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Building three-dimensional human brain organoids

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The organogenesis of the human central nervous system is an intricately orchestrated series of events that occurs over several months and ultimately gives rise to the circuits underlying cognition and behavior. There is a pressing need for developing reliable, realistic, and personalized *in vitro* models of the human brain to advance our understanding of neural development, evolution, and disease. Pluripotent stem cells have the remarkable ability to differentiate *in vitro* into any of the germ layers and, with the advent of three-dimensional (3D) cell culture methods, to self-organize into brain spheroids or organoids. These organoid cultures can be derived from any individual, can be guided to resemble specific brain regions, and can be employed to model complex cell-cell interactions in assembloids and to build human circuits. This emerging technology, in combination with bioengineering and other state-of-the-art methods for probing and manipulating neural tissue, has the potential to bring insights into human brain organogenesis and the pathogenesis of neurological and psychiatric disorders.

Brain organogenesis *in vitro* and *in vivo*

Methods for generating neural cells *in vitro* aim to recapitulate key stages of *in vivo* brain organogenesis. Folding of the ectoderm-derived neural plate gives rise to the neural tube, which becomes enlarged on the anterior side to form the forebrain in the central nervous system (CNS). Corticogenesis involves the sequential generation and positioning of layer-specific glutamatergic neurons from progenitors that line the ventricles in the dorsal forebrain, the migration of GABAergic interneurons that are born in the ventral forebrain, and waves of gliogenesis to form astrocytes and oligodendrocytes, which continue postnatally.

Pluripotent stem cells, derived from the inner mass of the blastocyst (embryonic stem cells) or from reprogrammed somatic cells (induced pluripotent stem cells), can be differentiated into neural cells in bi-dimensional (2D) cultures — where early on neuroepithelial cells position themselves into structures called rosettes — or in self-organizing three-dimensional (3D) brain spheroids or organoids[1]. Intermediate ('2.5D') cultures can be obtained when neural cells differentiated in 2D are lifted and cultured in 3D conditions to form cellular aggregates or when differentiated 3D aggregates derived from pluripotent stem cells are subsequently plated for culture in 2D.

Brain organoids can be generated from aggregates of pluripotent stem cells through undirected differentiation methods that lack inductive signals[2,3], or by patterning through directed differentiation methods to resemble specific brain regions[4-8] (e.g., forebrain, midbrain, retina).

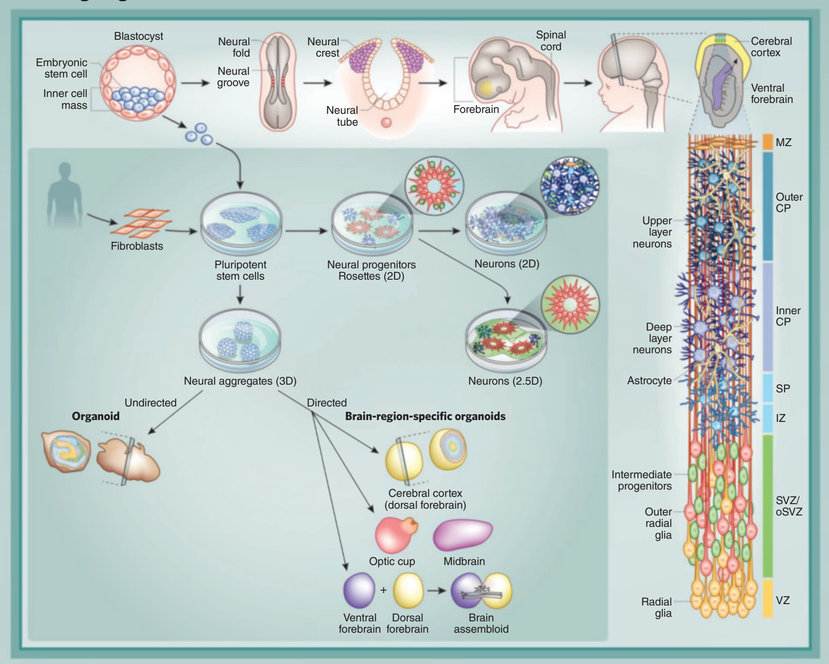
Brain assembloids

To model interactions between brain regions, organoids can be patterned to resemble specific regions of the nervous system and then can be fused to generate brain assembloids[1]. Another way to generate assembloids is by spatio-temporally controlling patterning within one 3D aggregate, by embedding organizer-like structures (i.e., cells, coated beads) that release or block developmental signals. A third method involves combining other single cells into brain organoids: for instance, by embedding yolk-sac-derived microglia to study neuroimmune interactions, by embedding mesoderm-derived vascular cells to study the blood-brain barrier, or by embedding tumor cells to study brain metastasis. These 3D cultures can be probed using genetic, anatomical and functional read-outs.

