CNS

Biochemistry

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هذا الشيت شامل الفايل الأول لمادة دكتورة ديالا (يعني المحاضرة الأولى وأول ثلث ساعة تقريبا من المحاضرة الثانية)

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Note: Anything in *blue italic* is extra for further clarification

Stem Cells: The New Therapeutics Era

*This lecture is about stem cells and their use in the treatment of neurodegenerative diseases. The lecture is educational and awareness-raising because we must have enough knowledge about stem cells in general, their use in the treatment of neurodegenerative diseases, and where the research has now reached on this topic (still at research stage). Now, this topic is being talked about a lot, and sometimes stem cells can be the last solution to treat a hopeless disease, but as a clinician you must pay attention to when to use this treatment and evaluate the situation well and make sure that the treatment you wish to use is approved by the Food and Drug Administration of the country in which you are practicing. Now let's start to talk about the biology of stem cells.

What are stem cells?

- **Unspecialized (undifferentiated)** cells that can differentiate to different cell types that can perform wide variety of functions (examples: can differentiate into leukocyte, hepatocyte, osteoblast). They are primal cells common to all multicellular organisms and all are unspecialized (undifferentiated) cells that are of the same family type (lineage).

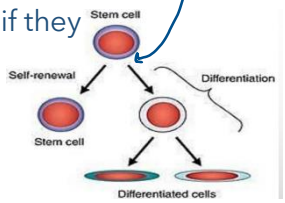
- Stem cells must maintain two opposite properties to be classified as stem cells:

  1-Able to **divide/split** (renew themselves) to be regenerated and maintain a population of stem cells through cell division.

  2-and at the same time, able to **differentiate** into a wide range of specialized cell types that can perform different functions.

  → That's why they divide asymmetrically.

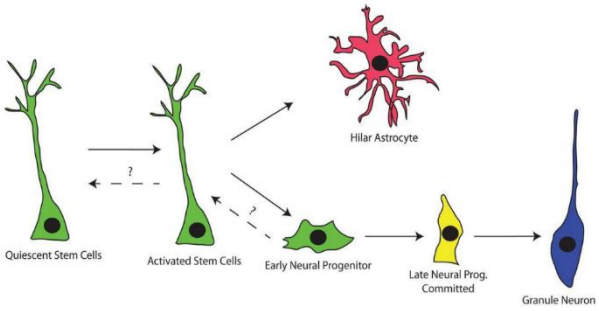
  - Note: All differentiated cells are unable to divide (such as adipocyte, hepatocyte, and any cell ends with "cyte" suffix), these are fully differentiated cells and if they die for any reason they cannot divide, and another cell must be brought.



Differentiation vs self-renewal

- **What is asymmetric cell division?** *Extra: **Asymmetric cell division** produces two daughter cells with different cellular fates. This is in contrast to symmetric cell divisions which give rise to two identical daughter cells of equivalent fates. Notably, stem cells divide asymmetrically to give rise to two distinct daughter cells with different fates: one copy of the original stem cell that maintains "stemness" as well as a second daughter that differentiates into a non-stem cell fate.*
- Asymmetric division is due to differential segregation of cell membrane proteins between the daughter cells.
- **How Does Asymmetric Division Occur? Differential segregation of cell membrane proteins** (such as receptors) between the two daughter cells. This means that during cell division and before dividing to two cells, the cell membrane proteins and the transcription factors that are important in keeping the 'stemness' and potency of a stem cell are gathered alone and going to be in the cell that regenerates the stem cell population. Whereas the cell membrane proteins and the transcription factors that are important for driving differentiation are also gathered alone and going to move to the second cell that goes into the differentiation pathway. So there are two different poles.
- **Self-renewal**: The ability to go through numerous cycles of cell division while maintaining the undifferentiated state.

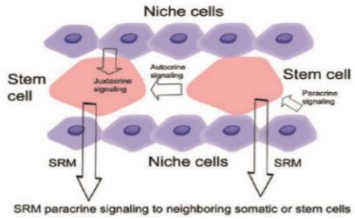
What does stem cell division produce?



Stem cells are the parent cells that will divide into progenitor cells. Stem cells do not undergo differentiation in a single step. They pass through **intermediate cellular steps** in which these intermediates are partially differentiated and produce different types of fully differentiated and mature cells. One step of these intermediate steps is the progenitor cells. An example is hematopoietic stem cells as they go into multiple steps until they reach the fully differentiated cells of multiple types.

