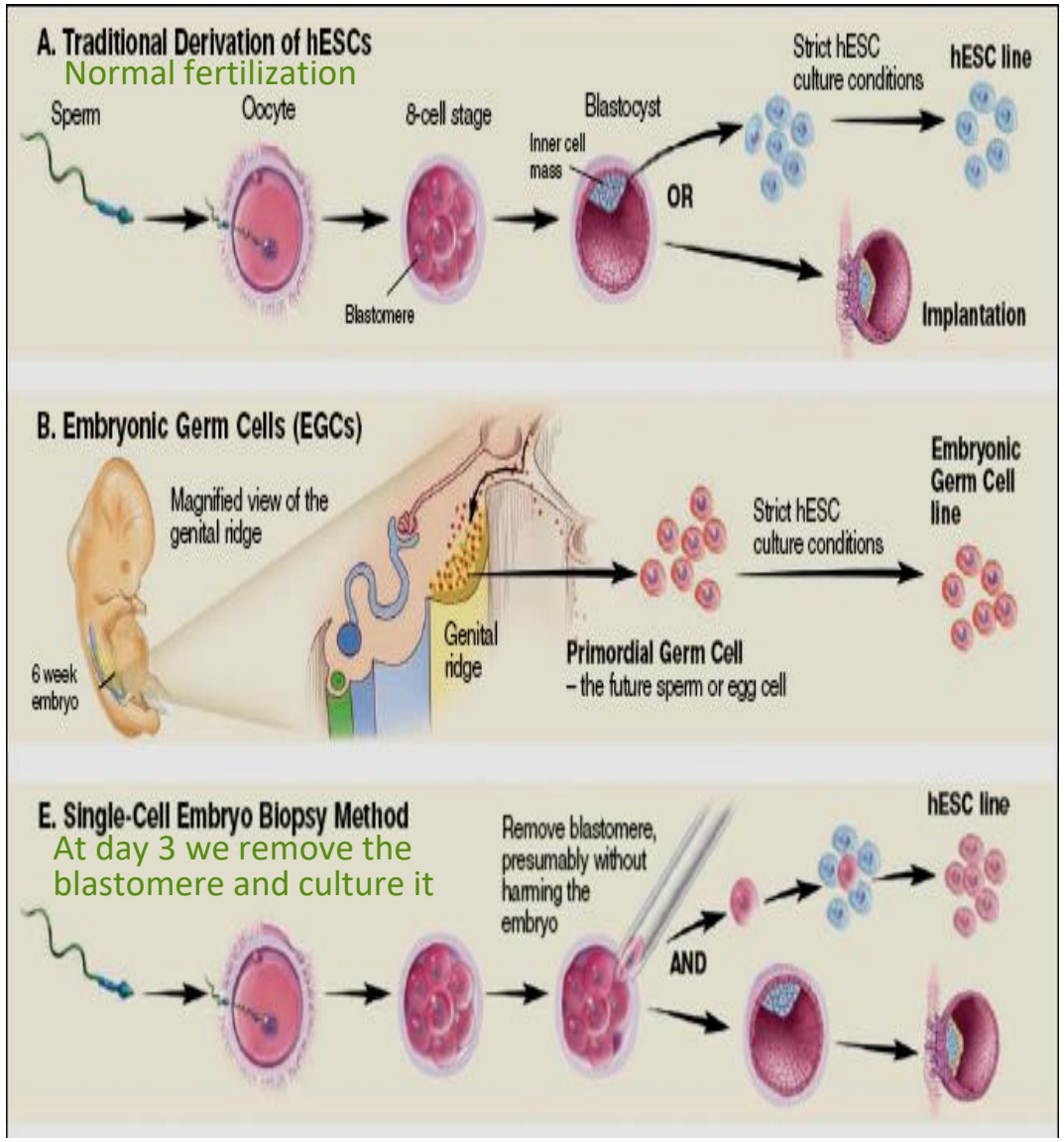


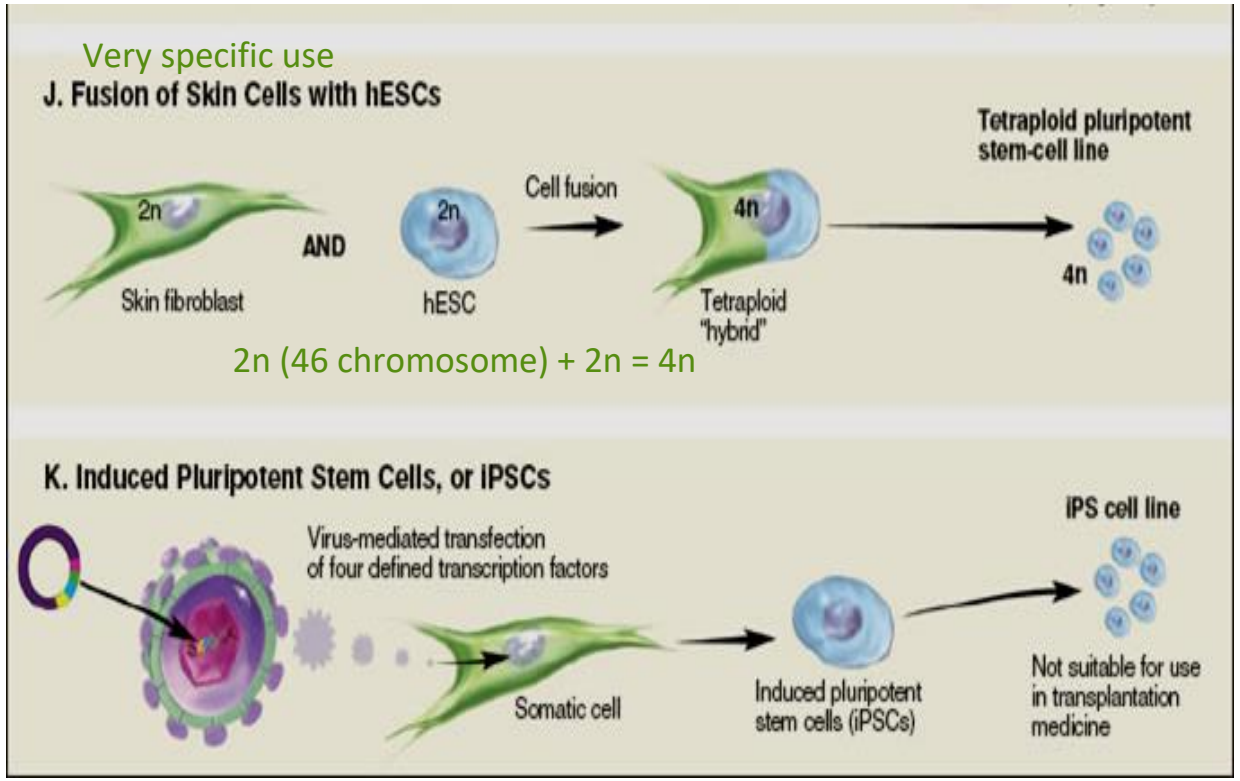


3- Induced

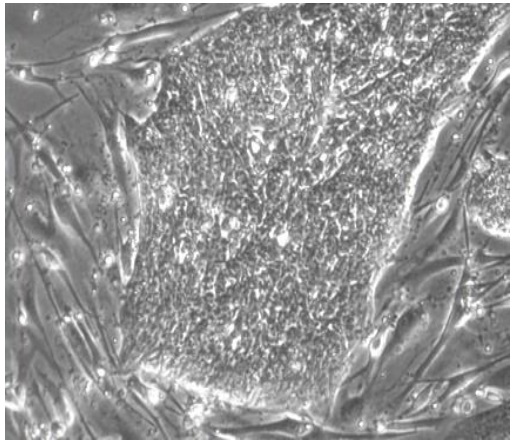


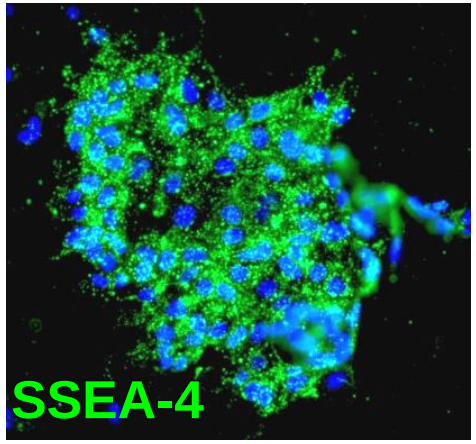
