NEUROSCIENCE

# Assembling human brain organoids

## Three-dimensional assembloids can be used to study human development and disease

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Brain development is a remarkable self-organization process in which cells proliferate, differentiate, migrate, and wire to form functional neural circuits. In humans, this process takes place over a long fetal phase and continues into the postnatal period, but it is largely inaccessible for direct, functional investigation at a cellular level. Therefore, the features that make the human central nervous system unique and the sequence of molecular and cellular events underlying brain disorders remain largely uncharted. Human pluripotent stem (hPS) cells, including those obtained by reprogramming somatic cells, have the ability to self-organize and differentiate when grown in three-dimensional (3D) aggregates rather than in direct contact with a flat plastic surface (*1*). Such 3D neural cultures, also known as organoids and organ spheroids, recapitulate many aspects of human brain development in vitro (*1*) and have the potential to accelerate progress in human neurobiology. Here, I discuss the emerging approaches to produce brain assembloids—the next generation of brain organoids that combine multiple cell lineages in 3D. These cultures can be used to model interactions between various brain regions in vitro, and ultimately may be applied to understand the assembly of neural circuits and to capture complex cell-cell interactions in the brain.

Brain organoids have been generated from hPS cells to resemble the dorsal forebrain, which generates the cerebral cortex (see the figure). These cerebral cortical organoids contain glutamatergic neurons of various layers that are synaptically connected, and their generation is followed by the production and maturation of starlike glial cells, called astrocytes (*2*). During development in utero, the formation of specific brain regions is shaped by interactions with other brain regions through long-distance projections as well as by wiring locally into microcircuits with neurons that have migrated from other regions in the central nervous system. To capture these cell-cell interactions in vitro, ventral forebrain organoids that contain γ-aminobutyric acid (GABA)–ergic cortical interneurons can be combined with a dorsal forebrain organoid (*3*). GABAergic cortical neurons migrate, modify their morphology, and make synapses with cortical glutamatergic neurons, generating a functional neural network in a 3D cellular structure called a multiregion assembloid (*3*–*5*). This process of cortical interneuron migration takes place in late stages of human development, up to 2 years after birth, and assembloids of the ventral and dorsal forebrain offer a unique opportunity to study primate-specific features of GABAergic interneuron development as well as investigate how their migration may go awry, using cultures from patients with epilepsy or autism spectrum disorders (*3*). Moreover, this approach can be used to combine other region-specific organoids, such as the striatum and midbrain, to study glutamatergic and dopaminergic inputs to the basal ganglia and the spinal cord to model cortico-spinal projections, or combinations with the thalamus to model cortico-thalamic interactions and how this cross-talk shapes cortical circuits. However, more work is needed to reliably assess connectivity in vitro and to learn to what extent this platform can capture more subtle inter-brain regional changes associated with complex psychiatric disease.

Besides input from other brain regions, neural development and function is shaped by interactions with other cell types, which are often specified outside the nervous system, such as yolk sac–derived microglia, which are resident immune cells in the central nervous system, or mesoderm-derived blood vessels. It is increasingly recognized that neuroimmune and neurovascular interactions are important in brain development. These interactions can be modeled in vitro by adding primary or hPS cell–derived microglia and pericytes and brain endothelial cells (that compose blood vessels) to brain region–specific organoids at various stages of differentiation to form multilineage assembloids. For example, hPS cell–derived microglia-like cells have been integrated in cortical organoids that display aggregates of the β-amyloid (Aβ) protein, which is the main component in amyloid plaques, and have subsequently been used to model features of Alzheimer's disease (AD) (*6*). More specifically, microglia-like cells carrying the AD high-risk allele, apolipoprotein E4 variant (*APOE4*), embedded into 3D brain organoids showed longer processes and took up less Aβ than cells expressing the low-risk *APOE3* allele. Alternatively, primary microglia isolated from patient postmortem cerebral cortex could be integrated into brain organoids to investigate their function in neurodevelopmental or neurodegenerative disease. Glial cells, such as microglia and astrocytes, actively sculpt neural circuits and regulate synaptic plasticity by engulfing synapses, and assembloids containing human glial cells as well as neurons could be used to study the role of immune-related pathways in synapse elimination, such as understanding how variants in the complement *C4* gene regulate synapse function in humans and how they contribute to schizophrenia risk (*7*). Ultimately, multilineage assembloids could be used to elucidate fundamental questions in neuroglial cross-talk, such as: How do glial cells regulate synapse number and function in humans during development and in disease? How did human glial cells evolve to control synapse formation and elimination compared with other mammals, and how does this contribute to the unique features of the expanded human cerebral cortex that controls higher-order cognition and complex behaviors? Neural-glial interactions and chronic exposure in the context of late-onset neurodegenerative disorders will be more challenging to study because most assembloid cultures resemble early stages of development, and strategies for accelerating maturation in vitro are required.