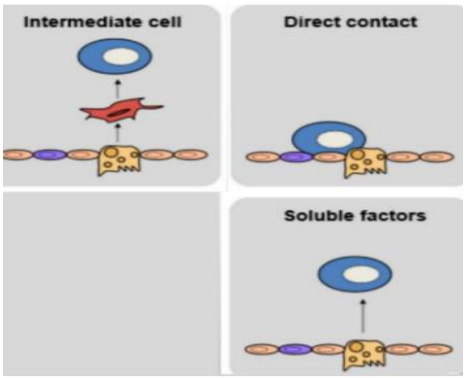
- **Progenitor cell**: Stem cells generate an intermediate cell type or types before they achieve their fully differentiated state. Progenitor cells are not completely stem cells but also not fully differentiated (they start to lose their stem properties towards differentiation but still they have some transcription factors and proteins that are expressed in the stem cells).

Stem cell niche:



Stem cells are very sensitive and can be affected easily, so they need a specialized cellular environment that provides stem cells with the support needed for self-renewal to be able to maintain the population of stem cells. Also, it optimizes the conditions necessary to drive the differentiation of a certain stem cell type into its fully differentiated functional form.

The niche can exist in several forms according to the type of stem cell:



1- Cells only: A single cell type, or a whole host of interacting cells. Cells outside the stem cell's lineage, or they may derive primarily from the stem cell's own descendants (stem cells specially in the embryonic stages can grow in **colonies** so they can interact with each other).
2- Cells and ECM components (like proteins and sugars). ECM is laid down by adjacent cells.
3- Cell surface soluble factors OR the transcription factors/ soluble factors that are secreted by adjacent cells to the stem cell niche, such as Notch, Wnt, FGF (fibroblast growth factor), EGF (epidermal growth factor), TGF-β, SCF (stem cell factor), and chemokine families. So this form of niche must be maintained to maintain the stemness of the stem cells, OR the opposite when other molecules are secreted, differentiation to certain cell type may occur.

*Stem cell niche can exist in one form or more than one. And any defect in the stem cell niche will cause the stem cells to start to differentiate. **Each stem cell type has its own stem cell niche**: ***more clarification from the doctor****: each stem cell that is dictated to provide a certain differentiation pathway has its own niche that provides it with certain factors that will help in conditioning this certain stem cell to end up in that particular lineage, for example the niche of the stem cells that Will become heart cells are different than the niche of the stem cells that will become skin cells for example, this is because the niche participated in conditioning and determining whether the stem cell will become heart cell or a skin cell for example, so by that we can summarize that the niche of stem cell is important for two things: keeping the stem cell as a stem cell and preventing it from differentiating, and when it is the time for differentiation, the niche will participate in determining what differentiation pathway will the stem cells go through. That's why stem cells divide asymmetrically.*

Why do stem cells need a special environment (Stem cell niche)?

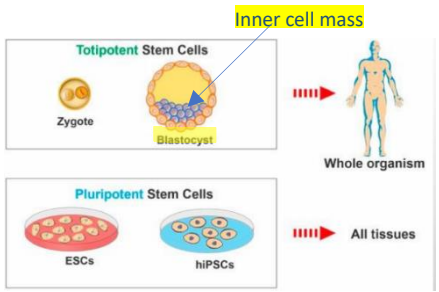
- Provide special **support** for their viability due to the demands on stem cells, and also keep their stemness so they do not differentiate (differentiated cells cannot be reversed back to stem cells in normal conditions, but it can be reversed experimentally in the lab).
- **Nutritive** function as niche has a role in supplying nutrients.
- Affect signaling and **communication** between these cells.
- Niches might be agents of **feedback control** (control of stem cell pool size so that it doesn't expand too much nor shrink).
- Niches are instruments of **coordination among tissue compartments.**
  - Communicate with each other and thus deliver the necessary messages needed to keep the stemness of stem cells.
- Niches are **hubs (center) of inter-lineage coordination.**
  - A stem cell may proceed into several differentiation pathways, thus there must be some sort of coordination between them so one doesn't dominate over the others. Lineage means that differentiation has started but cells are still not fully differentiated, so this certain lineage is what will make the cells to continue towards differentiation into certain type of cell, so we need special niches for the interaction of different lineages.

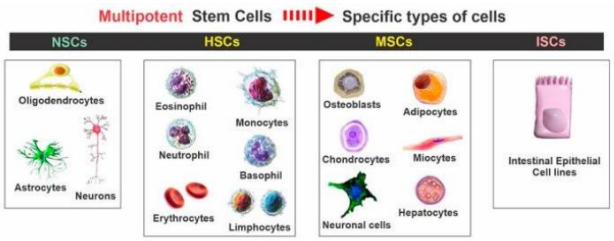
Potency of stem cells: (the differentiation potential of the stem cells)

Stem cells can be classified according to their potency (Their ability to differentiate and what types of tissues they can produce out of their differentiation) into:

1- **Totipotent**: unlimited ability, able to differentiate into ALL cells of the embryo and extraembryonic tissues (including the placenta), this happens in early embryonic stages.

***Extra**: extraembryonic tissue is the tissue that primarily give rise to those structures that support the embryo during its development (e.g. the placenta, umbilical cord, and the four extraembryonic membranes: (yolk sac, the allantois, the amnion, and the chorion)).





