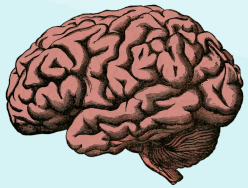

Anatomy Team
MED 439

Revised & Approved

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MED439
KING SAUD UNIVERSITY

Introduction to the Pluripotent Stem Cells

GNT Block

Don't forget to check the Editing File

Color index:

Content
Male slides
Female slides
Important
Doctors notes
Extra information, explanation

Contact us:
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Objectives

1. To know the difference between adult stem cells and pluripotent stem cells

2. To know different kinds of pluripotent stem cells and how they are created or isolated.

3. To know how iPSCs are created

4. To know what is stem cell research and what can it be used for

Stem cells (Introduction)

Definition

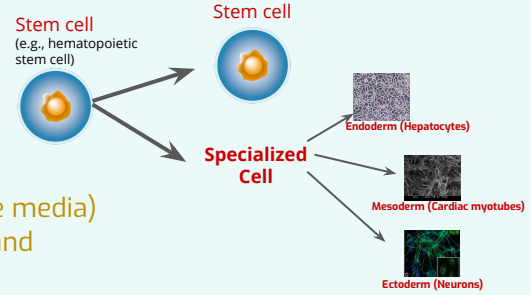
-A cell that has the ability to continuously divide and give rise to new copy of itself (self-renew).
-Has ability to differentiate into various kinds of cells/tissues:

1. Endoderm (Hepatocytes)
2. Mesoderm (Cardiac myotubes)
3. Ectoderm (Neurons)

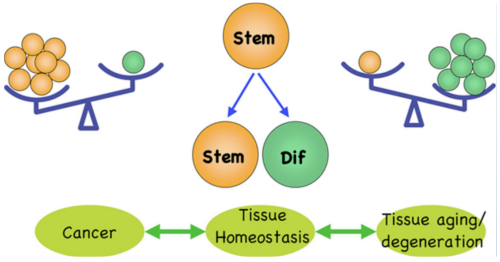
Unique characteristics

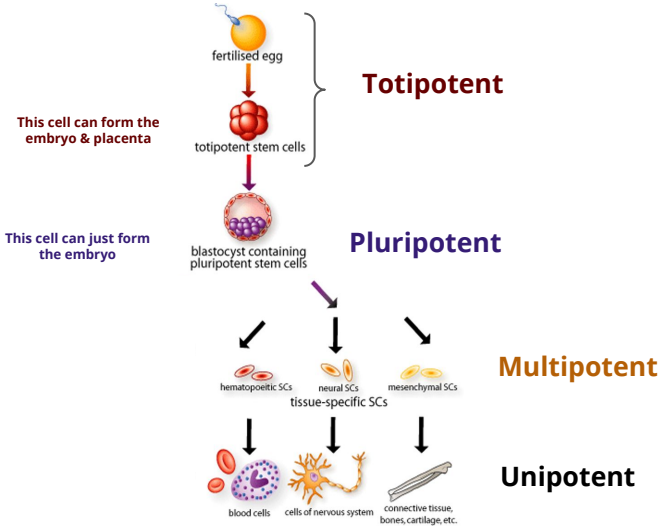
- Unlimited self renew (Regeneration) (Too much may result in cancer)
- They need to be in balance to maintain tissue homeostasis

- Differentiation (eg. beating cells of the heart muscles)
1-Internal signals (specific genes)
2-External signals (Growth factors, cytokines, supplements in tissue culture media)
Too much without enough regeneration may lead to aging & cell degeneration and senescence.



Imbalance can either cause aging or cancer





Main function within the body:

Continuous Repair of defective cell types and regeneration of tissues.

Classification of stem cells

A- Potency based

Potency based
Classification of stem cells

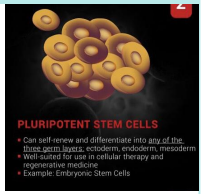
1
2
3
4
5

Totipotent

- embryo at 1-3 days, differentiate into embryonic and extraembryonic cell types
-Can form embryo and placenta .
Gives a total entire organism (can differentiate into anything)
extraembryonic (like placenta, amniotic fluid and umbilical cord)

Pluripotent

Descendants of totipotent cells and differentiate into cells of 3 germ layers.(ectoderm, mesoderm, endoderm)
-Can JUST form the embryo. Can't differentiate into placenta or extraembryonic tissue.

