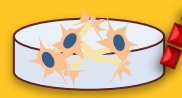
Induced Pluripotent Stem Cells (iPSCs) for Brain Research

In vivo:

Purpose:
Regenerative Medicine

Application:
- Transplant
- Patient Specific Treatment
- Gene Therapy

- iPSC derived midbrain-dopaminergic neurons were transplanted into the putamen of a Parkinson's Disease (PD) primate model which led to a decrease in the primates' PD scores.
- The results of the iPSC derived dopaminergic transplant were compared to L-DOPA treatment, suggesting a future for iPSC derived cells as a therapeutic alternative.
- Researchers in Japan will transplant 5 million dopaminergic precursor cells into 7 PD patients.

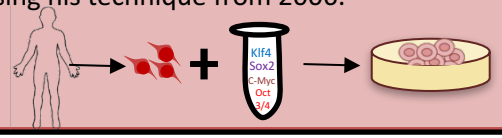


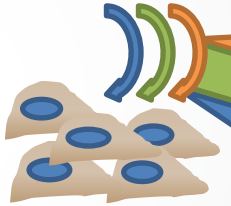
What is an iPSC?

Induced Pluripotent Stem Cell (iPSC): a stem cell made from a reprogrammed adult cell that has the ability to self renew, to differentiate into all 3 germ layers (Ectoderm, Mesoderm, Endoderm), and can undergo cell specific differentiation.

Discovery of iPSCs:

In 2006, Yamanaka successfully reprogrammed somatic cells of adult mice into pluripotent stem cells by supplying the adult cells with transcription factors that function in embryonic stem cell pluripotency, and cell division: Oct3/4, SOX2, c-Myc and Klf4.
In 2007, Yamanaka successfully made iPSCs from adult human dermal fibroblast cells using his technique from 2006.

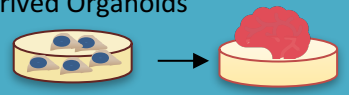




In vitro:

Models:
3D iPSC cultures

iPSC derived Organoids



Application:
Disease Models
Organoids can be used to understand the underlying mechanisms of diseases.

Drug Discovery
Once the anatomical and physiological changes caused by a disease are known, treatments can be tested on organoids and in 3D cultures. These are the closest models to human tissue, environment, and complexity.

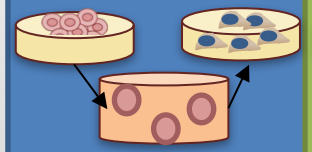
Drug Cytotoxicity
Models can be used to develop the dosage of drugs that would be tolerable for patients.

Phase 1 differentiation: iPSC → Neuronal Precursor Cell

Embryoid Body Formation
1. Place iPSCs into suspension culture
2. Wait for aggregates/ball-like cell formations

Pros: Multiple cell types formed

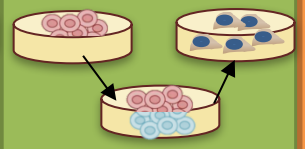
Cons: Require rigorous selection procedure for isolation of 1 cell type



Stromal Feeder Co-Culture
1. Combine iPSCs with stromal feeder cells

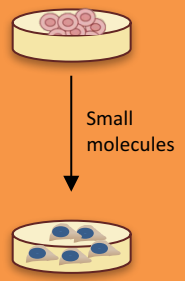
Pro: develop specific progenitor cell type

Cons: need specific stromal feeder cells for the desired differentiation; not effective for all types of differentiation.



Selective survival conditions
1. Combine iPSCs with chemical and patterning molecules

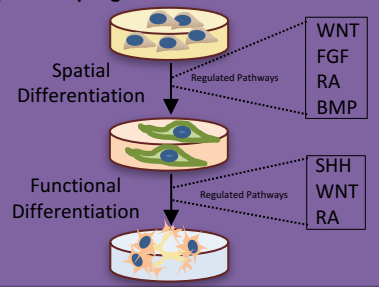
Pro: Effective for any type of differentiation



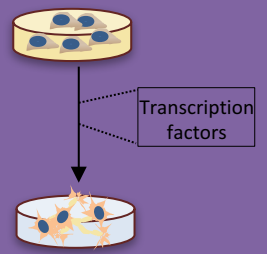
Phase 2 differentiation:

To further differentiate neuronal precursor cells into spatially and functionally specific neurons.

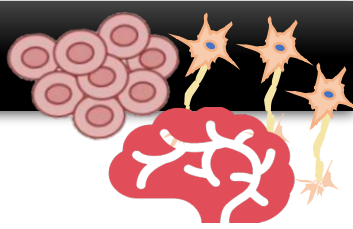
Technique 1: Morphogens:
Applying chemicals and small molecules to regulate pathways of morphogenesis.



Technique 2: Transcription Factors :
Introducing transcription factors to influence gene expression.