2- **Pluripotent**: less ability to differentiate when compared to totipotent, able to differentiate into ALL cells of the embryo but NOT to extraembryonic tissues, first example is embryonic stem cells (ESCs).

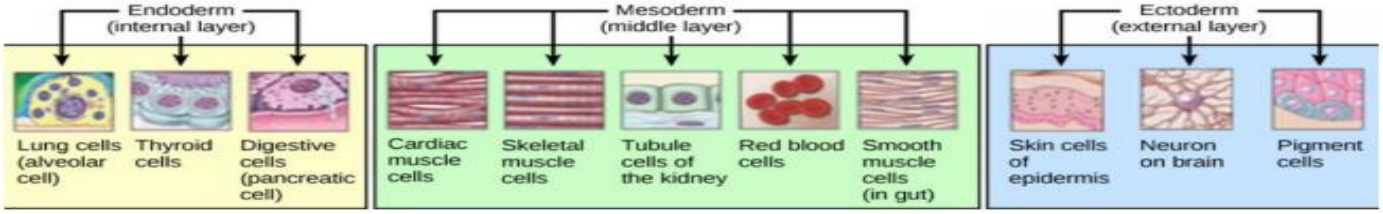
- A blastocyst has a layer of cells outside and is hollow from the inside except at one pole where there is a gathering of cells, called **inner cell mass** of the blastocyst. Inside this mass, the pluripotent embryonic stem cells are derived.
- The second example is the Induced Pluripotent Stem Cells (iPSCs).
  - Which are human-manufactured cells that were induced in the lab. How? In labs, scientists reprogram and reverse the differentiation of fully differentiated cells from the patient by expressing the transcription factors necessary to maintain stemness of cells, so they returned undifferentiated as pluripotent stem cells.

- What examples of fully differentiated cells that can be reversed to make iPSCs?
  Skin fibroblasts were used initially because it is easy to obtain skin biopsy, also some clinicians extract cells from urine with no need for biopsy.
- When iPSCs are made from fully differentiated cells, they can be differentiated to any cell type in your body (to any cell in the three germ layers).
- iPSCs are just like ESCs. One of the steps in preparing the iPSCs is to compare them to ESCs according to many variables, and it was found that both are the same.

3- **Multipotent**: able to differentiate into several/multiple cell types of the body, but not all, example: hematopoietic stem cells.
4- **Unipotent**: able to differentiate into a single cell type (cell is more characterized).

Three germ layers:

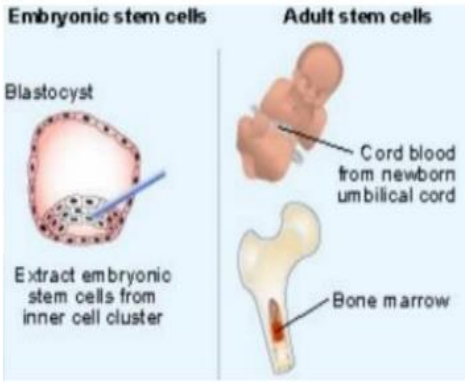
If we take a **pluripotent stem cell** population and expose it to differentiation conditions, they should be able to give rise to cells from all three germ layers, as seen in the figure.



Types of stem cells:

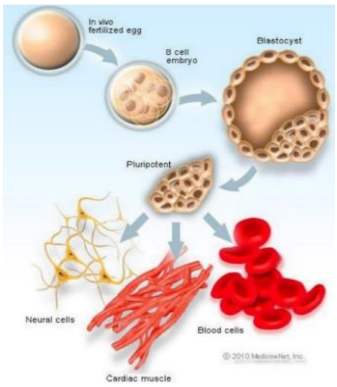
Another classification of stem cells depends on at which stage they appear:

- **Embryonic stem cells and induced pluripotent stem cells** (same ability to differentiate): appear during embryonic development and are important for fetus development. They **can differentiate into all the specialized embryonic tissue** and all cells of the body.



- **Adult stem cells:** appear after birth. They **act as a repair (regeneration) system for the body replacing specialized damaged cells.** Some examples are:
  - Intestinal cell regeneration by stem cells
  - Neural stem cells: Even though we know that neurons do not regenerate, but this is not completely true as they have a **limited** ability to regenerate but not a complete ability (not every cell that dies is replaced).
    *So in many tissues, there are very small populations of stem cells that are responsible for regeneration that occurs in adults.

Embryonic Stem Cells (ESCs)



- ES cells are derived from the **inner cell mass** of mammalian blastocysts.

> * A blastocyst has a layer of cells outside and is hollow from the inside except at one side where there is an accumulation of cells, called inner cell mass. Inside this mass, the ESCs (pluripotent stem cells) are derived.