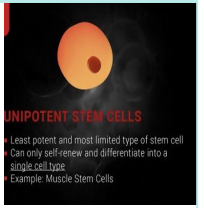


Multipotent

-Produce cells of closely related of cells (e.g. hematopoietic from bone marrow, Mesenchymal stem cells) family stem cells.
Differentiate into limited types

Unipotent

-Produce ONLY one cell type (e.g. muscle stem cells).

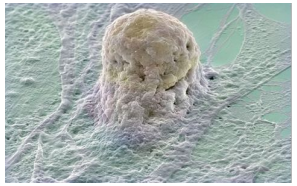


Oligopotent

-Differentiate into ONLY a few cells,
-such as: lymphoid or myeloid stem cells.

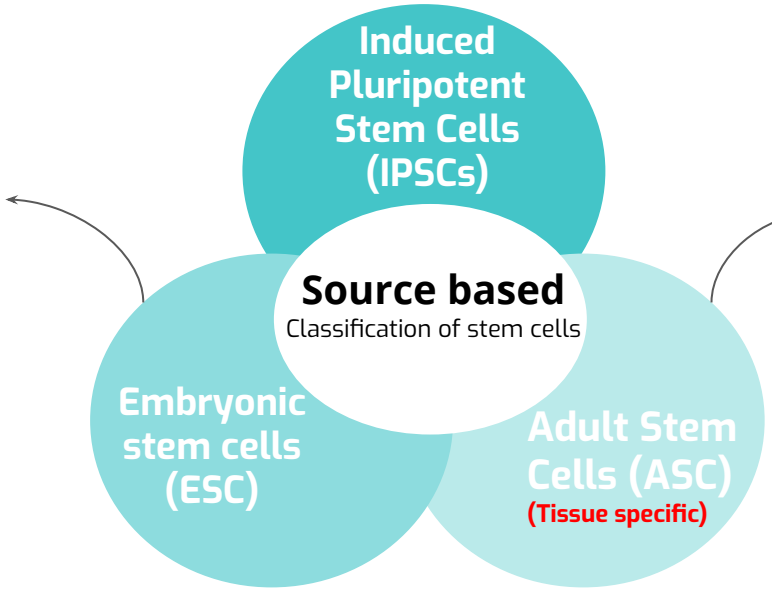
Classification of stem cells

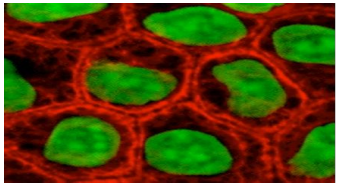
B- Source based



- Pluripotent .
- Large number can be harvested.
  Unlimited proliferation
- May cause immune rejection.
- Ethical concerns.

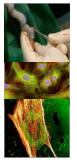
Sources :
1- IVF embryos.
2- Aborted embryos.
3- Cloned embryos.



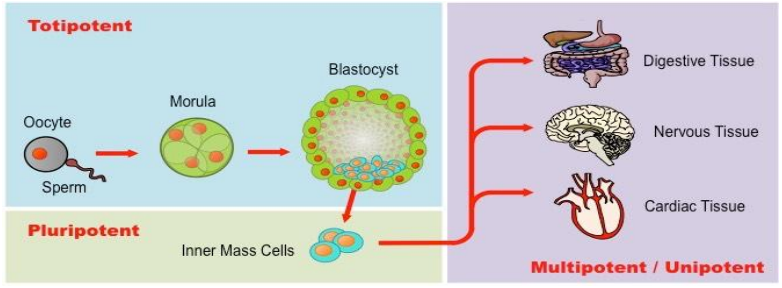


- Multipotent.
- Limited numbers and more difficult to isolate.
- No immune rejection and No ethical concerns.
- Found in specific mature body tissues as well as the umbilical cord and placenta after birth.

