CNS
BIOCHEMISTRY

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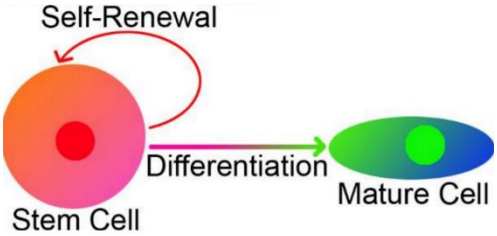
Stem Cells: The New Therapeutics Era

This lecture will discuss stem cells and their use in the treatment of neurodegenerative diseases.

What are stem cells?

- They are primal cells common to all multicellular organisms.
- They are characterized by 2 main features:
    1. The ability to renew themselves through cell division and maintain a population of stem cells.
    2. The ability to differentiate into a wide range of specialized cell types that can perform different functions.
- All stem cells are unspecialized (undifferentiated) cells that are of the same family type (lineage).

Differentiation vs self-renewal



- Stem cells can divide asymmetrically to be able to renew themselves on one side and differentiate into other cell types on the other side.
    - Asymmetric division is due to differential segregation of cell membrane proteins between the daughter cells.
- Self-renewal: The ability to go through numerous cycles of cell division while maintaining the undifferentiated state.

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, the cell membrane proteins that are important for keeping the 'stemness' of a stem cell are going to be located in the cell that renews the stem cell population. Whereas the cell membrane proteins that are important for driving differentiation are going to move to the second cell that goes into the differentiation pathway.

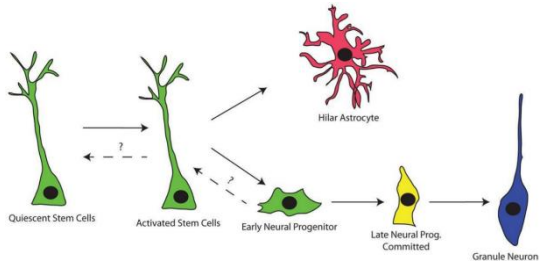
To simplify this concept more, here's an explanation from an online source:

"An **asymmetric cell division** produces two daughter cells with different cellular fates. Notably, stem cells divide asymmetrically to give rise to two distinct daughter cells: one copy of the original stem cell as well as a second daughter programmed to differentiate into a non-stem cell fate."

What does stem cell division produce?

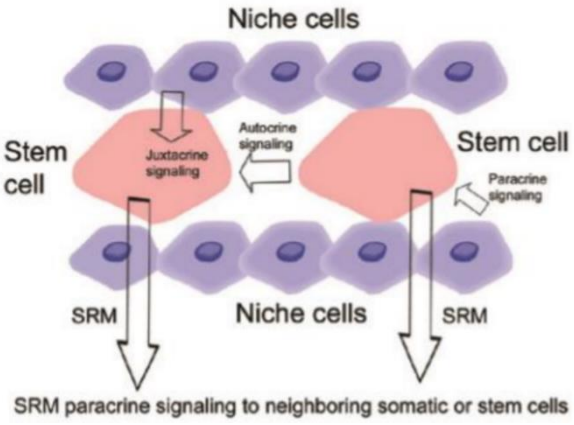
During differentiation pathways, stem cells do **not** undergo differentiation in a single step. They pass through intermediate cellular steps in which these intermediates are partially differentiated and can diverge the differentiation pathway into several ones producing different types of fully differentiated and mature cells. These intermediate cells are called **progenitor cells**.

- Progenitor cell: Stem cells generate an intermediate cell type or types before they achieve their fully differentiated state.



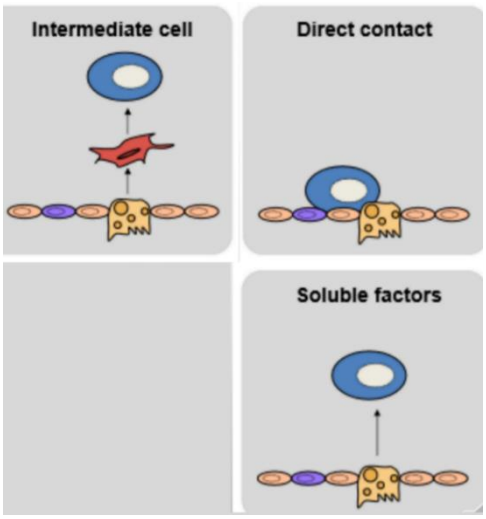
Stem cell niche:

- 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:
  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.

  2. Cells and ECM components (like proteins and sugars).
  3. Secreted or cell surface soluble factors, such as Notch, Wnt, FGF, EGF, TGF-β, SCF, and chemokine families.