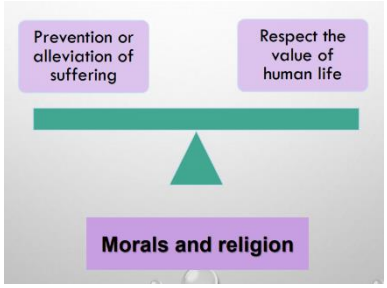
- Develop before implantation of embryo in the uterus wall (early stage).

Pluripotency of ESCs:

What makes stem cells pluripotent and able to differentiate to all cell types?

All cells whether they are differentiated or stem cells have the same DNA, however what makes a stem cell itself is its transcription profile. In other words, both types of cells have the same genes however they differ in genes that are expressed, in the case of stem cells it's gene expression profile what makes them able to activate and transcribe pluripotency genes and transcription factors, these transcription factors allow for synthesis of certain proteins which give stem cells their characteristics of asymmetric division, unlimited divisions, and ability to differentiate to any other cells. Some examples of such important transcription factors in this process are: Oct 4, Nanog, Wnt-β-catenin signaling, SOX2, and Other TFs (these are not the only ones).

The Ethical Dilemma of ESCs



- What is the problem in using ESCs from embryos? one of the problems presented with ESCs and their usage in the treatment of diseases is that we need to isolate them from embryos (extract them from the inner cell mass), which means we are basically going to kill the embryo after it started to develop. So there is an ethical dilemma, However, they will be used to alleviate the suffering of patients. This raises the need to find a balance between morals and religion.
  *When we talk about religion, the issue is not only related to Islam, but there is a consensus of all religions not to use ESCs.
- Thus, scientists needed to find another source of pluripotent stem cells. These are the Induced Pluripotent Stem Cells (iPSCs). Why not multipotent stem cells? Because even though they are easy to extract but they have limited ability to differentiate.
- Another problem to consider is that transplanting cells from one embryo into another patient (even if his brother) is introducing foreign cells into the patient's body which may cause immunological problems and immune rejection because ESCs have genetic and immune profile. So it is not just an ethical problem, there are other problems.

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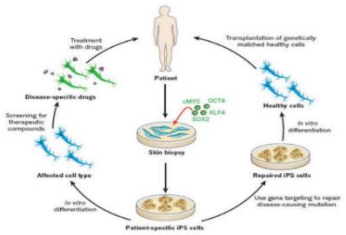
- **How are iPSCs derived?**
  - In labs, scientists reprogram and **reverse the differentiation of fully differentiated cells** from the patient by expressing the transcription factors necessary to maintain stemness of cells, so they are **reversed to stem cells.**



- **Advantages of iPSCs include:**

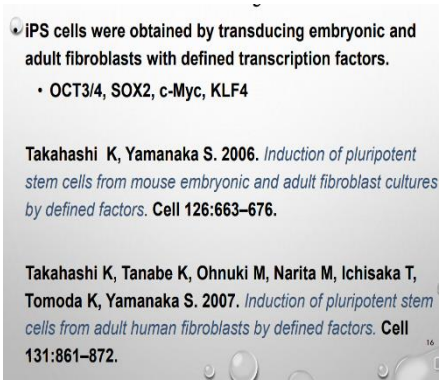
(These advantages are not only to replace damaged cells, but there are also other uses for stem cells).

  - Autologous: which come from the patient himself (take a small biopsy and reverse it to stem cells) → no immune rejection
  - Pluripotent: can differentiate to any cell type
  - No Ethical problems.
  - Safer
  - Patient-specific → meaning that they reflect your whole genetic and epigenetic profile (even the genetic diseases).

Generation of iPSCs

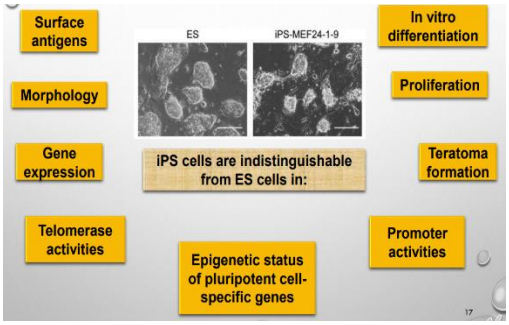
Here is everything the doctor said about generation of iPSCs:

- The idea of generation of iPSCs was achieved for the first time by two groups, the first is Japanese (Yamanaka) and the second is American. The Japanese group took the lead and win the Nobel prize.
- generation of iPSCs started by reversing fibroblasts back to stem cells using a large group of 24 pluripotent transcription factors expressed by viral vectors in fibroblasts, so fibroblasts begun to return to stem cells because their gene expression profile had changes.
- The 24 transcription factors are not used all together, but they reached a collection of 4 transcription factors that can give rise to a pluripotent stem cell (this collection differ between the 2 groups). This happened after pulling each factor one by one and trying its ability to reverse the differentiated cells to stem cells.
- In 2006 → by Yamanaka →induction of pluripotent stem cells from mouse **embryonic** fibroblasts using these 4 factors: OCT3/4, SOX2, c-Myc, KLF4.
  In 2007→Induction of pluripotent stem cells from **human** fibroblasts.