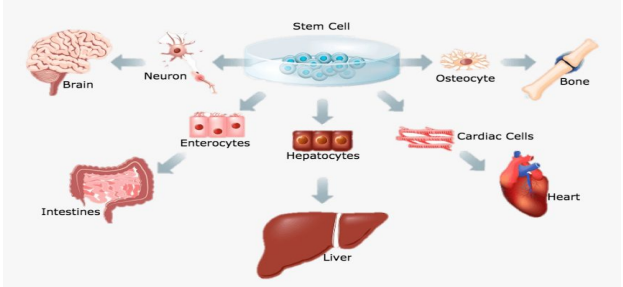
Sources :
1- Bone marrow.
2- Placenta cord.
3- Mesenchymal Stem cells.



Cause of immune rejection: They come from fertilized embryo that is not from the same transplant patient (different genetic material). E.g. transform them into beta cells then transfer them to a patient with diabetes



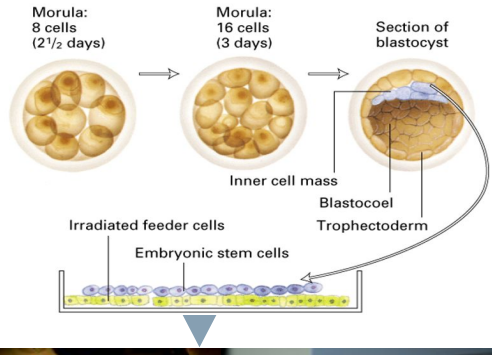
Cause of no immune rejection: it is the same genetic material. E.g. derive mesenchymal stem cells from adipose tissue of patients then differentiate them into osteoblasts and transplant them back to the same patient



Generation of embryonic stem cells

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

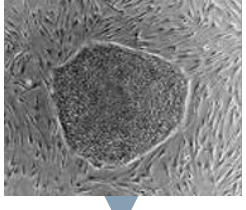
Isolate and transfer of ICS into culture dish in culture media at 37 C and 5% CO2.

3

Inner surface of culture dish is coated with inactivated MEFs (Mouse Embryonic Fibroblasts) as a feeder layer:
- Provides sticky surface for attachment
- Release nutrients. To maintain pluripotency

4

Cells divide and spread over the dish.



5

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

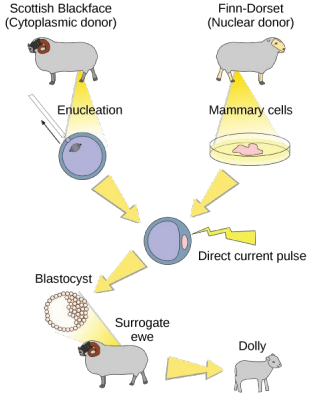
Therapeutic and Reproductive Cloning

Reproductive Cloning (B)