Different approaches for isolation of pluripotent stem cells

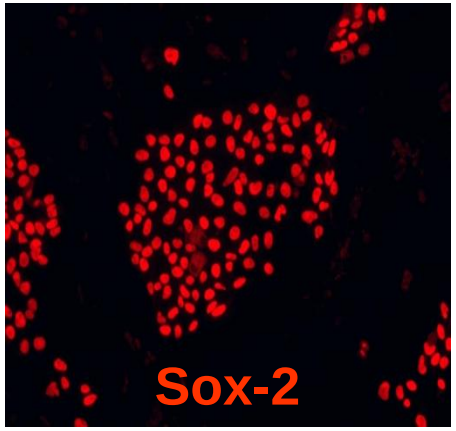


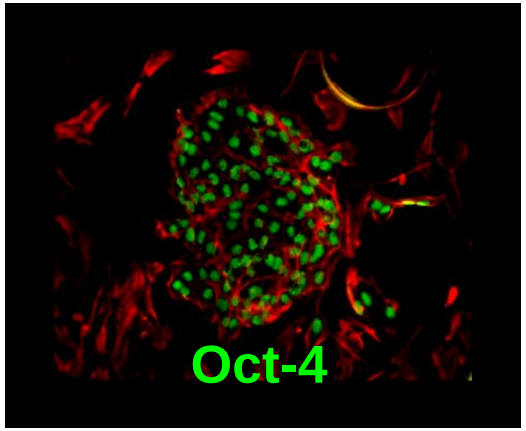


Characterization of human pluripotent stem cells (ESC)