Yamanaka's comparison of iPS and ES cells

Embryonic stem cells grow in colonies (clusters) that are visible under microscope, so they do not grow as a single cell because single cells will start to differentiate. So, under the microscope, growth morphology looked the same in both iPS and ES cells, but this is not enough to say that they are the same, therefore, they were compared according to other different factors, and it was found that they are both the same.



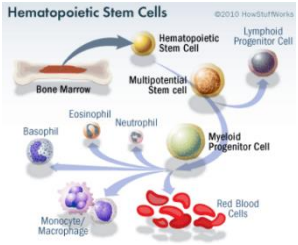
So what are the comparisons between them? They compared them according to factors mentioned below, and all was found to be the same in both iPS and ES cells:

1- **Surface antigens**, which are the cell surface proteins, it was found that cell membrane proteins that distinguish pluripotent stem cells are the same as the ones that distinguish iPSCs.
2- **Morphology**
3- **Gene expression profile**→ at m-RNA level, found that both have the same expression.
4- **Telomerase activity**, which is essential to maintain continuous cell division, so it makes sense that it will be different between a cell that has been differentiated (doesn't need any further divisions) and another cell that is pluripotent (will need continuous divisions), in which the one that divides such as the pluripotent stem cell will have higher telomerase activity.
5- **In vitro differentiation**→ there are well established differentiation profiles which are certain steps that should be made in order to attempt to make a cell differentiate into another specific cell, so there is a specific differentiation profile for neurons and hepatocytes, so we tried to use these differentiation profiles on different types of cells, and we tried different differentiation profiles from each of the three germ layers , the answer was that both of them successfully differentiated.
6- **Proliferation**: ability to divide
7- **Teratoma formation**→ are tumors of the three germ layers (since we are talking about pluripotent stem cells).
   * Form mixed tumors when injected to mice, this indicates that they are stem cells.
8- **Promoter activity**→reflect gene expression profile and activity of certain genes.
9- **Epigenetic status of pluripotent cell-specific genes**→ Epigenetic modifications are modifications that do not change the nucleotide themselves in the DNA, but they change the expression profile through modifications on nitrogenous bases, such as methylation and acetylation. Epigenetics involves either activation or silencing of certain genes which is different from one cell to another, in which all cells have the same DNA, however in some cells, certain genes are activated and in other cells the same gene may be silenced, this is determined by epigenetic modifications, the most important one being that methylation of certain genetic sequences will lead to silencing of these genes. After comparing the epigenetic status of both cell types, it was found that they have the same epigenetic status, in which they have silenced and activated the same genes.

Adult stem cells

- Undifferentiated cells found throughout the body (in adults).
- **Function**: they divide to replenish dying cells and regenerate damaged tissue (this process happens all the time). However, their ability to differentiate is limited in comparison to the pluripotent stem cells. Thus, they are multipotent, able to differentiate to multiple (3-6) cell types but not all cell types (NOT pluripotent).

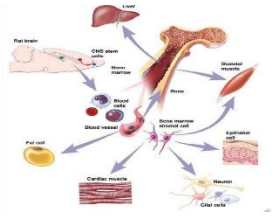
Types of adult stem cells: (there are more types than the mentioned)



1- Bone marrow stem cells (found in bone marrow). They are further subdivided into:

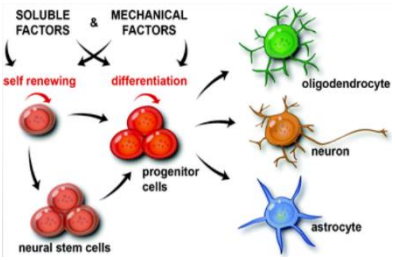
A) **Hematopoietic stem cells:** can give rise to all cells of the blood (RBCs, WBCs, platelets, etc.)

B) **Somatic stem cells,** such as mammary stem cells and mesenchymal stem cells, which are present in bone marrow but they can give rise to other group of cells other than the osteoblasts, like, chondrocytes, myocytes, adipocytes, and neuronal cells.



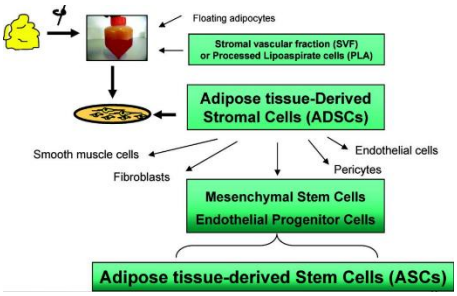
2- Neural stem cells: can generate some types of neurons. Even though we know that neurons do not regenerate, but this is not completely true as they have a limited ability to regenerate.

