A Brain in a Dish

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Abstract Ohio State University researchers have made a leap forward in disease research by creating an eraser sized human "brain" in a petri dish[1]. Although lacking a circulatory system their brain model includes spinal cord, cortex, midbrain, brain stem, and even the beginnings of an eye- aiding in the effectiveness of research on complex neurological disease. To create their new brain model, the researchers converted adult skin cells into pluripotent stem cells, which afforded the opportunity to build the multiple nervous cell types required for such a complex system. Having this tissue model will assist researchers in developing new disease models, and thus, facilitate the development of novel clinical interventions.
Keywords spinal cord, cortex, midbrain, brain stem, brain

The brain is the most sophisticated and complex organ that nature has devised. Neurons are the functional basic component of the nervous system. Cell bodies of cortical neurons are arranged in layers and each layer has complex diversity of neuronal subtypes. This complex specialization follows a coordinated temporal pattern that emerges through the specification of different subtypes of cortical neurons. This in turn populates the various cortical layers, where these neurons exhibit specific patterns of gene expression and connectivity.

An organoid is a three-dimensional organ-bud that is grown in vitro. There are various types of organoid, one of which is cerebral organoid, which is a miniature organ resembling the brain. These organoid are created using human pluripotent stem cells (cells that have ability to form any adult cell type). The purpose of creating these organoids is to be able to study various disease models in a simple, variable space, free of various in-vivo limitations (especially working with humans).

Some basic steps to creating cerebral organoid start with taking human pluripotent stem cells, cultivating them, inducing various nerve growth factors, fixating in a gel environment and then using bioreactor to spin. There are variety of ways to do testing of these organoid, using gene expression and functional characterization.

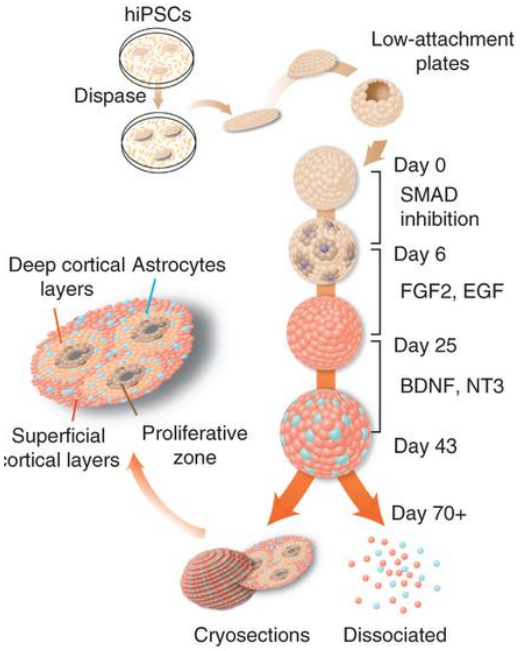

Figure 1 Generation of human cortical spheroids [Image courtesy of Dr. Pasca [2]]

The first demonstration of efficient generation of cortical neurons in vitro took