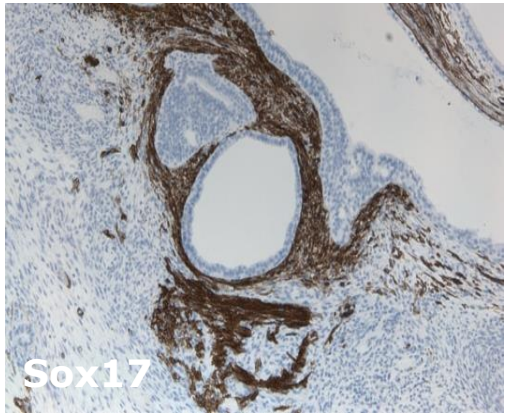




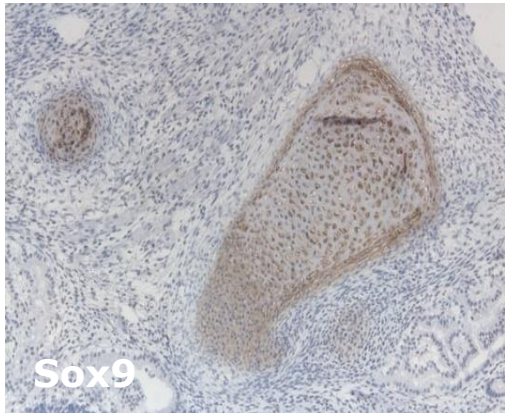


To check that the cell we have is a pluripotent stem cell we use special stains or markers (most common are **SOX-2** and **OCT-4)**