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

In addition to providing nutrition and support to the stem cells' self-renewal, it also:

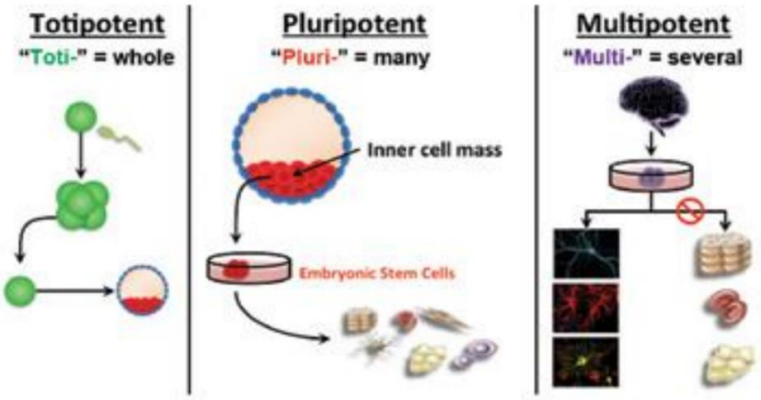
- Provides special support for their viability due to the demands on stem cells.
- Nutritive function.
- Niches might be agents of feedback control (control of stem cell pool size so that it doesn't expand too much nor shrink).
- Niches are instruments of coordination among tissue compartments.

    - By interacting and providing feedback mechanisms, they can coordinate the size, differentiation into a certain pathway, and production of a certain cell type.
- 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.

Potency of stem cells

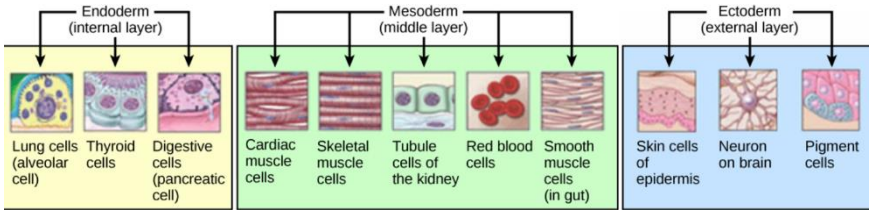
Stem cells can be classified according to their potency (ability to differentiate, or potential to produce several cell types, or how many cell types can be produced from a certain stem cell type) into:

1. Totipotent: able to differentiate into **ALL** cells of the body and extraembryonic tissues, including the placenta.
2. Pluripotent: able to differentiate into **ALL** cells of the body but **NO** extraembryonic tissues.
3. Multipotent: able to differentiate into several cell types of the body, but not all.
4. Unipotent: able to differentiate into a single cell type.



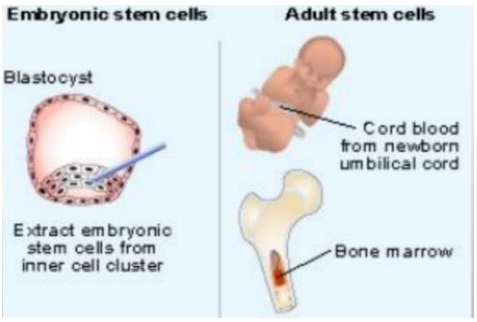
Three germ layers

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

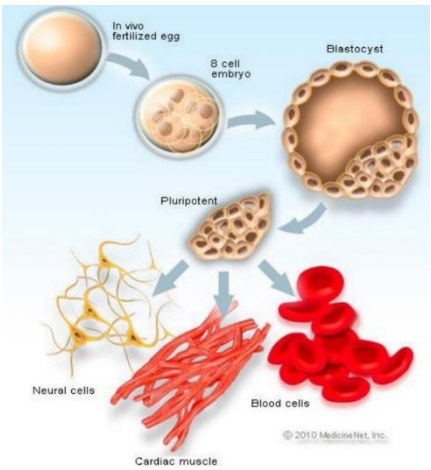
Another classification of stem cells depends on their time of presence during development:



1. Embryonic stem cells: appear during embryonic development and are important for the development of a fetus. They can differentiate into all the specialized embryonic tissue and all cells of the body because during development all cells, tissues, and organs must form.

2. Adult stem cells: appear in adult life or after birth. They act as a repair (regeneration) system for the body replacing specialized damaged cells.

Embryonic Stem Cells (ESCs)



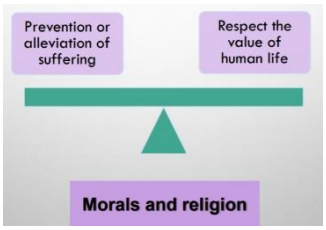
- ES cells are derived from the inner cell mass of mammalian blastocysts.
  - Fetal development starts by the formation and fertilization of an egg to form a zygote → then, the zygote starts to divide until it reaches the eight-cell stage → after that a blastocyst is going to form.
  - A blastocyst has 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 in the uterus.