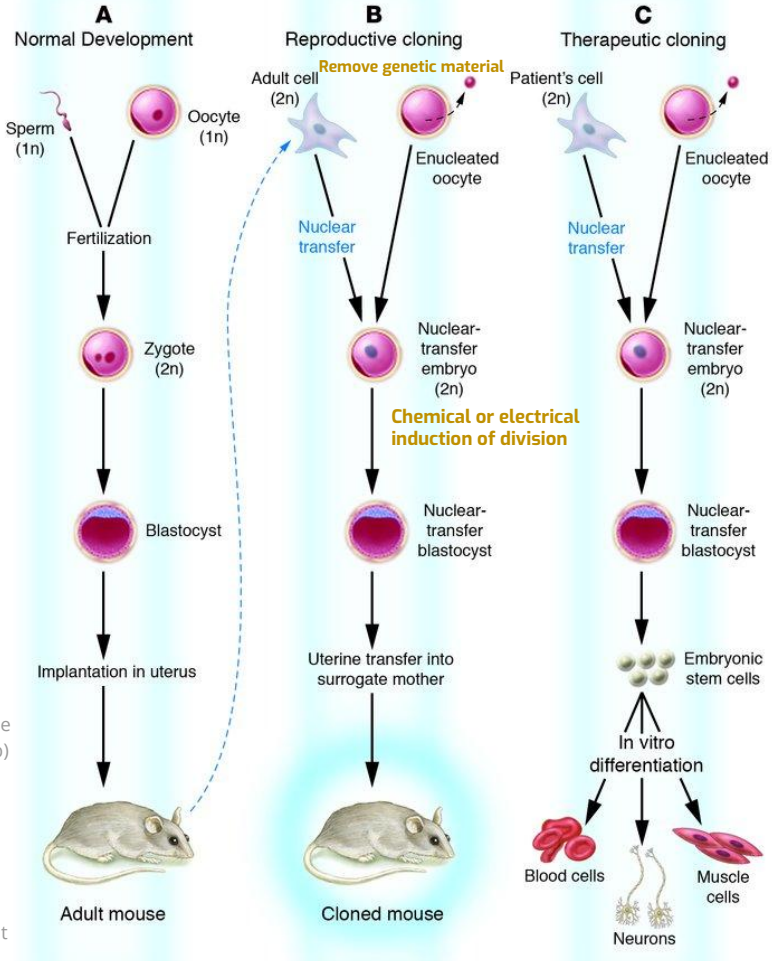
-An oocyte was deprived of its nucleus & a different nucleus was inserted and the blastocyst was re-inserted into a surrogate mother.

-Through somatic cell nuclear transfer

-It produces an animal that is genetically identical to the donor animal (the one you took the nucleus from).



1-They took oocyte from the 1st donor (black face sheep) and removed it's nucleus (enucleated)
2-They took the DNA from the mammary cells of the 2nd donor.
3-They stimulate the cells with an electrical shock so it thinks it's being fertilized.
4- Put it back in the black face sheep.
5-We have dolly :)



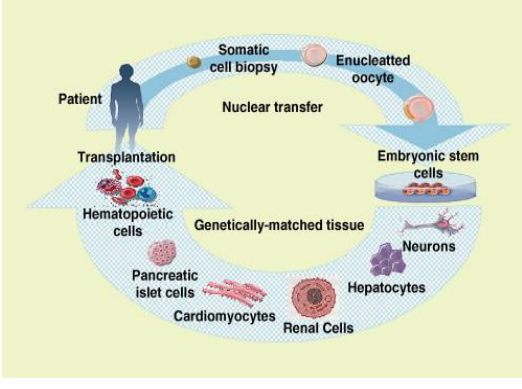
Therapeutic Cloning (C)

-We use the patient's cells to reduce rejection.

-We culture the embryonic cells and differentiate them.

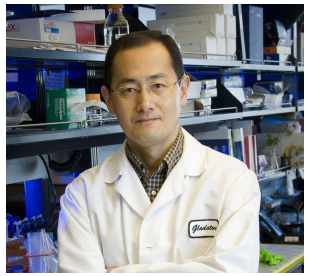
-It's not used to produce a full human, rather uses stem cells to correct diseases and other health problems that someone may encounter.

Advantage: no immune problem
Disadvantage: ethical concern

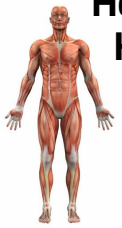


The first iPSCs

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.

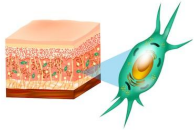


iPS Cells



Healthy or diseased Human or mouse

Adult cells (e.g. skin fibroblasts)



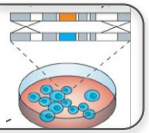
Express **Yamanaka** Factors:
- **OCT4**
- **SOX2**
- **KLF4**
- **(Myc)**

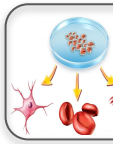
Express **Thomson** Factors:
- **OCT4**
- **SOX2**
- **NANOG**
- **Lin28**

Self renewal

iPS Cells

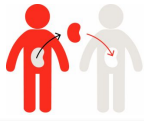
Genetic repair by homologous recombination (if necessary)





Differentiation (into target tissue)