- **Neurospheres** – floating heterogeneous aggregates of cells, containing a large proportion of stem cells responsible for adult neurogenesis. They are found in subventricular zone, which lines the lateral ventricles of the brain, and the dentate gyrus of the hippocampal formations.



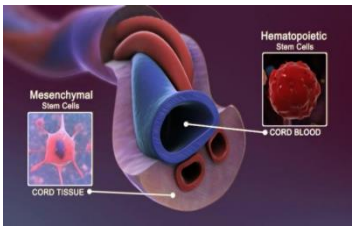
3- Adipose stem cells (ASCs): They are found in adipose tissue.

- They can be obtained after **liposuction** operation (in which a part of adipose tissue is removed for the purpose of reducing weight, but we can use this fat to extract some stem cells that **give rise to different cells other than adipocytes** such as fibroblasts, endothelial cells, pericytes that can be used for regeneration and can be transplanted into eye, liver…or other places).

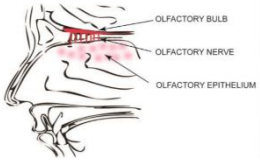


- Pay attention! The issue of the differentiation of stem cells into multiple cell types that can be transplanted is STILL UNDER STUDY, and it is not allowed ethically and legally for you as a medical practitioner to perform this procedure even if there are positive results or promising results on animal models or clinical trials on human, UNTIL it is FDA approved (food and drug administration approved, which is our reference here in Jordan, as every new chemical drug or cell transplant or any food should be checked by FDA and approved to be able to be used.
- So until now **there is NO STEM CELL TREATMENT APPROVED BY FDA EXCEPT FOR BONE MARROW TRANSPLANTATION**, it is the only one allowed to be used, otherwise all types of therapy are only given to certain patients enrolled in clinical trials as a part of a research study, so the certain patient in this case should know that this treatment is given as a part of an experimental research study and is not approved and not guaranteed to work. Many researchers try to attract patients with certain conditions that affect their lives such as sterility, spinal cord injuries, and neurodegenerative diseases to be able to try these different methods of therapy.

4- Umbilical cord stem cells: The **blood of the umbilical cord contains stem cells** that can be stored nowadays to be used in the future. In Jordan we have a certain private practice which is called "baby cord" which does this, and in other countries there are other private and even public companies that also do this. And regarding to the use of these stem cells, currently it is most accepted that these stem cells will work for the same person who donated the stem cells, however, some other scientists believe that the close family may benefit from such stem cells, but the problem is the immune rejection. Another method which is much cheaper which is to store dental stem cells which are found when the babies just loose their teeth, so we can get the tooth and put it in a container in the freezer which may be useful in the future if applications for such types of stem cells has been discovered.

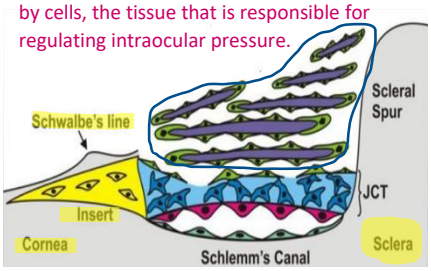


5- Olfactory adult stem cells: found in olfactory mucosal cells (in the olfactory bulb). They are responsible for regenerating the sensory neuronal cells in the olfactory system which are easily damaged, especially after being exposed to certain strong odors such as fuel derivatives odor.



6- Tissue stem cells in cornea. They are a very small population of stem cells. The figure below shows a representation of a trabecular meshwork which is a thin tissue found in between the sclera and cornea and is **responsible for regulating the intraocular pressure**. In a very small region (in yellow) underneath Schwalbe's line, there is a region (Insert) that contains a small population of hundreds of stem cells that can regenerate this trabecular tissue, because intraocular pressure is always changing, for example it can be changed with posture, this can damage cells of the trabecular meshwork when they try to adapt to certain changes such as increasing intraocular pressure more than a certain limit.

Trabecular meshwork: Trabeculae surrounded by cells, the tissue that is responsible for regulating intraocular pressure.



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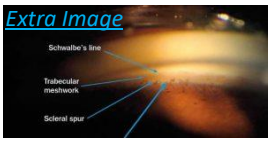

A comparison between different stem cell types:

If we compare generally between the different stem cell types:

- Pluripotent stem cells can give rise to more cell types compared to multipotent stem cells such as mesenchymal or neural stem cells. This is an advantage for pluripotent stem cells over multipotent stem cells.

Comparison between the various types of stem cells. This side-by-side comparison includes their origin and the inherent clinical advantages and disadvantages of using these cells.