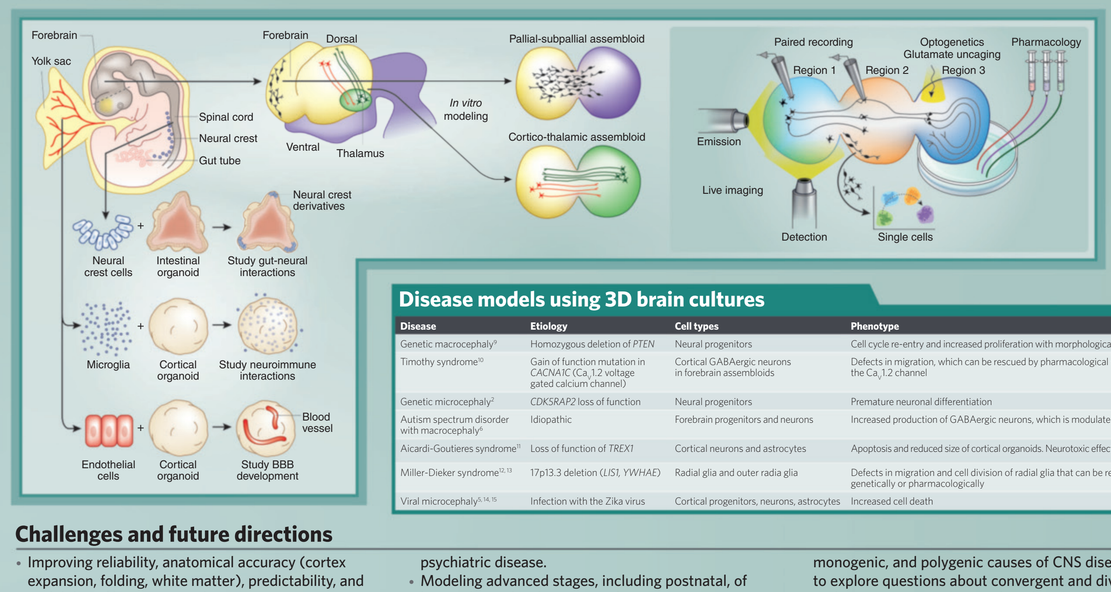
Applications of brain organoids

Brain organoids and assembloids can be used to ask questions about evolutionary innovation in human and non-human primates and to understand the developmental program and maturation of the nervous system (e.g., the programs underlying astrocyte transition from a fetal to a postnatal state). Brain organoids derived from patients or that have been genetically engineered to carry genetic variants associated with disease (i.e., isogenic lines) can be used to investigate disease pathogenesis in the nervous system. Lastly, as these 3D cultures become more scalable and assays probing 3D tissue improve, drug and CRISPR-Cas9-based screens can be used to identify therapeutic targets.