Pluripotency of ESCs

Pluripotency depends on the expression of a group of transcription factors, including:

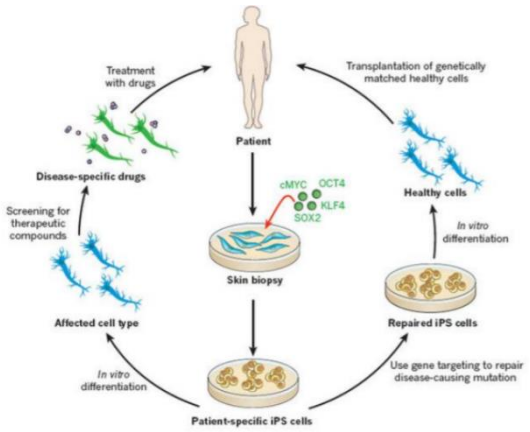
1. Oct 4
2. Nanog
3. Wnt-β-catenin signaling
4. Other TFs

The Ethical Dilemma of ESCs



- One of the problems presented with ESCs and their usage in the treatment of diseases is that we need to isolate them from embryos, which means we are basically going to kill the embryo after it started to develop. However, they will be used to alleviate the suffering of patients. This raises the need to find a balance between morals and religion.
- Another problem to consider is that transplanting cells from one embryo into another patient (even if they are both from the same family) is introducing foreign cells into the patient's body which may cause immunological problems and an immune rejection.
- Thus, scientists needed to find another source of pluripotent stem cells. These are the **Induced Pluripotent Stem Cells (iPSCs),** which come from the patient himself (endogenous cells) and in which the ethical dilemma can be avoided.

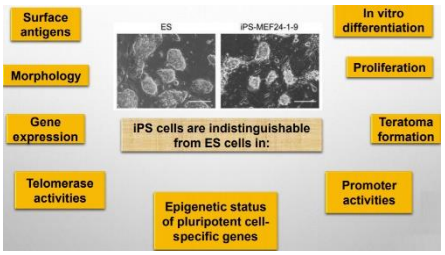
- How are iPSCs derived? In labs, scientists reverse the differentiation of fully differentiated cells from the patient by expressing the transcription factors necessary to maintain stemness of cells. Only a few of these cells that are being reprogrammed or de-differentiated can go back to stem cells and be pluripotent again.



- Advantages of iPSCs include:
  - No Ethical problems.
  - Safer (because they are endogenous).
  - Patient-specific (so no immunological problems) or Autologous.

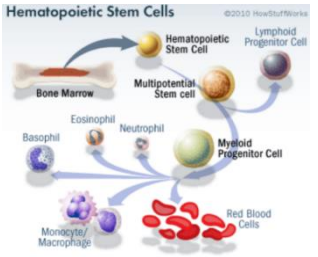
Generation of iPSCs

- In 2006, Yamanaka was the first scientist to generate iPSCs. He obtained iPS cells by transducing embryonic and adult fibroblasts with defined transcription factors (OCT3/4, SOX2, c-Myc, KLF4).
  - Takahashi K, Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676.
- Another group of scientists were able to do it using a different set of four transcription factors.
  - Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872.
- **Yamanaka's comparison of iPS and ES cells**: After they collected the reprogrammed cells, they tested them and checked whether they are really acting like embryonic stem cells. Under the microscope, they looked morphologically the same and grew in colonies just like ESCs. They compared their morphology, surface antigens, gene expressions profiles, telomerase activities, epigenetic status of pluripotent cell specific genes, promoter activities and the active genes inside them, in-vitro differentiation ability to several cell types, proliferation ability, and Teratoma formation ability (after injecting them into mice, these cells were able to form teratomas (tumors of the three germ layers)).

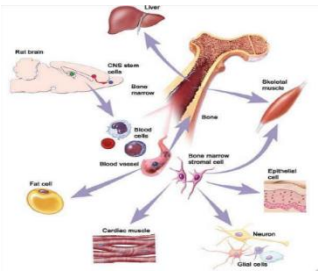


Adult stem cells

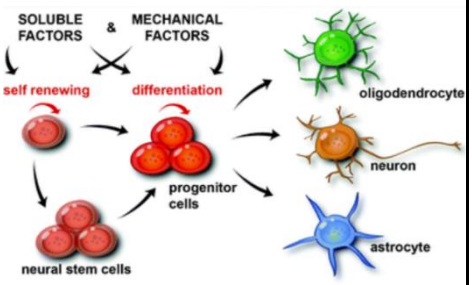
- Undifferentiated cells found throughout the body.
- Function: they divide to replenish dying cells and regenerate damaged tissue. However, their ability to differentiate is limited in comparison to the pluripotent stem cells. Thus, they can be multipotent or unipotent, but NOT pluripotent or totipotent.
- Types of adult stem cells: (there are more types than mentioned)



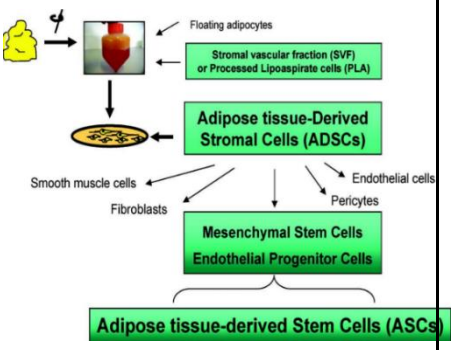
1. **Bone marrow stem cells** (found in bone marrow). They are further subdivided into:
   a) **Hematopoietic stem cells**: can give rise to all cells of the blood (RBCs, WBCs, platelets, etc.).
   b) **Somatic stem cells**, such as mammary stem cells and mesenchymal stem cells. They can give rise to a group of cells, like osteoblasts, chondrocytes, myocytes, adipocytes, and neuronal cells.