Stem Cell Type	Origin	Advantages	Disadvantages
ESCs (pluripotent)	Embryo (blastocyst)	✓ Unlimited proliferation	✓ Ethical problems ✓ Risk of immune rejection ✓ Unpredictable differentiation ✓ High risk of tumor formation
IPSCs (pluripotent)	Reprogrammed adult cells: fibroblasts, hepatocytes, circulating T cells, and keratinocytes	✓ No ethical problems ✓ Low risk of immune rejection ✓ High accessibility	✓ High risk of tumor formation ✓ Risk of susceptibility to the original pathology of the patient ✓ Genetic and epigenetic abnormalities
MSCs (multipotent)	Adult tissues (bone marrow, skin, blood, umbilical cord, etc.)	✓ No ethical problems ✓ High accessibility ✓ Easy isolation methods ✓ Autologous cells generation ✓ Self-renewal capacity ✓ Low risk of immune rejection	✓ Risk of tumor formation
NSCs (Multipotent)	Embryo, human fetal brain and brain tissue of adults (SVZ and SGZ of hippocampus)	✓ Low risk of tumor formation	✓ Risk of immune rejection ✓ Limited differentiation ✓ Low self-renewal capacity ✓ Limited proliferation and expansion ✓ Limited availability ✓ Difficult isolating methods

- **Induced pluripotent stem cells** are ethical and safer in terms of immune rejection in comparison to embryonic stem cells, even multipotent stem cells if they are extracted from the same patient there will be no immune rejection, but if they are given to other patient there will be an immune rejection.
- Sometimes stem cells are easy to extract from the patient, then they are given to the same patient to be used in another place, BUT some cells are less accessible and need invasive procedures. It is better to avoid invasive procedures as we don't want to improve the patient's status and at the same time worsen it. So a clinician must consider all these factors.

Uses of stem cells:

Stem cells can be used in many ways other than replacement of damaged cells, such as:

- **To study specific signals and differentiation.**

- **Genetic therapy:**

Since the origin of the idea of genetic therapy which is based on **replacing a certain defective gene with a normal one**, there hasn't been a certain application of gene therapy on a well-established disease. Regarding the concept of genetic therapy we really don't need to replace the defective gene in all tissues of the body, but we need to replace it in **certain tissues** where the genetic mutations is most effective, so for example if we have certain genetic defect in the insulin gene, we don't need to replace it everywhere, but we need to replace it most importantly in the pancreas.

- The method we use to deliver the certain gene and replace the pathogenic gene is by using **viral vectors**, so we use a virus that is emptied from the inside, but we only keep the fusion proteins and the proteins needed to incorporate the genetic material and to replace the pathogenic genes. The most used viral vector is adenoassociated viruses, which are not pathogenic by themselves, and they need assistants to cause disease, that's why they are very suitable for this process.

- However, this process has certain limitations, The first is the limited capacity of genetic material that can be carried by the virus, for example the maximum capacity that has been established to be transferred is about 5000 nucleotides, however, for example if we want to treat hemophilia, the defective gene in hemophilia is about 18000 nucleotides, so in this case we cannot put the 18000 nucleotides in the virus due to its limited capacity.
- Another limitation is that sometimes our body recognizes these viruses and the genetic material as foreign and gets rid of it, for example in the treatment of diabetes, it was reported that the liver recognized the genetic material and the virus as foreign and got rid of it.
- That's why currently genetic therapy hasn't really shown good results when it is given through the bloodstream because of identification and removal by the liver, and now the focus is on the genetic therapy that is targeted to be inserted at a specific tissue, for example intraocularly, to inject the genetic therapy directly to the eye and not to the bloodstream to treat eye diseases such as retinoblastoma.

- **Drug testing:** with regards to drug testing, it is well known that drugs work in certain individuals better than others, this **depends on their genetic profile which includes epigenetic changes and single nucleotide polymorphisms or genetic mutations**, so if we want to test a certain drug to work on hepatocytes, we can use stem cells and differentiate them into hepatocytes and test the drug on these liver cells to check whether they are effective or not, and we can also choose that this certain cell has a certain genetic makeup to see if a certain drug will work better including this genetic makeup or not, which will of course simulate how drugs work in real life in which sometimes they are more effective in certain individuals with certain genetic makeup and they are less effective in others, for example in the case of chemotherapy, if we have 2 persons diagnosed with the same type of cancer with the same stage, sometimes they will not have the same treatment because certain treatments are effective only against certain mutations, so we test these individuals for these mutations and accordingly we choose the specific treatment that is most effective.

Note: We should know that these experiments are usually done in the labs (ex-vivo), and we do these experiments on specific cells, which will definitely give an indication of how the drug will work inside our body and will also give an indication if the treatment is going to be successful or not, however the response of certain cells is not identical to the response of the whole body in reality because when we deliver the drug to the body it goes to all cells and all systems, but despite all this it is very good to give an indication.