Brain organogenesis *in vitro* and *in vivo*



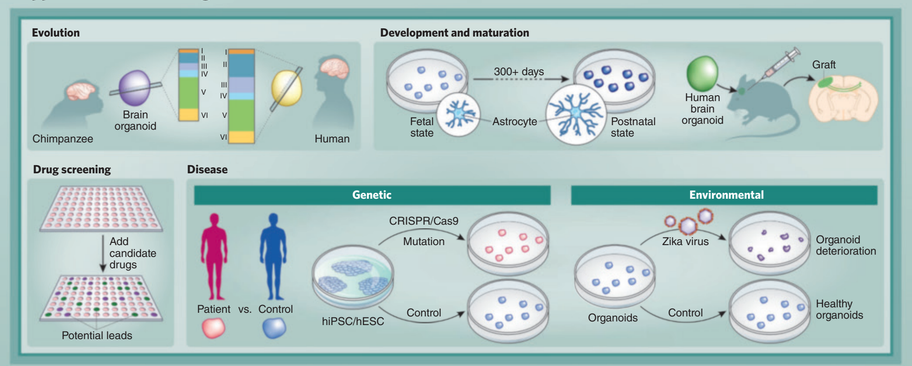
Brain assembloids



Disease models using 3D brain cultures

Disease	Etiology	Cell types	Phenotype
Genetic macrocephaly[9]	Homozygous deletion of *PTEN*	Neural progenitors	Cell cycle re-entry and increased proliferation with morphological changes
Timothy syndrome[10]	Gain of function mutation in *CACNA1C* ($Ca_v1.2$ voltage gated calcium channel)	Cortical GABAergic neurons in forebrain assembloids	Defects in migration, which can be rescued by pharmacological manipulation of the $Ca_v1.2$ channel
Genetic microcephaly[2]	*CDK5RAP2* loss of function	Neural progenitors	Premature neuronal differentiation
Autism spectrum disorder with macrocephaly[6]	Idiopathic	Forebrain progenitors and neurons	Increased production of GABAergic neurons, which is modulated by FOXG1
Aicardi-Goutieres syndrome[11]	Loss of function of *TREX1*	Cortical neurons and astrocytes	Apoptosis and reduced size of cortical organoids. Neurotoxic effects of astrocytes.
Miller-Dieker syndrome[12, 13]	17p13.3 deletion (*LIS1, YWHAE*)	Radial glia and outer radia glia	Defects in migration and cell division of radial glia that can be restored genetically or pharmacologically
Viral microcephaly[5, 14, 15]	Infection with the Zika virus	Cortical progenitors, neurons, astrocytes	Increased cell death

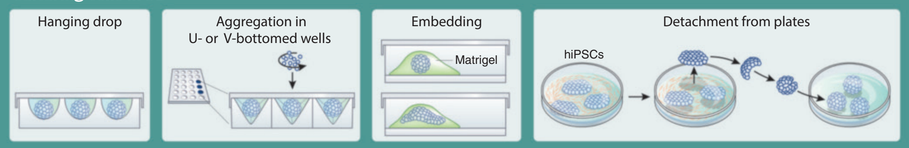
Applications of brain organoids



Challenges and future directions

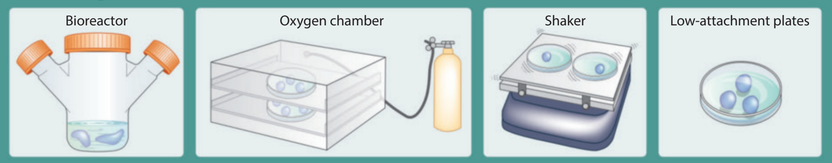
- Improving reliability, anatomical accuracy (cortex expansion, folding, white matter), predictability, and scalability of brain organoids.
- Developing methods for transplantation of organoids into rodents or other species to obtain circuit-wide integration and oscillatory activity, to study sensory input, and to develop more accurate models of psychiatric disease.
- Modeling advanced stages, including postnatal, of human brain development and incorporating missing cell types, e.g. glia, endothelial cells and pericytes, to study neuroimmune and neurovascular interactions or cancer cells (e.g., oncogenesis and metastasis).
- Developing reliable models of environmental, monogenic, and polygenic causes of CNS disease to explore questions about convergent and divergent pathogenesis in psychiatric disorders (e.g., autism spectrum disorders, schizophrenia).
- Building large-scale platforms for drug discovery and genetic screens.

Culturing cells in 3D



Methods for culturing cells in 3D include hanging drop cultures attached to a slide, cell aggregation by centrifugation in U- or V- bottom wells, embedding into extracellular matrices, and detachment of intact pluripotent colonies that are then moved to ultra-low-attachment dishes. These 3D cultures can be subsequently maintained in low- or high-oxygen conditions, shaken or spun in a bioreactor, or maintained in low-attachment plates without shaking.

Maintaining 3D cell cultures



Abbreviations

BBB: blood–brain barrier; CP: cortical plate; ENS: enteric nervous system; IZ: intermediate zone; MGE: medial ganglionic eminence; MZ: marginal zone; SP: subplate; SVZ/oSVZ: subventricular zone/outer subventricular zone; VZ: ventricular zone

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