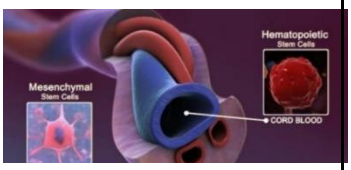
2. **Neural stem cells**: can generate some types of neurons. Even though we know that neurons do not regenerate, but this is not completely true as they have a limited ability to regenerate.
   - Neurospheres – floating heterogenous aggregates of cells, containing a large proportion of stem cells responsible 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.



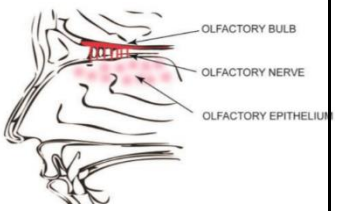
3. **Adipose stem cells (ASCs)**. They are found in adipose tissue.
   - They are mesenchymal stem cells which can differentiate into other cells like fibroblasts, endothelial cells, pericytes etc.
   - They can be obtained after liposuction operation (in which a part of adipose tissue is removed for the purpose of reducing weight, but we can use this fat to get some mesenchymal stem cells).



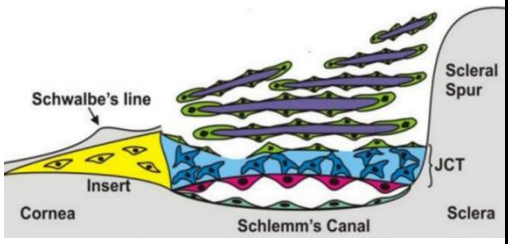
4. **Umbilical cord stem cells**. They are found in the umbilical cord. The umbilical cord contains two types of stem cells:
   - The blood of the umbilical cord contains hematopoietic stem cells.
   - The core tissue contains mesenchymal stem cells.



5. **Olfactory adult stem cells**. They are found in the olfactory mucosal cells. They are responsible for regenerating the sensory cells, which are easily damaged especially after being exposed to certain odors.



6. **Tissue stem cells of the cornea**. They are a very small population of stem cells. They are found in the cornea, trabecular meshwork, etc. The figure below shows a representation of a trabecular meshwork which is a small tissue found in between the cornea and iris and is responsible for regulating the intraocular pressure. In a very small region (in yellow) underneath Schwalbe's line, there is a region inserted between the cornea and iris and it contains a small population of stem cells that can regenerate this trabecular tissue.



Uses of stem cells

Stem cells can be used in many ways other than treatment, such as:

- To study the specific signals of differentiation and the differentiation process.
- Genetic therapy by changing the stem cells' genetic material if mutations are present (so if there is a disease caused by a mutation, you could treat it by changing the genetic material of the stem cells).
- Drug testing.
- Cell based therapies (personalized medicine): We can generate iPSCs from any individual and then differentiate them to the cell of interest, such as hepatocytes and pancreatic cells. Then we can test the drug that we are developing or those already present in the market on the iPSCs to see if this patient is going to respond, how the response appears, if any resistance is taking place, and so on.
- Stem cells for cancer treatment by activation of chemotherapeutic agents.

Stem cell therapy limitations

Limitations to using stem cells as therapeutic agents include:

- Carcinogenicity: Since stem cells have the ability to divide, these cells can develop cancers in the organs they are transplanted into.
    - However, this disadvantage is mainly when they are transplanted as stem cells. If you differentiate them beforehand and then transplant them, their ability to divide is limited and carcinogenicity will not be a limitation.
- 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.
- Infection: 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.

Limitations of using adult stem cells

- Lack of information about the stem cell markers that are specific to them results in difficulties to separate and identify cells.
- In-vitro systems for manipulating adult stem cell populations and maintaining their stemness after isolation are often not well defined.
- Our understanding of how adult stem cells are regulated within their niche in-vivo is in its infancy. We need to transplant cells into the proper niche to guarantee they differentiate into the required cell type.
- ASCs are multipotent, thus their ability to differentiate is limited in comparison to the pluripotent stem cells.

Stem Cells and Neurodegenerative Diseases

Now we will focus on the use of different types of stem cells in treating neurodegenerative diseases.

Neurodegenerative Diseases

- A wide range of acute and chronic conditions in which neurons and glial cells in the brain and spinal cord are damaged and lost. This damage can occur through different mechanisms leading to different functional losses.
- The use of stem cells is determined by whether the disease is acute or chronic and whether the disease involves one cell type or several.
- Acute: Ischemic stroke or spinal cord injury
- Chronic: Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), or Alzheimer disease (AD).