ENDODERM



MESODERM



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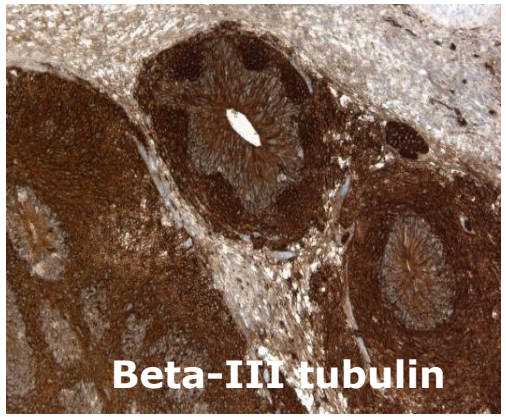
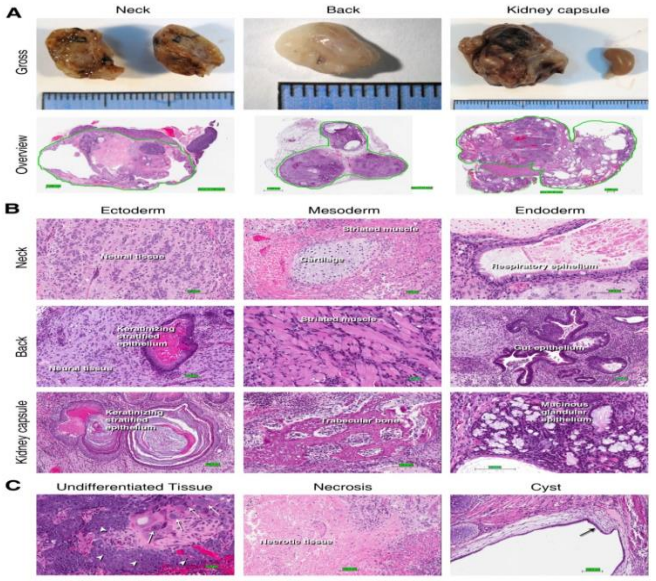
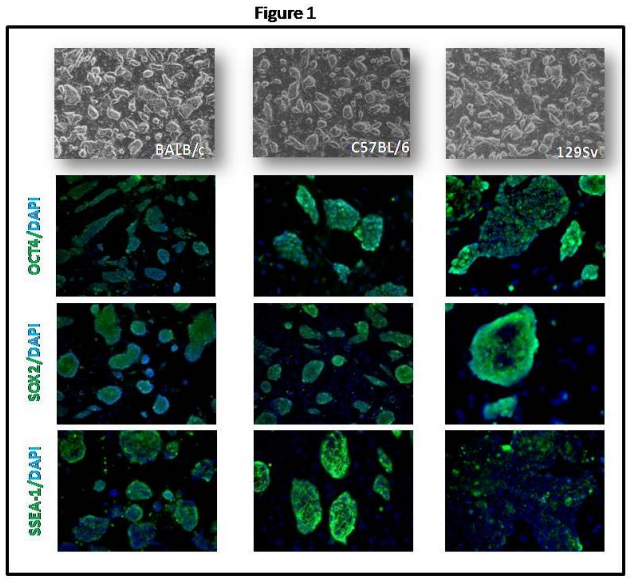
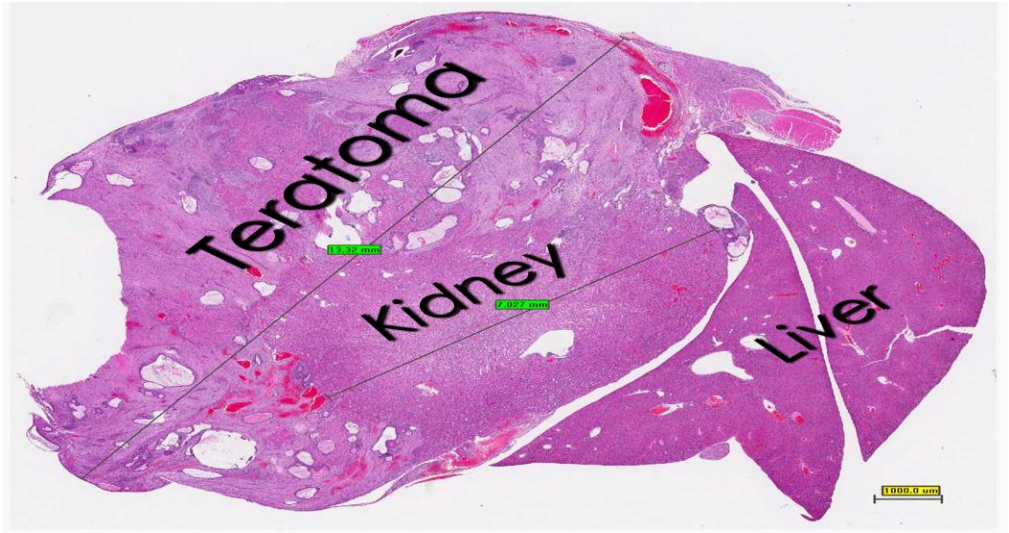