Mutations in genes expressed in myeloid cells, such as monocytes and microglia, have been associated with neuropsychiatric diseases, but how dysfunction in immune cells influences brain function is unknown (*8*). Recently, a triculture 3D assembloid system that includes neurons, astrocytes, and microglia has been used to study neural-glial interactions and mimic neuroinflammation-like processes (*9*). Building on these efforts, multilineage brain assembloids could model cell-cell interactions using autologous immune cells, which carry the immunological history of the individual, such as exposure to various antigens. For example, how neurons in the hypothalamus producing the arousal-associated neuropeptide hypocretin are specifically lost in the severe sleep disorder narcolepsy is still unclear. Epidemiological studies and strong genetic association with human leukocyte antigen (*HLA*) and T cell recep-

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tor (*TCR*) genes suggest immune involvement (*10*). Hypothalamic organoids that contain neurons that produce hypocretin and other neural and glial populations could be assembled with immune cells harvested from the same narcolepsy patients to elucidate the sequence of cellular events leading to cell loss. Similarly, immune cells from patients with multiple sclerosis could be included in an autologous assembloid system that also includes myelin-producing oligodendrocytes to understand the pathological process of demyelination.

Other examples of multilineage assembloids are gut organoids that were combined with neural crest cells, which migrate into the organoid and differentiate to form a neural plexus that gives rise to rhythmic waves of activity (*11*), or cancer cells that can be assembled with brain organoids to investigate tumorigenesis in the nervous system. For instance, genetic manipulation of oncogenes and tumor suppressor genes was recently used to initiate and directly image tumorigenesis in a brain organoid system (*12*). Moreover, patient-derived tumor organoids were fused to brain organoids to study the cell biology of tumor invasion. This approach could be used to ask questions about metastasis and why some tumors are more aggressive than others.