Main considerations when using stem cells to treat neurodegenerative diseases:

- What is required for the stem cell-based approach to be clinically competitive?
    - Stem cells need to be very competitive compared to other treatment modalities already available to clinics (e.g. there needs to be a reason why you and your patient would choose this treatment over others, especially if other treatments are cheaper). They should improve the situation and disease in the patient to a similar or greater degree. They also shouldn't cause problems that could be worse for the patients.
- **Risks** to the patient that are acceptable, depending on disease **severity**. Animal models may not fully predict their **toxicity**, occurrence of **immune** and other biologic responses, and risk for **tumor formation** after implantation in patients (which we have discussed previously).
- The **variability** between neurodegenerative diseases in the **degree of disability** that they cause and in the therapeutic options that are available.
    - Some neurodegenerative diseases are very disabling for the patient so that even a small percentage in reduction of disability would be an acceptable reason for the patient to undergo stem cell treatment.
    - The available treatments for some diseases just treat the symptoms (such as in Parkinson's), so stem cells might be preferable if they were able to significantly improve the patient's life and reduce the degree of disability.
- The **cell type** that needs to be regenerated and transplanted in the following neurodegenerative disease:
    - PD – Dopaminergic neurons
    - ALS – Motor Neurons
    - Stroke, spinal cord injury and Alzheimer's Disease – Several cell types

        - Treatment would require replacing synapse, remyelinating tissue, and other changes.
- The stem cell-based approach should show **substantial improvement** of functional deficits in animal models before their use in clinical application.
    - For example, PD patients can live with their disease with the available treatment. Thus, stem cell therapy would have to provide substantial improvement to be considered as a treatment option.
    - On the other hand, in a spinal cord injury with a high degree of disability, even a small percentage of improvement would be acceptable.
- To determine the **biological mechanism** underlying the observed effects of a stem cell-based treatment in an animal model (e.g. reconstruction of neuronal circuity, replacement of loss neurons, or induction of neurological repair).

Common considerations when translating stem cell therapies to neurodegenerative disease patients

Inclusion/exclusion criteria	Enrolling late-stage patients may prevent loss of quality of life Late-stage patients may mask any positive effects due to the intervention occurring too late in the disease course
Realistic expectation	Informed consent forms must clearly illuminate the goals of the study Safety trials vs. efficacy trials Expectations of therapeutic effects based on disease state at intervention
Controlled study	Ideal study is a double-blind placebo study Late-stage patients may mask any positive effects not observed due to the intervention occurring too late in disease Original PD studies offered control arms treatment after a 1-year follow-up which confuses interpretation of efficacy
Immunosuppression	While the brain remains an immunologically privileged site due to the blood-brain-barrier, there is evidence that this barrier can be compromised in disease Studies into cell graft survival demonstrate that immunosuppression increases that survival of graft tissue
Potential side effects	Prevent/minimize potential side effects (i.e. meningitis, fever) Avoid exacerbation of disease and tumor formation Risk vs. quality of life
Safety of cellular therapy administration	Consider CNS accessibility and safety of delivery methods Pros/cons of systemic delivery, lumbar puncture or stereotactic injection are important

Abbreviations: PD, Parkinson's disease; CNS, central nervous system.

Notes on the Table:

- We need to consider if the patient needs to be immunosuppressed before the treatment begins, the potential side effects (could future problems be more dangerous than the disease itself?), the safety of administration, and inclusion and exclusion criteria (are there certain conditions that must be present for the patient to be considered for this type of treatment).
- Double-blind placebo studies are considered the ideal. In this case, both the patient and operator do not know whether the patient is receiving the actual treatment or a placebo.
- Ensure that expectations are realistic and explained to the patient, and informed consent must be taken.

Now we will look into examples of neurodegenerative diseases that are targeted by stem cell therapy (or therapy was at least attempted).

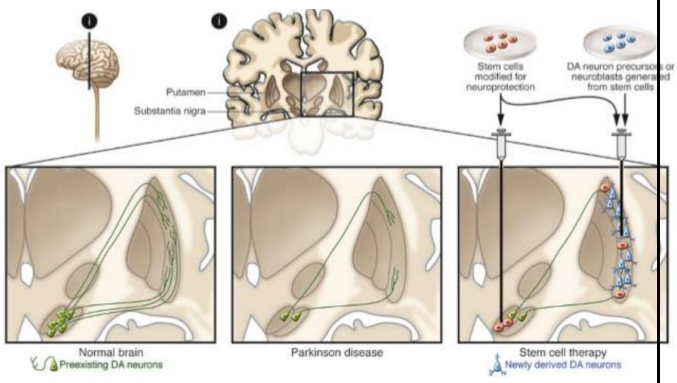
Parkinson's Disease (PD)

- Degeneration of nigrostriatal dopaminergic neurons is the main pathology.
- Characteristic symptoms are rigidity, hypokinesia, tremor, and postural instability.
- Different treatment modalities are available including L-dopa, dopamine agonists, enzyme inhibitors, and deep brain stimulation. Their main purpose is to replace the loss of dopamine.
- There is no treatment for dementia.
- iPSCs for modelling the genetically complex PD. (we emailed the professor about this point, since it wasn't clarified in the lecture. What this statement means is that iPSCs can be differentiated into neuronal tissue and then can either be subjected to reduced dopamine (as in, cells that need dopamine receive less than required) or dopaminergic neurons are killed or suppressed. Using these in vitro models, we can study Parkinson's disease. This method can also be used to study other diseases.)