- **Cell based therapies.**
- **Stem cells for cancer treatment by activation of chemotherapeutic agents.**

Stem cell therapy limitations:

stem cell therapy has disadvantages such as:

1-Carcinogenicity: ability to cause cancer especially **pluripotent stem cells**. However, this disadvantage is mainly when they are transplanted as stem cells (this is a risk that must be avoided). If you differentiate them before and then transplant them, their ability to divide is limited and carcinogenicity will not be a limitation.

2- Immune rejection: This can be seen when transplanting stem cells from one individual to another. This problem is not present for an autologous source of stem cells.

3- Infection and contamination: Because we are using cells as therapy, we need to **prevent any cross infection** from happening and control it when transplanting stem cells from one individual to another.

4- Genetic instability following a prolonged time in culture: When stem cells are taken from the donor and then cultured ex-vivo, this may cause change in the genetic profile due to changes in environment (the culture environment differ from the environment of human body, as it does not contain all the reactions, cells, and blood supply that are present around in human body).

These factors make the usage of stem cells limited.

Limitations of using adult stem cells (ASCs):

- Their limited ability to differentiate (cannot give rise to all cell types), **multipotency of ASCs.**
- Lack of **stem cell markers** resulting in difficulties to separate and identify cells. Not all types of multipotent stem cells have known cell markers, a cell marker is a cell membrane protein that is specific to cell type, and it is the best way to identify a cell type more than by their genetic profile or gene expression profile, so by the cell markers I can be sure 100% in vivo that this is that specific type of cell. Each cell has its own cell marker, some are discovered, and some are still undiscovered, so a lot of adult stem cell types in our body still don't have known specific markers.
- In-vitro systems for manipulating adult stem cell (multipotent or pluripotent) populations are often not well defined, since there is no consensus of how to deal with them, culture them, and how long they are allowed to stay outside the body.
- In-vivo: Our understanding of how adult stem cells are regulated within their niche is in its infancy. The certain **niche** required to maintain the stemness for each cell type is still unknown, which may be composed of cells, ECM, or soluble factors, it is possible that part of it was discovered but not all.

Note1: The challenge when dealing with stem cell experiments and trying to induce their differentiation to a specific type of cells is by discovering the **differentiation procedure** or the differentiation steps that we should do in order to make the stem cell differentiate into a specific type of cells and tissue, for example, there are some types of cells that have a known differentiation procedure for example bone has a known differentiation procedure which depends on a factor called BMP2 (Bone Morphogenetic Protein 2), and skeletal muscles have a differentiation procedure that depends on a factor called myogenin, in both cases as soon as we add these factors to the stem cells, they will start differentiating into these types of tissues, however not every type of tissue has an established differentiation procedure, that's why this is a big challenge to discover the differentiation procedure for other types of cells.

- Regarding the time frame for such types of therapy, we should keep in mind that the time frame is extended for many years, and the process for certain genetic therapy or even any type of therapy, goes through many years of development, research and approval, that's why researchers should be patient through the process and we should also know that clinicians are **the last step of the chain (the end users of technology)**, so they just apply what hundreds of experts have worked on throughout years.

Note 2: In this lecture only understand the main concepts. **What topics did the doctor focus on in the lecture?**

- The biology of stem cells (adult vs embryonic stem cells, pluripotent vs multipotent...) and just know that there is a collection of transcription factors that make the cell pluripotent without memorizing them.
- The concept of induced pluripotent stem cells
- The ethical considerations (very important)
- The challenges of the use of stem cells in treating these 4 diseases (this is the topic of the next sheet): (Alzheimer, Parkinson, stroke, and spinal cord injuries), and know how complex is the process and type of cells that can be used in each disease without knowing the clinical trials that have been carried and where the attempts have reached, because none of these treatments reached the point of clinical application (all are under research).

Past papers:

1- The statement that describes stem cells is:

a. Changes in the niche have no effect on the behavior of stem cells.

b. They can be used for cell- based therapy and modelling human diseases.

c. Their niche drive their differentiation and does not keep their stemness

d. They have a limited ability to asymmetrically divide.

e. We can use them as a cell- based therapy directly after we test them in tissue culture disease models and they show an improvement of the disease.

2- You have recently heard that stem cells may have a potential in regenerating damaged lung tissue caused by SARS-CoV-2 in COVID-19. Before they can be used in clinic, the following has/have to be checked:

a. Carcinogenicity specifically if pluripotent stem cells are used.

b. The mechanism by which stem cells repair the lost pulmonary function.

c. All experimental stages starting with ex vivo experiments, animal stage, clinical trials of 3 stages.

d. Food and drug administration approval in the country of practice.

e. All points have to be verified before stem cell can be used as a treatment for COVID.

3-Which stem cell is the most potent, genetically engineered and causes no immune reaction:

a. iPS

b. embryonic

c. adult neural

4- True about stem cells

▪ embryonal stem cells have more potency that adult

Answers:

1- B
2- E
3- A

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