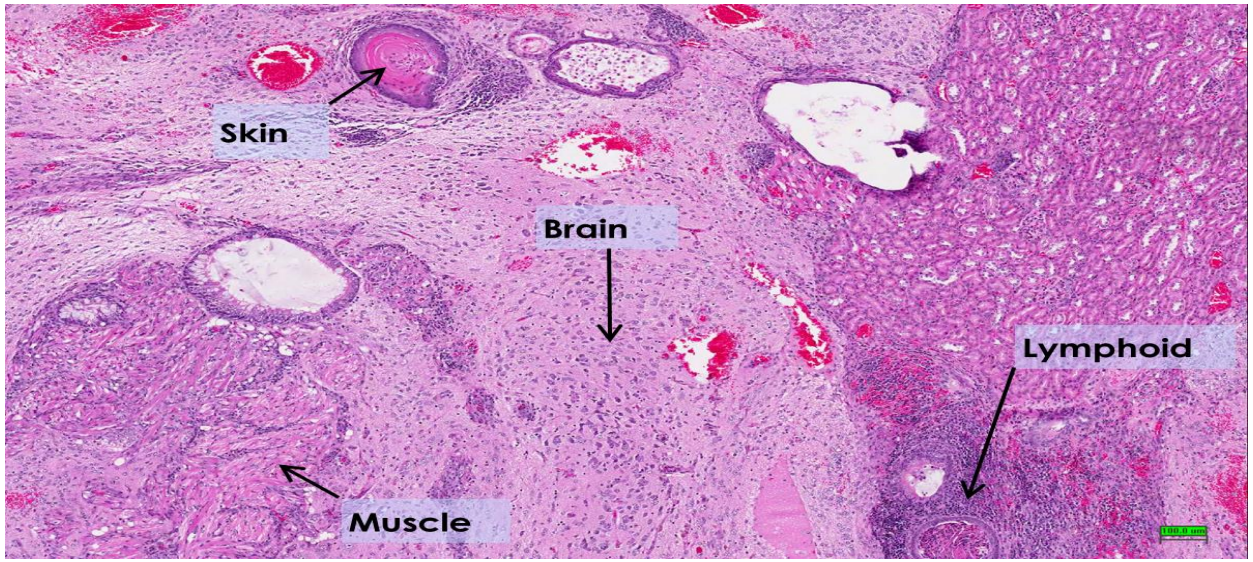
Here we can see some pictures showing teratoma which is a serious complication of stem cells. Before it was believed that teratomas only developed in people who were immunocompromised but our lab did a study and used 10 healthy (immunocompetent) samples. And in 9 out of 10 a teratoma developed. So we should always keep it in mind as a serious complication.







A large tumor mass measuring twice as the kidney is compressing it.