Stem Cell-Based Therapies for PD

Clinical trials used intrastriatal transplantation of cells derived from human embryonic mesencephalic tissue (rich in postmitotic dopaminergic (DA) neuroblasts).

Extra: The slides mention the phrase "proof of principle". A study that provides proof-of-principle has been able to prove that their treatment has shown potential.



Pros	Cons
The DA neurons that formed from the transplanted tissue were able to reinnervate the denervated striatum and become functionally integrated into the tissue. They were able to restore striatal DA release and gave rise to clear symptomatic relief in some patients.	A small fraction of graft-derived DA neurons contained Lewy bodies (the hallmark of PD) (i.e. the disease was transferred to these neurons). Availability of human embryonic mesencephalic tissue is limited.
11-16 years after transplantation, the cell replacement remained a viable therapy (the cells lasted for a long time)	There was high variability of functional outcome (in terms of restoration of function) after transplantation. Some patients improved a great amount while others showed very little.
The progression of pathology in graft-derived neurons is slow (in comparison to the original cells), and they are still functional after a decade.	Poor standardization of the transplanted cell material contributes to high variability.

Other sources of DA Neurons

These are other possible sources of stem cells, but so far none of them have moved to the clinic as treatment options.

- Embryonic stem cells (ESCs)
- Cloned ESCs
- Neural stem cells (NSCs) and progenitors of embryonic ventral mesencephalon
- Adult NSCs from the subventricular zone (SVZ)
- Bone marrow stem cells
- Fibroblast-derived iPS cells.

Human stem cell-derived DA neuron precursors/neuroblasts can survive in animal models of PD and can be functional after maturation.

Hurdles that prevent stem cell therapy for PD to jump from bench (laboratory) to clinic

- PD is a **multisystem disorder**. If **nondopaminergic systems** are affected, they will not improve by intrastriatal DA grafts (which improve dopamine release).
- **Substantial re-innervation** of striatum has not been demonstrated (i.e. the amount of re-innervation was not verified to be substantial).
- **Restoration of DA release** in vivo has not been demonstrated.
- **Marked improvement** (50-70%) in the deficits and symptoms experienced by PD patients has not been demonstrated. Not reaching 50-70% improvement makes it less convincing for patients.
- Risk of **tumor formation**, even if minor, is not acceptable. This is because patients can live with Parkinson's and available treatments, they should not be subjected to the risk of cancer.
- The need to inject cells at **all sites of injury** (which may be practically difficult).

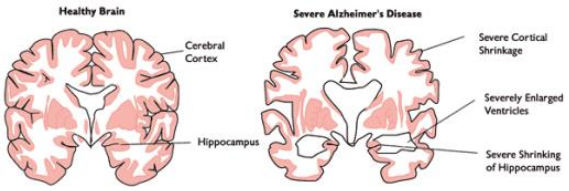
Clinical Trials

One clinical trial was conducted by the international stem cell corporation (ISCO). They used parthenogenetic cells derived from unfertilized oocytes after suppression of the second meiotic division.

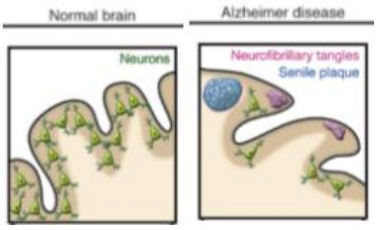
The drawback of this trial was that the transplanted cells were PAX6-**positive** (PAX6 is a transcription factor important for development) suggesting that they are of a dorsal neural fate. The problem is authentic midbrain dopaminergic neurons are derived from a PAX6-**negative** ventral midbrain neural precursor (i.e. dopaminergic neurons are derived from a PAX6-negative neural precursor). Thus, using dopaminergic neurons derived from PAX6-positive cells may lead to future complications.

Alzheimer's Disease (AD)

- Memory impairment, cognitive decline, and dementia occur due to widespread and progressive pathologic changes in several tissues.