Transplantation



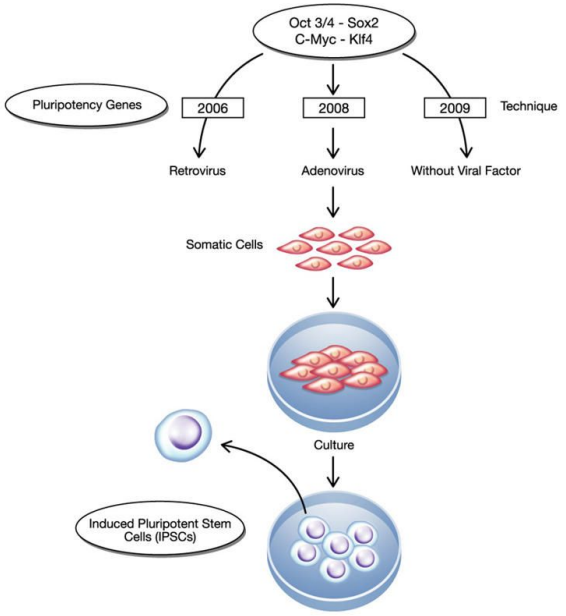
In vitro screening of drug candidates on healthy & diseased cells

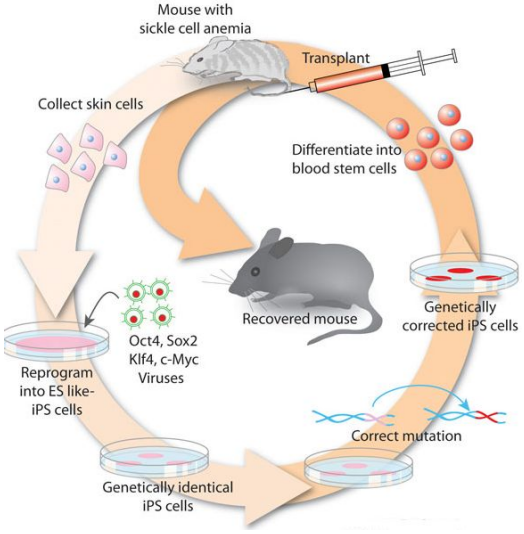
Induced Pluripotent stem cell (iPS) cells

- The method was described by Yamanaka in which the skin cells of laboratory mice were genetically manipulated and returned back to their embryonic state.
- 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.
- Currently a virus (adenovirus more specifically) is required to express the needed genes to revert the somatic cell back into iPSCs, while research on techniques without the usage of a viral factor is still being conducted.

An example of the experiment (image to the right):

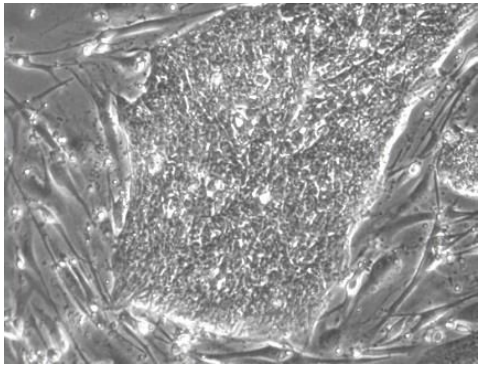
- Skin cells from the tip of the tail of a sickle-cell mouse were taken and reverted back into iPSCs and differentiated into hematopoietic cells with gene correction, then it was transfused back into the mouse.
- The mouse started recovering after that.



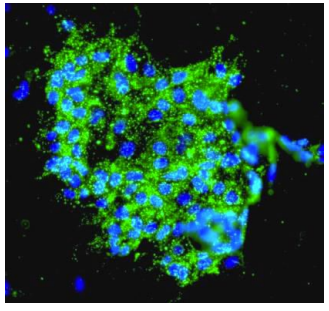


Characterization of human Pluripotent Stem cells (ESCs)