Snap Shot: Induced Pluripotent Stem Cells (iPSCs) for Brain Research



Induced pluripotent stem cells (iPSCs) are derived from adult cells. iPSCs have three characteristics: pluripotency, self renewal and the ability to undergo directed differentiation. The ability of iPSCs to form three germ layers allows scientists to harness these cells for studying disorders that effect cells throughout the human body. Of interest for Brain research iPSCs ability to differentiation into the ectoderm is critical because this is the beginning of the nervous system. Utilizing the potential of directed differentiation of iPSCs has taken time because deciphering which differentiation protocol is most effective has been difficult. Regardless, protocols are becoming further defined, and iPSCs are being used as disease models, therapies, and in regenerative medicine.

iPSC Generation

There are three steps to generate iPSCs. First, the adult cell source needs to be isolated and cultured. Second, the adult cells are reprogrammed. To reprogram the adult cells, the reprogramming factors (OCT3/4, SOX2, Klf4 and c-Myc) need to be introduced via integrating or non-integrating delivery methods to the cell culture and incubated on a feeder cell layer (note that these 4 factors may be substituted depending on experimenters' protocol). Third, once the cells express the 4 factors they can be classified as iPSCs which are characterized via morphological and physiochemical methods.

iPSC Differentiation

Differentiation of iPSCs can be split into two phases. The techniques used will depend on experimental aims. Of interest is the differentiation of iPSCs into functionally specific neurons that can be used for brain research. Therefore, in phase 1 differentiation, the iPSCs will become neuronal precursor cells (NPCs) using techniques such as embryoid body formation, stromal feeder co-culture, and selective survival conditions. NPCs can differentiate into different cell types of the central nervous system, and carry a rostral identity. To further specify the neuronal cell population the NPCs can undergo phase 2 differentiation. Two techniques that can be used to obtain spatial and functional specificity are morphogens and transcription factors. Morphogens can be used to manipulate the pathways involved in development to achieve a desired neuronal outcome. Transcription factors known to be active in a subset of mature neurons can be delivered into NPCs which will lead to the expression of those genes.

Current and Emerging Applications

iPSCs can be used *in vitro* as 3D cultures or organoids to model diseases. Specifically, in 2013 Lancaster et al. studied organoids derived from a microcephaly patient. These organoids were examined using immunofluorescence, to study the anomalies that occurred at the cellular level during development in these patients. In addition, *in vitro*, iPSC derived tissues can be maintained under human physiological conditions to study potential therapeutics and to test drug cytotoxicity, as many of the treatments tested in animal models seem to fail in humans.

iPSCs can also be used *in vivo* largely for regenerative medicine. iPSCs can be used to derive particular types of neurons (ex. dopaminergic neurons), which can be transplanted into an animal model to verify the efficacy of the transplant prior to using iPSC derived tissue in humans. In 2017, Takahashi et al. transplanted midbrain dopaminergic neurons derived from human iPSCs into *Macaca fascicularis,* a primate model of Parkinson's disease (PD). Based on the results of the 2017 study, Takahashi is leading a group of researchers to transplant iPSC derived dopaminergic neurons into 7 human PD patients.

Advantages and Disadvantages

iPSCs are simple to use because there are multiple products available, due to high interest in the field. The results are reproducible, and most importantly iPSCs can be patient specific cells which reduces the risk of immuno-rejection. Unfortunately, there is a very low reprogramming efficiency rate (~0.001- 4%)[4]. Depending on the method used to generate iPSCs, there can be viral mutations in the genome, progenitor cell tumor formation, and variable epigenetic influences.

Ethical Considerations

Some of the concerns with iPSCs include how similar they are to embryonic stem cells, their possible misuse as gametes, patient consent for transplant, change in "self" identity after transplant, and that the benefits must be greater than the risks if iPSCs derived tissue is transplanted into humans.

References

1. Cyranoski D. (2018) 'Reprogrammed' stem cells to be tested in people with Parkinson's. Nature News
2. Lancaster, M.A., et al. (2013) Cerebral organoids model human brain development and microcephaly. Nature 501, 373-379
3. Fung, R.K.F., and Kerridge, I.H. (2013) Uncertain translation, uncertain benefit and uncertain risk: ethical challenges facing first-in-human trials of induced pluripotent stem (IPS) cells. Bioethics 27 (2), 89-96
4. Singh, V.K., et al. (2015) Induced pluripotent stem cells: application in regenerative medicine, disease modeling and drug discovery. Front Cell Dev Biol 3(2), 1-18
5. Takahashi, K., and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4), 663-676
6. Takahashi, K., et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5), 861-872
7. Takahashi, K., et al. (2017) Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. Nature 548, 592-596
8. Tao, Y., and Zhang, S. (2017) Neuronal Subtype Specification From Human Pluripotent Stem Cells. Cell Stem Cell 19(5) 573-586