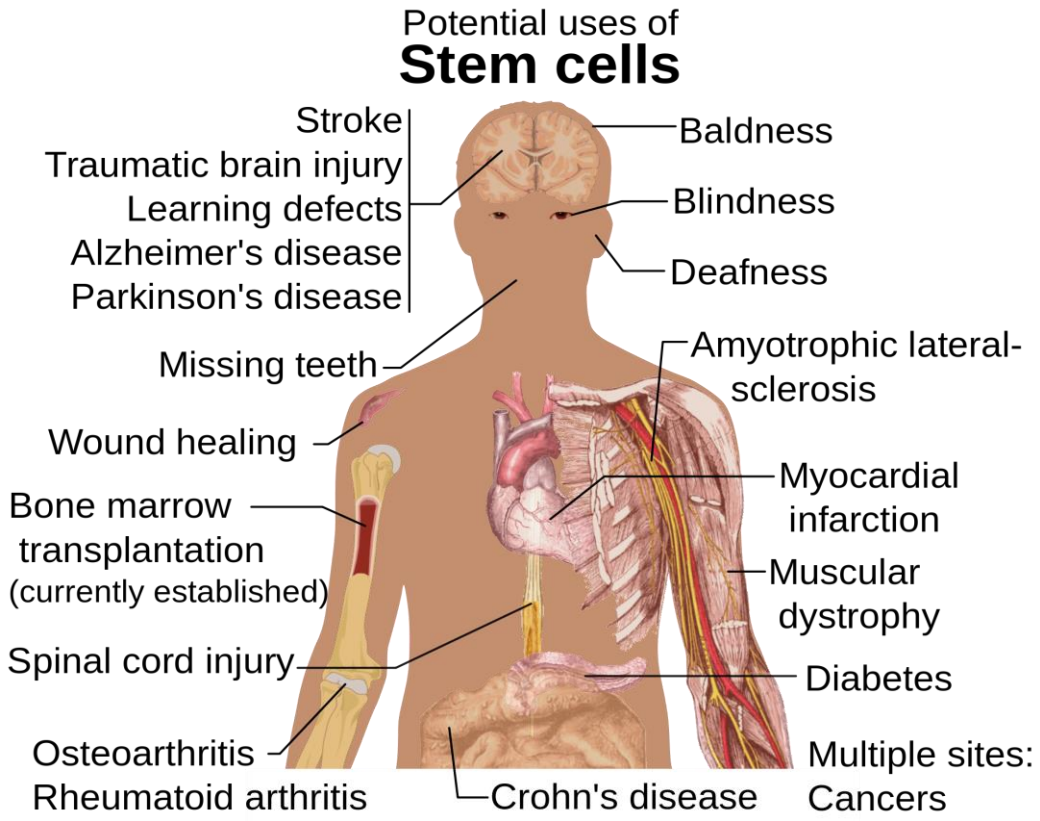


What is a teratoma? The teratoma was composed of mixed tissue patterns: skin with keratin, brain tissue, striated and smooth muscle, lymphoid tissue ……..

Only on the girls' slides

Goal of Stem Cell Therapies

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 (treat diseases)



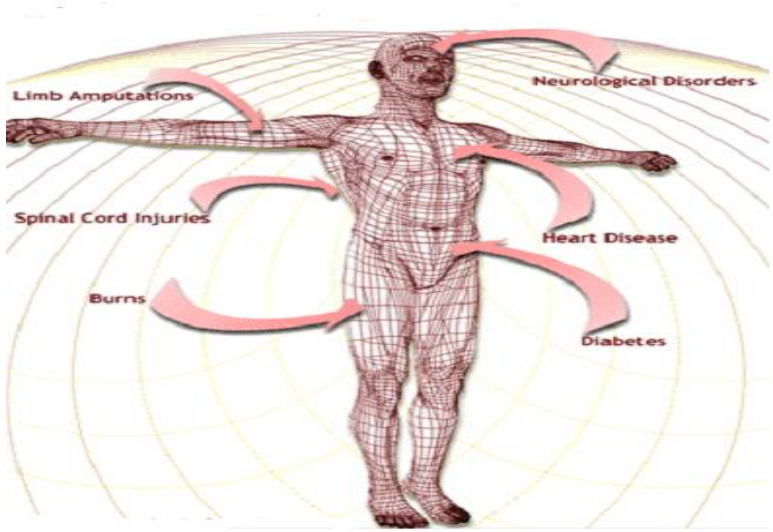
People in the US affected by diseases that may be helped by stem cell research:

Condition	Number of Persons Affected
Cardiovascular diseases	58 Million
Autoimmune diseases	30 Million
Diabetes	16 Million
Osteoporosis	10 Million
Cancer	8.2 Million
Alzheimer's disease	4 Million
Parkinson's disease	1.5 Million
Burns (severe)	0.3 Million
Spinal cord injuries	0.25 Million
Birth defects	150,000 (per year)
Total	128.4 Million

Data from the Patients' Coalition for Urgent Research, Washington, DC (according to Perry, Ref. 267).