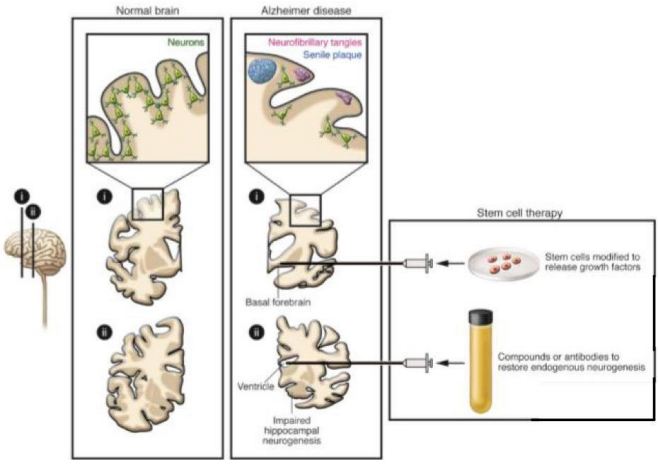


- Neuronal and synaptic loss, neurofibrillary tangles, and deposits of β-amyloid protein involve the basal forebrain cholinergic system, amygdala, hippocampus, and cortical areas.



Therapies for AD

1. Cholinergic neurons: Acetylcholinesterase inhibitors, which enhance cholinergic function, induce some temporary improvement in AD patients.
2. Neurogenesis or maturation of hippocampal neurons as the formation of immature hippocampal neurons was reported in AD.
3. Nerve growth factor (NGF) releasing stem cells to stimulate regeneration and repair of neurons.
4. Anti-β-amyloid antibodies or β-amyloid-degrading protease neprilysin.



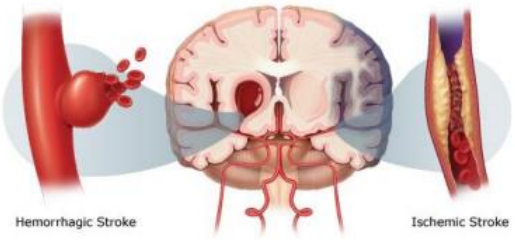
Hurdles that prevent stem cell therapy for AD to jump from bench (laboratory) to clinic

- Stem cells have to be pre-differentiated in vitro to **many different types of neuroblasts** (remember several cell types are involved) for subsequent implantation in **many brain areas**. This is difficult to do.
- For a long-lasting symptomatic benefit, **cholinergic cell replacement requires intact target cells** (host neurons that the new cholinergic neurons can act on), which are damaged in AD.
- Stem cell-based replacement strategies are very far from clinical application in AD.

Clinical Trials

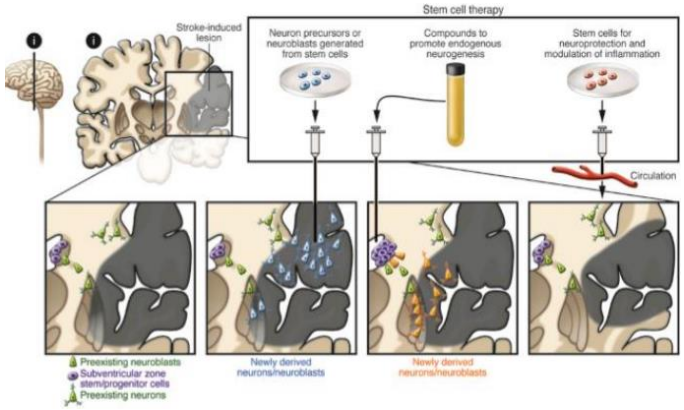
- One clinical trial was done by Stemedica cell technologies.
- They provided stem cells from healthy people to mild to moderate AD patients to test if stem cells work for AD.

Stroke



- Ischemic stroke, caused by occlusion of a cerebral artery, leads to focal death of multiple neuron types, as well as oligodendrocytes, astrocytes, and endothelial cells. Additionally, synapses are damaged and functions of the brain are interrupted. Region affected and infarct size depends on which artery was affected.

- Neuronal plasticity and reorganization of neural circuitries contribute to spontaneous recovery to varying degrees, but most patients exhibit persistent motor, sensory, or cognitive impairments.



Stem Cell-Based Therapies for Stroke

- Human ESC-derived NSCs and MSCs (mesenchymal SCs) were grafted into the rat stroke site, migrated toward the lesion, and improve forelimb performance.
- IV injection of human NSCs induced improvements after hemorrhagic stroke in rats, probably through anti-inflammatory actions.
- However, no substantial clinical improvements were detected after IV injection of autologous MSCs in patients with an ischemic lesion in the regions supplied by the middle cerebral artery (MCA).
- Several clinical studies using intravenous or intraarterial infusion (into damaged territory) of autologous bone marrow-derived stem cells in stroke patients are ongoing. However, nothing has moved to the clinic as a definitive treatment.
- A clinical trial in stroke patients involving transplantation of clonal, conditionally immortalized NSCs isolated from human fetal cortex is being tested.
- 80% of neuroblasts and neurons die during the first two weeks after formation from the stem cells at the stroke site in rats.