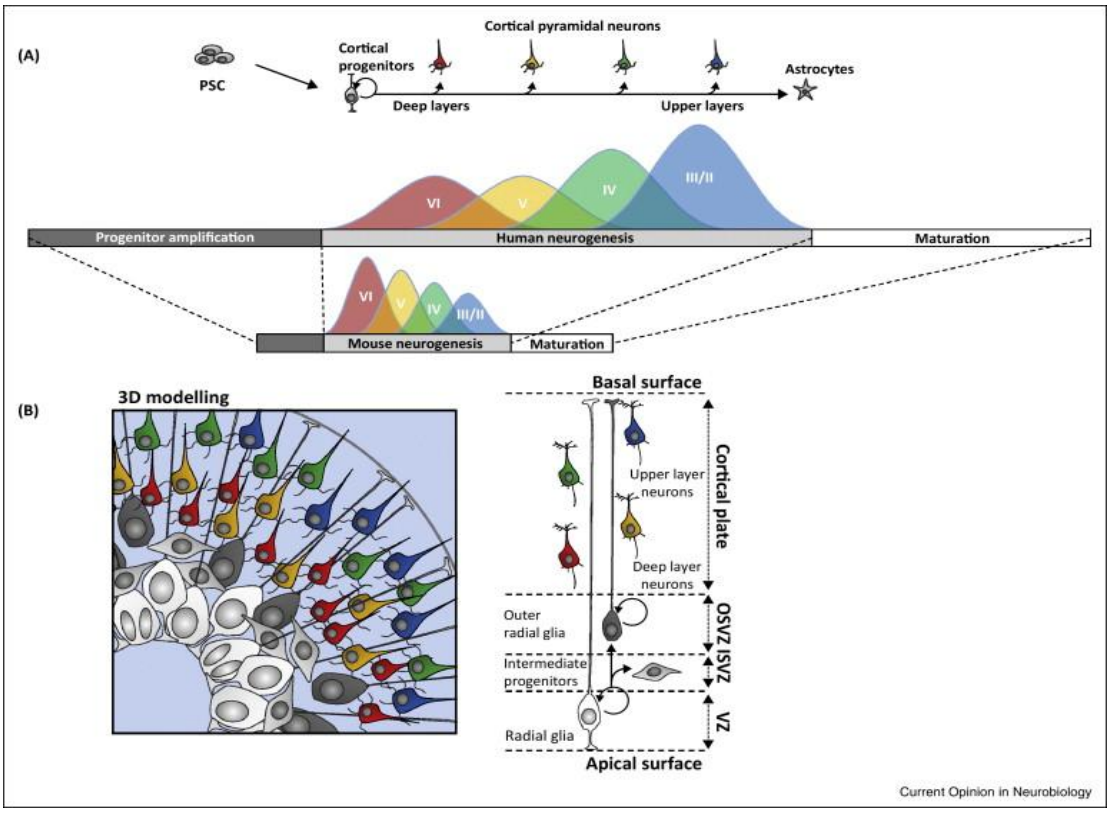

Figure 2 Temporal and Spatial modeling cortical neuron neurogenesis [Image courtesy of Dr. Anderson[5]]

place in 2008, where authors presented the evidence that a complex self-organized cytoarchitecture can emerge in a purely in vitro setting[3,4]. There has been amazing progress in the field of organoids over past few years.

In a recent Nature methods paper relating to "Brains in a petri dish", the authors developed a new streamlined method for inducing pluripotent stem cells to form cortex-like organoids (Figure 1), which include neurons supported by network of glial cells. Authors used an in vitro neural differentiation approach, where human cortical spheroids (hCSs) are maintained in floating conditions on low-attachment plates with biweekly changes of regular serum-free media. This system can be easily maintained for up to 9 months in vitro. Thus, authors were able to create functional and realistic layers of neurons that talk to each other in complex networks.

Figure 1 is the schematic representation of the main stages during the process of creation of hCS. Authors used low attachment plates for the suspended colonies. Authors used both BMP and TGF signaling pathways to achieve rapid and efficient neural induction. At 6th day, EGF was added and at 25th day, BDNF was added. Medium was changed very frequently. These hCS grew to 300 microns in diameter by 2 weeks of culture and then reached 45 mm in diameter by 2.5 months. To help characterize these hCS, the authors used many functional classification techniques [2]. They studied the functional

characterization of the neurons by using fura-2 (calcium) imaging and panels of antibodies in the human fetal cortex. This system of 3D network was also amenable to slice physiology techniques. The authors performed whole cell recordings and found that almost 80% of the cells fired action potentials in response to depolarizing current steps.

There have been other techniques used in the past for developing differentiating pluripotent stem cells into cortical neurons. Some examples include neural induction in high-density monolayer cultures, embedding clusters of hiPSCs in gelatinous protein mixtures (such as Matrigel) and later culturing them in a spinning bioreactor, using embryoid bodies derived from hiPSCs that are plated on coated surfaces to generate neural progenitors[2,5–7].

In 2013, Lancaster et. al. in order to recapitulate features of human cortical development, namely characteristic progenitor zone organization with abundant outer radial glial stem cells, developed cerebral organoids that had similar development as in human brain[6]. The authors also used these cerebral organoid to model microcephaly defect (which has been difficult in model in mice) that could help explain various disease phenotypes.

In 2015, Maguruma and colleagues developed a method to generate neurons of the cerebellum by 3D culture of human embryonic stem cells with sequential addition of growth factors[7].

In the paper on Cortical Neurogenesis (complexity emerging from simplicity)[5], authors modeled spatial and temporal patterns of corticogenesis (see figure 2). These 3 D models could recapitulate similarity to in-vivo organization of cortical structure.

Following paragraphs talk about the potential applications of organoid for various disease models.

Timothy syndrome (TS), a development delay disorder caused by a mutation in a L-type voltage-gated calcium channel, can cause autism. The iPSC derived cortical cells from TS patient led to revelation about defects in calcium signaling and neuronal activity, and defects in the generation of specific types of neurons[8].

Pluripotent stem cells were used to identify the lysosomal alterations in **Gaucher disease** (GD) neurons (genetic disease in which fatty substances accumulate in cells and certain organs). It was found that the lysosomal alterations described were caused by the GBA1-associated neurodegeneration[9].

