Brain in a dish

By Lev Osherovich, Senior Writer

Austrian researchers grabbed headlines last month when they coaxed cultured human induced pluripotent stem cells into forming brain tissue,[1] but in actuality the approach does not offer applications beyond studying very early brain development.

Engineering stem cell–derived *in vitro* organ models for studying development and disease has made great strides for relatively simple organs such as the intestine,[2] lung[3] and liver. For example, Japanese researchers have generated liver organoids from induced pluripotent stem (iPS) cells and shown that the *in vitro*–grown tissue was functional when transplanted into mice.[4]

Compared with other organs, the brain is much more complex and thus is considered harder to grow from scratch. Instead, efforts have focused on growing individual tissue types such as retinal[5] and cerebellar[6] precursors.

Now, a team led by Jürgen Knoblich has combined a variety of iPS cell culture and differentiation methods to create brain organoids—well-organized clusters of brain tissue containing the major cell layers found in embryonic brains. Knoblich is deputy scientific director of the **Institute of Molecular Biotechnology of the Austrian Academy of Science**.

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> ***—Aaron Chuang, GlaxoSmithKline plc***

The group started with off-the-shelf iPS cells and treated them with differentiation-inducing cell culture medium to yield neuroectoderm tissue, which is the embryonic precursor to the nervous system.

When implanted into a 3D growth matrix and transferred to a liquid culture bioreactor, the neuroectoderm began to separate into a brain-like, layered tissue termed neuroepithelium.

After 20–30 days of growth, the neuroepithelium formed globular organoids with layers of distinctive neurons and glial cells similar to those seen in developing human brains.

The team used RT-PCR and immunohistochemistry to show that the miniature brains had a semblance of the organization found in full-sized brains, including localized expression of markers associated with specific brain regions such as the hippocampus and choroid plexus.

Microscopy and electrophysiological studies revealed that neurons inside the brain organoids had sprouted axon-like projections and could respond to stimulation by glutamate, an excitatory neurotransmitter. The team did not report the efficiency of the organoid growth protocol or the consistency of resulting organoids.

Knoblich's team next used the brain organoids to characterize how early steps in brain development go awry in certain hereditary diseases.

The researchers grew brain organoids from iPS cells derived from a patient with a genetic form of microcephaly, a rare birth defect characterized by stunted cortical development. The cells had a loss-of-function mutation in *CDK5 regulatory subunit associated protein 2* (*CDK5RAP2*), one of several genes linked to microcephaly.

When the patient's cells were put through the brain organoid–growing protocol, the cells initially formed a seemingly normal neuroepithelium. But the neurons within this cell layer stopped dividing prematurely, leading to thinner neuronal layers and smaller organoids than were seen in a healthy control cell layer. The team obtained similar results with small hairpin RNA knockdown of *CDK5RAP2*.

Results were reported in *Nature*.

Limited development

Knoblich's findings are a step toward developing *in vitro* models of neurological disease, but the precise conditions in which the model should be applicable is up for debate.

"This system allows one to selectively up- and downregulate genes and transcripts of interest and directly observe their impact on the 3D interactions and developmental processes across cell types," said Magali Haas, CSO and CTO of **One Mind for Research**, a not-for-profit organization that advocates for research into mental illness and brain injury.

"There are literally thousands of independent loci that seem to contribute to heritability of schizophrenia and other conditions such as autism and bipolar disorder," she said. "Many of these conditions are known to be associated with neurodevelopmental abnormalities, but the full mechanism of these is unknown. This type of culture model may help elucidate the early roles of these genes in the neurodevelopment process."

Mriganka Sur, a professor of neuroscience and director of the Simons Center for the Social Brain at the **Massachusetts Institute of Technology**, cautioned that brain organoid tissue is more primitive than the highly interconnected neuronal networks found in real brains.

He noted that most common neuropsychiatric and neurodegenerative diseases involve compromised connectivity between neurons or abnormal interactions between neurons and surrounding glial cells, but neither phenomenon is evident in brain organoids.

"They show that these neurons can fire, but not that they form normal synaptic networks," said Sur. "iPS cells are good for studying early developmental steps in neurons but not diseases of brain connectivity."

"The system has clear limitations including the fact that other cell/tissue types are not co-developing alongside the neuronal ones," added Haas. "Cell-cell signaling is a very important part of the development process, and this system would not fully recapitulate all those components."

"It would be a bit premature to talk about the use of our system in an industry setting," acknowledged Knoblich.

Aaron Chuang, research director of regenerative medicine at **GlaxoSmithKline plc**, said brain organoids appear to model only the earliest steps of embryonic brain development, whereas the complex tissues often involved in brain disease develop much later.

"These organoids developed for up to two months only," said Chuang. "These represent part of early fetal brain, but the majority of glial cells would not have been developed at this early stage. There is also no vascular system, which is known to play significant roles in brain function. Therefore, as the authors point out, their system captures only the very early stages of development."

Chuang and Sur advocated for refining the culture method to yield more developmentally advanced brain tissue.

"Improving the technology to generate 3D brain tissue with greater maturity would improve potential utility; focusing on the generation of specific brain regions such as the hippocampus with mature state could have greater utility," Chuang said.

"The next step is to grow synapses in a reasonable time frame and to grow different cell types," added Sur. "At the very least there should be excitatory and inhibitory neurons."

Knoblich said that his team is focusing on helping other laboratories learn how to make brain organoids.

"Industry should not yet be adopting this technology in its R&D pipeline procedures, but all serious CNS companies should have their discovery teams educated on these techniques to become intimately familiar with their advantages and limitations," concluded Haas.

Patent and licensing status were not disclosed.

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COMPANIES AND INSTITUTIONS MENTIONED

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