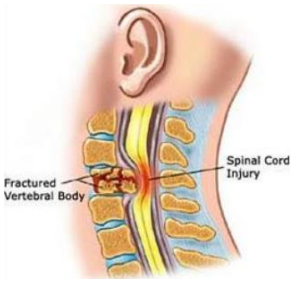
Clinical Trials

Two methods are being tested:

- Clinical trials have used transplanted ESCs, iPSCs, and NSCs to replace the missing/damaged brain cells in the infarcted area.
- Non-neuronal adult stem cells, such as MSCs, were used to provide trophic support to enhance self-repair systems such as endogenous neurogenesis.

Spinal Cord Injuries

These injuries may occur after various types of traumas. Pathological changes after spinal cord injury are complex and include:



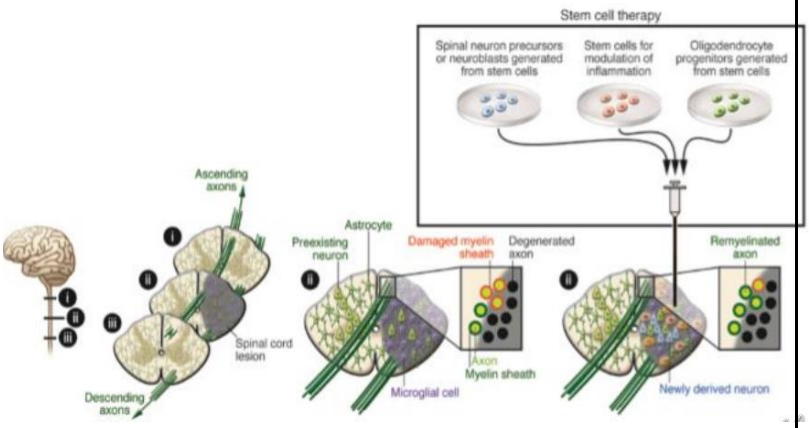
1. Interruption of ascending and descending pathways
2. Loss of neurons and glial cells
3. Inflammation
4. Scar formation
5. Demyelination.

So, there is loss of tissues which need to be replaced (and their function restored) through stem cells.

- Patients experience loss of movement, sensation, and autonomic control below the level of the injured spinal segment.
- Available treatments are ineffective.
- Different types of stem cells were tested and improved functional outcomes of animal models through secretion of neurotrophic factors, remyelination of spared axons, or modulation of inflammation.

Stem Cell-Based Therapies for Spinal Cord Injuries

- Therapy should correct the lost cells, including neurons, oligodendrocytes, and astrocytes.
- Synapses and axons should be re-established.
- Therapy should be able to induce remyelination. High purity oligodendrocyte progenitor cells (OPCs) generated from human ES cells in vitro can differentiate into oligodendrocytes (in clinical trial).



So far, the trials for using stem cells to treat spinal cord injuries have not been moved to be used in clinical practice. Before doing so, we should be able to:

- Determine how to control the proliferation of transplanted stem cells and their progeny. We need the amounts of these cells to remain within a certain limit.
- Determine how to enhance the differentiation of these cells to the specific types of neurons that have been lost.
- Determine how the resulting neurons can be directed to format appropriate synaptic contacts to perform the required lost function.

Other Stem Cell Types

Umbilical cord blood stem cells, bone marrow-derived hematopoietic and mesenchymal SCs have already been applied in patients with spinal cord injury, with claims of partial recovery. However, none of these have been moved to the clinic as a definitive treatment.

Problems in These Trials:

1. The implanted cells were often poorly characterized. This means we don't know much about them or how to differentiate them into their required cells.

2. The preclinical evidence of efficacy for several of these approaches was insufficient.
   (Extra: In drug development, preclinical studies is a stage of research that begins before clinical trials (testing in humans) can begin, and during which important feasibility and drug safety data are collected, typically in laboratory animals.)
3. The therapeutic benefit was reported from open-label trials where patients had been subjected to physiotherapy. Since both stem cell treatment and physiotherapy were occurring at the same time, we cannot be sure the improvement was due to the stem cells.
4. The mechanisms underlying observed improvements were unclear.

To summarize and comment on what we have taken for all of these neurodegenerative diseases:

- Clinical trials using stem cells have already been performed or initiated (e.g., for the rare, fatal, autosomal recessive neurodegenerative disorder Batten disease).
- No stem cell-based therapy has yet been proven beneficial for any neurodegenerative condition.
- Despite this fact, unproven treatments for several neurodegenerative diseases are offered at "clinics" around the world without rationale with poor scientific and clinical basis. As a clinician you should 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.
- Ethical, regulatory, societal, and economic issues need to be addressed. The use of autologous or non-autologous stem cells should be considered. The cost of treatment is usually high, which is a burden on the patient, especially if it did not really improve their situation.

Translating a stem cell-based treatment from the bench to bed:

For any stem cell-based treatment to move from bench to bed, it must pass through a long path of research, starting with in vitro characterization studies, then animal studies with small animal models then large animal models. Then it moves into three phases of clinical trials. This trial may take 10 or even 20 years of investigation until final evidence can be gained on whether the treatment is beneficial. Therefore, patients should be properly informed of what they are getting into and ensure they don't face complications worse than their current condition.