The **Promises** of Stem Cell Technology

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



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.

Only on the girls' slides

Summary

Stem cells	
definition	Cells that have the ability to continuously divide and differentiate to other kinds of cells
function	Repair and regeneration of tissues
classification	**1.potency based** A. Totipotent : from embryonic and extra embryonic cells B. Pluripotent : form 3 germ layers C. Multipotent : form related cells D. Oligopotent : form few cells E. Unipotent : form one cell type F. Nullpotent : terminal cell **2.Sourced based** A. Embryonic (pluripotent ,may cause immune reaction) B. Adult (multipotent , no immune reaction) C. IPSCs (no immune reaction or ethical dilemma)

1. The goal of stem cell therapies is to:

A. Reduce the Possibility of immune rejection
B. Promote cell replacement in organs that are damaged and do not have Ability for self-repair
C. To make full humans

2. Which of the following is The Promise of Stem Cell Technology:

A. Toxicity testing
B. Understanding prevention and treatment of birth defects
C. Study of drugs therapeutic potential
D. All are true

3. Mesenchymal stem cells are example of:

A. Pluripotent stem cells
B. Multipotent stem cells
C. Totipotent stem cells
D. Induced pluripotent stem cells (iPS cells)

4. What are yamanaka factors:

A. OCT3/4, SOX2, KLF4, c-Myc
B. Growth factors
C. Cytokines
D. OCT3/4, SOX2, Nanog

5. 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

6. 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

7. Induced Pluripotent Stem Cell (iPS) cells are:

A. Cells have limited potential to form only multiple adult cell types
B. Cells are Potential to form all differentiated cell types
C. somatic cells that have been reprogrammed to a pluripotent state
D. cells are potential to differentiate into any adult cell type

8. Stem cells need to be differentiated to the appropriate cell types before they can be used clinically because it may produce teratomas "undifferentiated cells":

A. True
B. False

9. adults stem cells differ from embryonic stem cells in:

A. it's can produced all cell types
B. have no major ethical concerns
C. risk of creating tumors

Answers:

1: B, 2: D, 3: B, 4: A, 5: C, 6: D, 7: C, 8: A, 9: B

10. If the stem cells differentiated into many stem cells and only few other types of cells this will lead to :

A. Homeostasis
B. Tissue aging
C. Tissue death
D. Carcinogenic tissue humans

11. Which of the following forms embryonic and extraembryonic cell types:

A. Unipotent
B. Multipotent
C. Oligopotent
D. Totipotent

12. All of the following are source of embryonic stem cells except:

A. IVF embryos
B. Cloned embryos
C. Placental cord
D. Aborted embryos

13. Which of the following is a feature of adult stem cells :

A. Large number can be harvested
B. Cause immune rejection
C. Ethical concerns
D. Multipotent

14. The Blastocyst is formed of each of the following except:

A. Trophoblast
B. Morula
C. Blastocoel
D. Inner Cell Mass (ICS)

15. Hematopotic stem cells gives:

A. Cells of the nervous system
B. Connective tissue
C. Cartilage
D. Blood cells

Answers:

10: D, 11: D, 12: C, 13: D, 14: B, 15: D

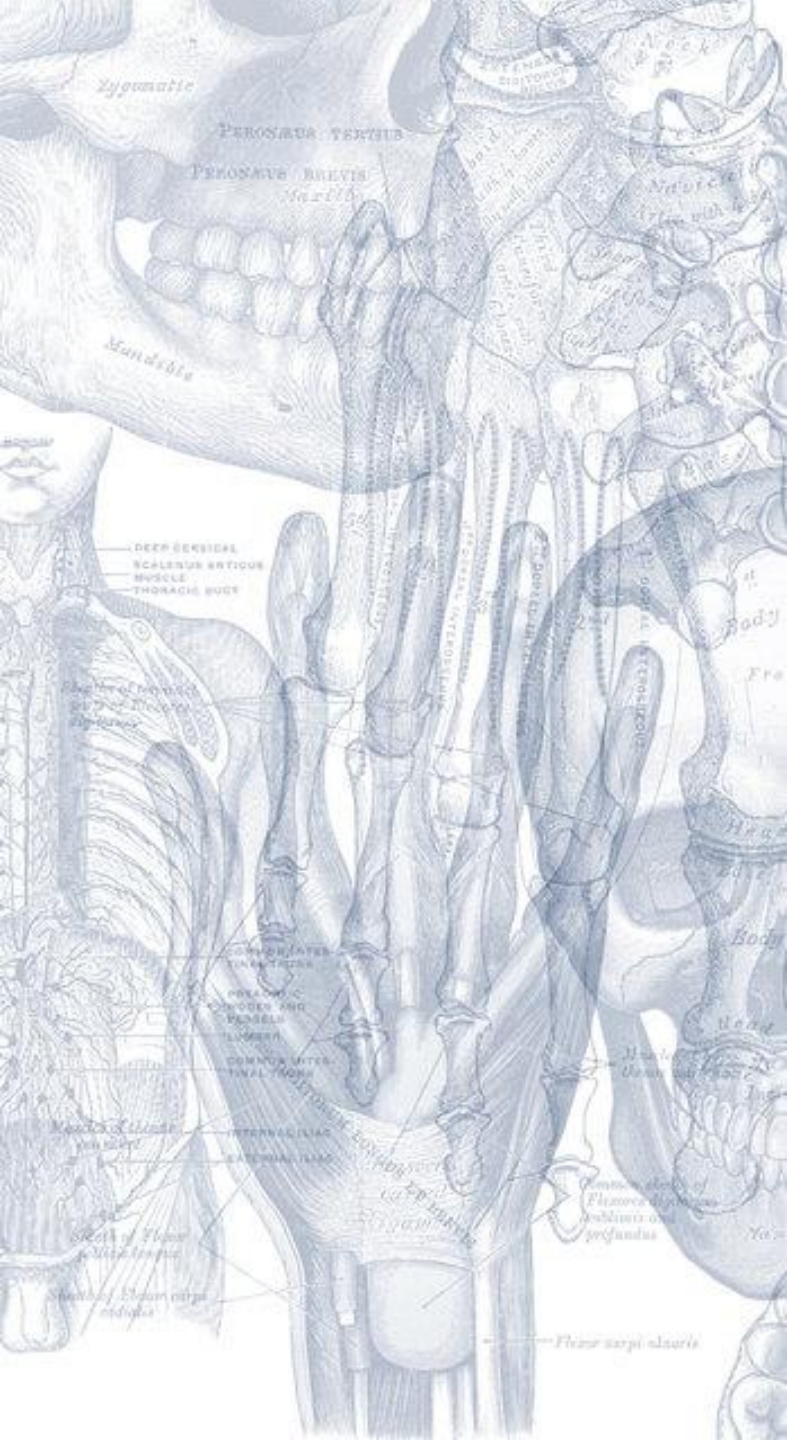
1-What are stem cells ?

2-What is the difference between oligopotent and unipotent stem cells?

Answer :

1-A cell that has the ability:
to continuously divide and give rise to new copy of itself (self-renew) and other specialized (differentiated) cells/tissues.

2-Oligopotent: differentiate into only few cells such as lymphoid or myeloid stem cells
Unipotent : differentiate into only one cell type such as muscle cell



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References:

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2- Greys Anatomy for Students

3- TeachMeAnatomy.com