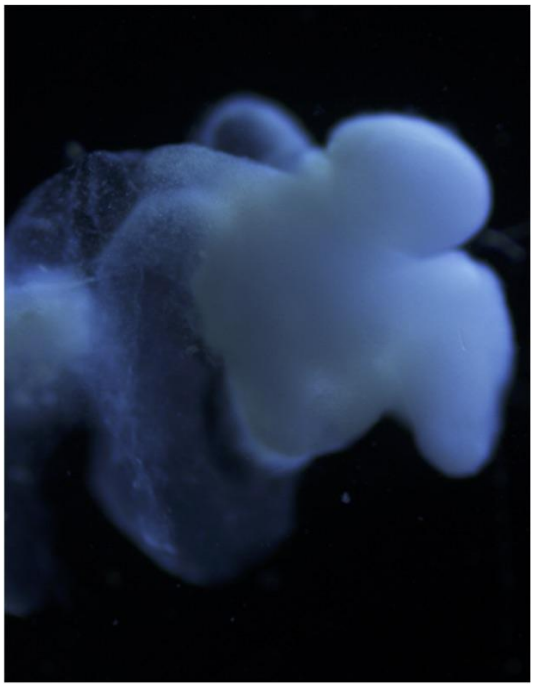

Figure 3 Image of an organoid [Courtesy of The Ohio State University[1]]

Anand and his group have already taken the application of these corticoids to **Alzheimer**'s and **Autism research**[1]. Authors

have filed an invention disclosure and are seeking an IP, so they were unable to disclose the methods. Figure 3 shows an image of an organoid. When, I asked the author about the potential of this work and timeline, his response was "*Potential is enormous. The models will accelerate research and drug discovery and more accurately predict efficacy of drugs in human clinical trials, and a lot lower cost. Timelines will vary based on the subtype of a disease under consideration (most brain diseases and disorders are syndromes with different causes, though some may converge on a pathway)*".

Gulf War illness is considered to be the outcome of the use of organophosphate pesticides [permethrin (PM) and N,Ndiethyl-m-toluamide (DEET)], daily prophylactic anti-cholinesterase pyridostigmine bromide (PB), and stress. It is also shown to cause reduction of hippocampal column and brain gray matter. Organoid can be used to evaluate the pathobiology of these noxious chemical on human brain development.

PTSD (Post Traumatic Stress Disorder) – There is a potential to develop organoid models from people with PTSD, controls and controls exposed to same traumatic conditions as PTSD patients to be able to study what can be effective remedy to treat this disorder.

TBI (Traumatic Brain Injury) – TBI normally involves the damage to axons and white matter tracts. Oligodendrocites and myelin are important in recovery process from the trauma. Organoids can be used to study the process of myelination and demyelination, after TBI.

MS (Multiple Sclerosis) – MS only develops in humans, thus making it very hard to study this disorder in mice, or any other species. Organoids can be used as tool study the development and treatment of this disorder.

There are a lot of next steps that could be taken in the field of organoid research to take them to a mode advanced level. Brain in the petri dish is not conscious and doesn't have any input attached to it. The first and foremost step would require blood vessels, i.e., a working heart to help brain grow further in development (Figure 4). Next, various different types inputs (ranging from light, current, etc) can be attached/modeled, and their effects could be monitored. Definitely the ultimate goal being to be able to study the effect of diseases on the growth of organoid brain.

In the end, you must marvel that the most complex and mysterious organ may be being grown in 89 cents petri dish.

Figure 4 Schematic representation of creating network of neurons to form BRAIN in a petri dish.

References

1. Anand R, Mckay S. Human Brain Organoids Derived from Induced Pluripotent Stem Cells. *Mil Heal Syst Res Symp*. 2015:43210.

2. Paşca AM, Sloan S a, Clarke LE, et al. Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat*

Methods. 2015;12(7):671-678. doi:10.1038/nmeth.3415.

3. Gaspard N, Bouschet T, Hourez R, et al. An intrinsic mechanism of corticogenesis from embryonic stem cells. *Nature*. 2008;455(7211):351-357. doi:10.1038/nature07287.

4. Eiraku M, Watanabe K, Matsuo-Takasaki M, et al. Self-Organized Formation of Polarized Cortical Tissues from ESCs and Its Active Manipulation by Extrinsic Signals. *Cell Stem Cell*. 2008;3(5):519-532. doi:10.1016/j.stem.2008.09.002.

5. Anderson S, Vanderhaeghen P. Cortical neurogenesis from pluripotent stem cells: complexity emerging from simplicity. *Curr Opin Neurobiol*. 2014;27:151-157. doi:10.1016/j.conb.2014.03.012.

6. Madeline A. Lancaster, Magdalena Renner1, Carol-Anne Martin, Daniel Wenzel1, Louise S. Bicknell MEH, Tessa Homfray, Josef M. Penninger APJ& JAK. Cerebral organoids model human brain development and microcephaly. *Mov Disord*. 2014;29(2):185-185. doi:10.1002/mds.25740.

7. Muguruma K, Nishiyama A, Kawakami H, Hashimoto K, Sasai Y. Self-Organization of Polarized Cerebellar Tissue in 3D Culture of Human Pluripotent Stem Cells. *Cell Rep*. 2015;10(4):537-550. doi:10.1016/j.celrep.2014.12.051.

8. Paşca SP, Portmann T, Voineagu I, et al. Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. *Nat Med*. 2011;17(12):1657-1662. doi:10.1038/nm.2576.

9. Awad O, Sarkar C, Panicker LM, et al. Altered TFEB-mediated lysosomal biogenesis in Gaucher disease iPSC-derived neuronal cells. *Hum Mol Genet*. 2015;24(20):5775-5788. doi:10.1093/hmg/ddv297.