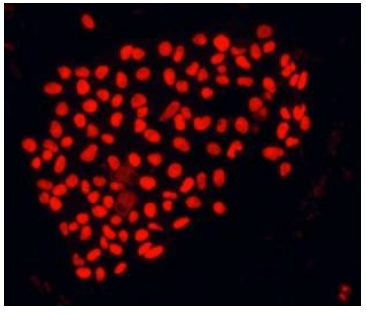
pluripotent Stem cells



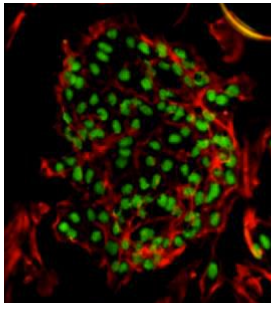
Here is the pluripotent stem cells expressing certain genes



SSEA-4

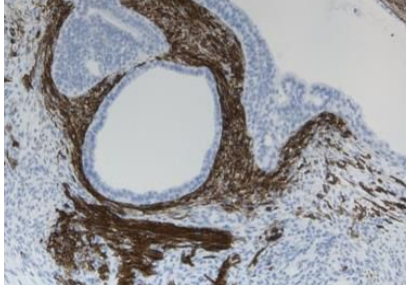


SOX-2

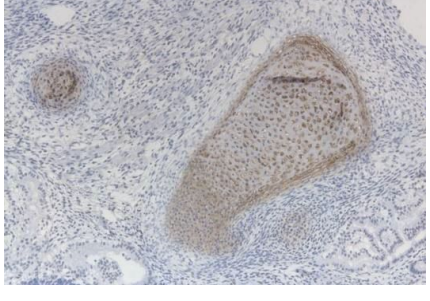


OCT-4

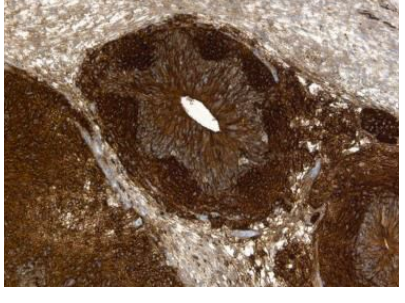
Pluripotent stem cells can differentiate into the cells of the three germ layers by exhibiting specific genes:



Endoderm (sox-7)

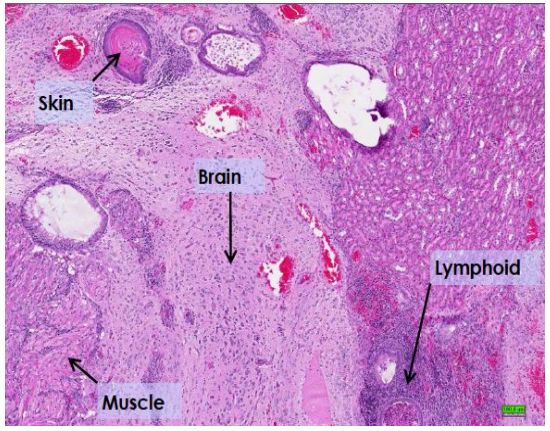


Mesoderm (sox-9)

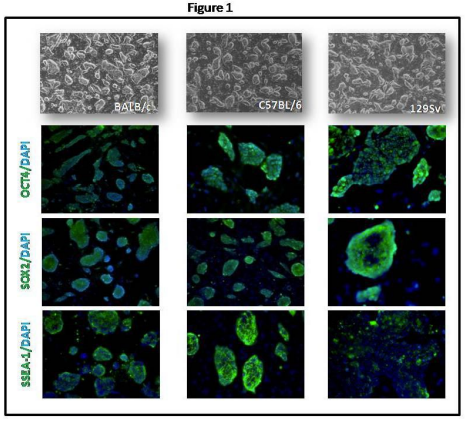


Ectoderm (Beta-III tubulin)