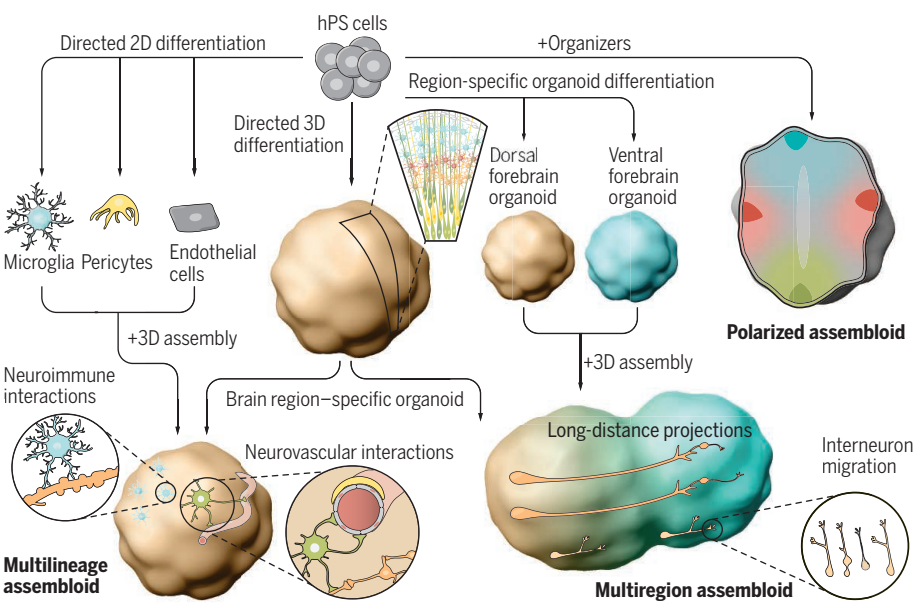
Moving forward, a key challenge in building and assembling human brain organoids in vitro is the ability to control polarity and obtain predictable 3D cellular architectures that recapitulate anatomical and functional aspects of the human brain. To form anteroposterior (head-to-tail) and dorsoventral (back-to-belly) axes in vivo, the neuroepithelium progressively subdivides into distinct regions under the influence of specialized groups of cells called organizers. These organizers instruct the cell fate and morphogenesis of neighboring cell populations by emitting molecular signals, and once this polarity has been established, the interactions necessary to form the central nervous system become possible. Future brain assembloids could include gel beads or tubes carrying morphogens or populations of organizer-like cells derived from hPS cells or isolated from animals to generate polarized assembloids. For example, cells resembling the floor plate region of the developing neural tube and expressing the morphogen sonic hedgehog (SHH) could be included to induce ventral cell identities, whereas roof plate–like cells that secrete the morphogens bone morphogenetic proteins (BMPs) and WNTs, could be used to specify dorsal fates.

Such strategies could allow the development of a polarized 3D spinal cord in vitro, for example, which would enable the exploration of regional diversity of neural and glial cells and how their specification is shaped by neural activity. This organoid could also be assembled with cortical organoids to study ascending and descending neural pathways in motor processing and with muscle tissue to develop human models of neuromuscular function and cortical control. Similarly, other morphogen-secreting regions in the developing central nervous system, such as prechordal plate–like cells and cortical hem-like cells, could be used to specify and spatially organize within the same organoid subdomains of the forebrain. Lastly, the recent differentiation in vitro from hPS cells of groups of cells that can organize axes in the early embryo (*13*) will enable the dissection of the mechanisms behind the formation of embryo body plan and neural axis in the human nervous system. However, as 3D cell cultures develop polarity, the use of in situ molecular biology will be essential for identifying cell fates and cell states with high resolution over time and for avoiding biases due to cell dissociation and other processing steps during assembloid manipulation.

Although a comprehensive understanding of the programs driving the development of all cells and regions in the central nervous system is lacking, identifying the minimal signals necessary for specification and self-organization will be essential in developing other region-specific organoids as well as polarized multiregion brain structures in vitro. However, even if this is achieved for a number of domains or regions of the nervous system, the sensory input that shapes the developing brain is missing, and this aspect may be critical to capture subtle functional imbalances underlying psychiatric disorders. Transplantation of intact brain organoids into mammalian brain tissue (*14*) could have applications for regenerative medicine and constitutes one way by which integration into larger neural networks that receive sensory input could be achieved in the future. Understanding the mechanisms of psychiatric disorders will require more accurate models of the human brain, but as approaches for deriving brain tissue in the lab are further refined to recapitulate in vivo function, more discussions about the ethical implications will be needed (*15*). ■

## Generating assembloids

Brain region–specific organoids are generated from human pluripotent stem (hPS) cells and can be assembled with other cell types (multilineage assembloids), with other organoids (multiregion assembloids), or with morphogens or organizer-like cells (polarized assembloids). Brain organoids and assembloids can be used to model complex cell-cell interactions and neural circuit formation in the human nervous system.

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