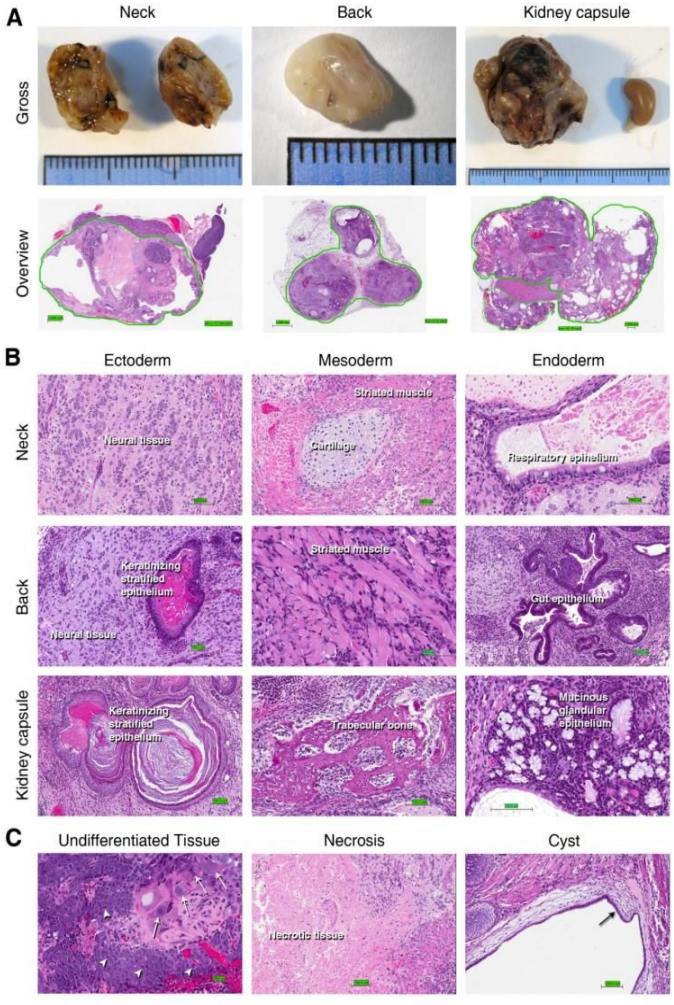
Teratoma



Teratoma is a tumor composed of mixed tissue pattern such as:
skin with keratin, brain tissue, striated and smooth muscle, lymphoid tissue etc...



On the image we see a teratoma formation in an immunocompetent mouse after syngeneic and allogeneic implantation of germline capable mouse embryonic stem cells (conducted in 2013) showing signs of the three germ layer cells in each of the neck, back and in the kidney capsule



Stem Cell Therapy Goal, promise, obstacles, uses and challenges

The Goal:

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

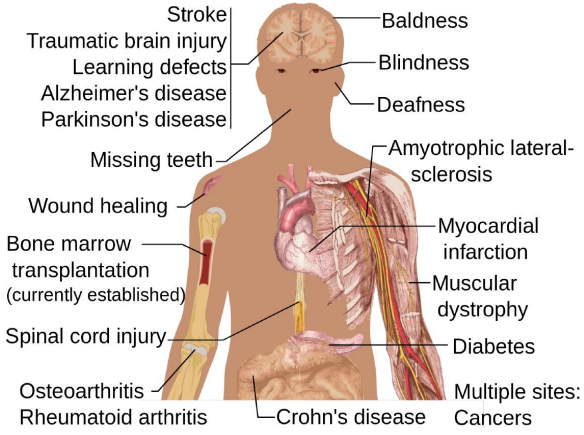
The Promise of Stem Cell Technology :

- 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

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

Potential uses of stem cells:



Challenges with embryonic stem cells:

- 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. If transferred before differentiation there is risk of Teratoma.
- Stem cell development or proliferation must be controlled once placed into patients (risk of teratoma formation).
- The use of mouse "feeder" cells to grow ESC could result in problems due to xenotransplantation. (we overcame this problem By using new culture media that contains nutrients which feeder cells produce)
- Possibility of rejection of stem cell transplants as foreign tissues is very high. (most important)

Questions from slides

1: Which of the following are pluripotent stem cells?

a) Cells that have the potential to differentiate into any adult cell type forming an entire organism.
b) Cells that have limited potential to form only multiple adult cell types.
c) Cells that don't have the ability for self renewal.
d) Cells that have the potential to form all differentiated cell types except placenta.

2: 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 laboratory

3: What are yamanaka factors?

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

4: Mesenchymal stem cells are examples of:

a) Pluripotent stem cells
b) Multipotent stem cells
c) Totipotent stem cells
d) Induced Pluripotent stem cells (iPSCs)

1-d
2-c
3-a
4-b

MCQ

Q1: Which of the following is one of the classification of stem cells on basis of potency ?			
A: adult stem cells .	B: Totipotent .	C: IPSCs.	D: ESC.

Q2: Differentiate into ONLY a few cells			
A: Totipotent .	B: Oligopotent.	C: Unipotent	D: Multipotent.

Q3: Which of the following of sourced based classification of stem cells is Tissue specific ?			
A: ESC.	B: Pluripotent	C: IPSCs	D: none

Q4: Which of the following is correct about ESC ?			
A: Pluripotent .	B: No immune rejection.	C:No ethical concerns.	D: Limited numbers and more difficult to isolate.

Q5: What cells do we use for reproductive cloning through nuclear transfer?			
A: Hematopoietic cells	B: Somatic cells	C: Embryonic	D: Both A & C

Q6: Reproductive cloning produces genetically identical clones of the animal?			
A: True	B: False	C:	D:

Answer key:
1 (B) , 2 (B) , 3 (D) , 4 (A) , 5 (B) , 6 (A)

MCQ

Q7: To reduce the chances of a rejection we use the cells of?			
A: The same person	B: Different person	C: A Sheep	D: All of them

Q8: Therapeutic cloning can produce full organisms?			
A: True	B: False	C:	D:

Q9: which one of the following is specifically a thomson factor:			
A: OCT4	B: SOX2	C: NANOG	D: SOX7

Q10: The goal of stem cell research and technology is:			
A: To promote cell replacement in organs that are damaged and do not have the ability for self repair.	B: To promote cell replacement in organs that are healthy and do not have the ability for self repair.	C: To find better technology for body enhancement.	D: Improve the testing methods of drugs.
Q11: Define teratoma			
A: A tumor composed of a single tissue pattern	B: A tumor composed of two, mixed, tissue patterns only	C: A tumor consisting of multiple, mixed, tissue pattern.	D: An inflammation of the gonads
Q12: select the true statement, Currently adult cells:			
A: Require both retrovirus and adenovirus to turn to iPSCs	B: Don't need viral factors at all to turn into iPSCs	C: Require retrovirus only to turn to iPSCs	D: Use adenovirus to turn to iPSCs

Answer key:
7(A) , 8(B) , 9(c) , 10(A) , 11(C) , 12(D)

SAQ

Q1: What is the definition of stem cells ?

Q2: Enumerate the sourced based classification of stem cells

Q3: mention the yamanaka factors:

Q4: Enumerate the obstacles facing stem cell research:

Answers

1 : Slide 3

2 : Slide 5

3 : - OCT4
- SOX2
- KLF4
- (Myc)

4: - 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

Team leaders
Rayan Jabaan
Abeer Awwad

Reviser
Norah Alasheikh

Organizer
Abdulaziz Alkraida

Note taker
Shahad Almezel

Team Members

- Aljoud Algazlan
- Arwa Alqahtani
- Asma Alamri
- Bodoor Almubarak
- Deemah Alotaibi
- Fatimah Saad
- Ghada Aljedaie
- Ghaida Alassiry
- Joud Alnujaidi
- May Barakah
- Norah Alasheikh
- Nouf Alsubaie
- Raghad Alasiri
- Raghad Soaeed
- Sarah Almuqati
- Sarah Alqahtani
- Shaden Alsaiedan
- Shahad Almezel
- Shayma Alghanoum
- Sumo Alzeer

- Abdulaziz Alghuligah
- Abdulaziz Alkraida
- Abdulaziz Alrabiah
- Abdulaziz Alsuhaim
- Ahmed Alkhayatt
- Bader Alrayes
- Basel Fakeeha
- Faisal Alotaibi
- **Hadi Alhemsi**
- **Hesham Alsqabi**
- Mohammed Aldehaim
- Mohamed Alquhidan
- Mubarak Alanazi
- **Osama Alharbi**
- Saad Aldohaim
- Saleh Algarni