NEUROSCIENCE

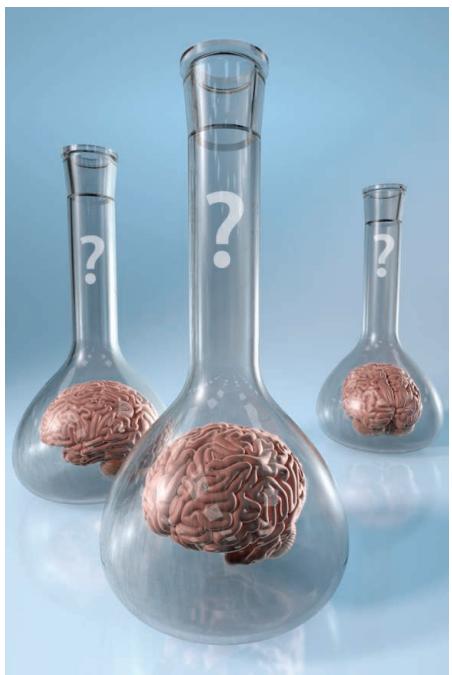
# What Are Mini-Brains?

Human cerebral organoids grown in the lab may quickly advance our understanding of brain development and disease.

**Byoung-il Bae and Christopher A. Walsh**

The human cerebral cortex defines us as who we are. Its development and function underlie complex human cognitive behavior, while its malfunction or degeneration causes countless neurological and psychiatric diseases. It has evolved markedly in humans compared to other animals and, therefore, no animal model truly recapitulates these human-specific features (*1*). We are currently limited to identifying genetic causes of abnormal brain development and function, observing brain shape and activity through imaging, and examining postmortem brain tissues. You simply cannot analyze human brain development directly. The more we try to model human disease in the mouse—with its miniscule cerebral cortex one-thousandth the size of a human's—the more we recognize the limitations of animal models. Lancaster *et al*. (*2*) have provided a major leap by developing a method to grow miniature human brain-like structures (cerebral organoids) from embryonic stem cells in vitro (*2*). The "mini-brains" recapitulate a surprising number of features of human embryonic brain development, heralding a new phase of modeling human disease.

Human and mouse embryonic stem (ES) cells, when cultured in special conditions, can produce organoids with a strong resemblance to precursors of the eyes, pituitary gland, and other brain structures (*3*). Remarkably, these organoids self-organize and display key features of the target organs, suggesting that many aspects of nervous system development are intrinsic to populations of stem cells and can be activated under the right growth conditions, even in the absence of many growth factors that had been thought to be essential. Lancaster *et al*. built upon this idea, developing a culture system for human ES cell–derived three-dimensional cerebral cortical organoids. This culture system introduces ES cells embedded in droplets of Matrigel (a gelatinous protein mixture) within a spinning bioreactor, providing the structural support and the nutrient/oxygen exchange that allows growth of larger, more complex organoids (up to 4 mm in diameter). Most growth factors are omitted from the medium, but retinoic acid, which is critical for cortical neurogenesis (*4*), is added to expedite development. In only 8 to 10 days, neurons appear, and in just 20 to 30 days, defined brain regions form. It takes months for this to happen in an actual human embryo.



**Homegrown.** A potential application for generating human cerebral organoids (brainlike structures) will be the ability to study brain development, model disease, and gain a better understanding of actual brain physiology.

Although a cerebral organoid is far smaller than the brain of an early fetus, it recapitulates characteristics of normal brain development, giving rise to a wide range of discrete brain regions, including forebrain, midbrain, hindbrain, meninges, choroid plexus, hippocampus, and retina. The cerebral cortex region is further subdivided, at least crudely, in a fashion analogous to division of the normal human brain into early motor, visual, and other areas. These regions seem to be interdependent, as markers of forebrain, for example, are adjacent to markers of hindbrain, suggesting mutual repression between regions.

Even regions within a cerebral organoid show the normal process of neocortical development intriguingly well. For example, the horizontal, oblique, and vertical orientations of dividing stem cell progenitors closely resemble the trend in the human brain rather than in the mouse brain. Specialized progenitor cell types of the normal developing human brain also are recognizable, including outer radial glial (oRG) progenitors (*1*). Like the developing human brain, a cerebral organoid contains abundant oRG progenitors, whereas the developing mouse brain and cerebral organoid do not. Neurons in the organoids also show some (albeit not all) of the migration seen in a normal brain: Organoid neurons form both a "preplate" scaffold that regulates later-migrating neurons, and an intermediate, cell-sparse zone through which neurons migrate from deep in the brain to more superficial regions. As neurons assemble in the organoid cortex, they do so in a stereotypical manner that is considerably rougher than, but surprisingly similar to, what is seen in a normal brain. Interneurons migrate especially long distances from outside the cortex in vivo, and a similar type of migration is observed in organoids. As neurons differentiate in the organoids, they exhibit spontaneous $Ca^{2+}$ surges or "action potentials," which are the hallmark of functioning neurons in the brain.

Lancaster *et al*. further show that human cerebral organoids model some human diseases better than do mice. For example, the authors model microcephaly ("small brain"), which is caused by mutations in the gene *CDK5RAP2*. *CDK5RAP2* encodes a centrosomal protein that controls centriole replication (and therefore cell signaling and proliferation). Unlike humans, mice with *Cdk5rap2* mutations show only mild brain defects or more severe defects, depending upon the mouse strain (*5*, *6*). When induced pluripotent stem (iPS) cells derived from skin fibroblasts from a microcephaly patient (with an aberrant *CDK5RAP2* gene) were cultured, cerebral organoids resembled human microcephaly—they were far smaller compared to control organoids due to premature neuronal differentiation at the expense of progenitor proliferation.

It is astonishing that human cerebral organoids can generate multiple distinct brain parts and functional cortical neurons

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Division of Genetics and Genomics, Manton Center for Orphan Disease, and Howard Hughes Medical Institute, Children's Hospital Boston, Broad Institute of MIT and Harvard, and Departments of Neurology and Pediatrics, Harvard Medical School, Boston, MA 02115, USA. E-mail: christopher.walsh@childrens.harvard.edu



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intrinsically in vitro in such a short period of time, and display normal human cerebral cortex development broadly, if not perfectly. They do not grow beyond a 4-mm-diameter size, apparently because the lack of a blood supply limits access to nutrients. They lack many brain parts and cell types. And it is not yet clear how close the electrical potentials in organoids are to brain potentials, nor whether organoid neurons connect with the regions seen in an actual brain. Ethicists need not worry just yet, and may never need to worry, about the philosophical implications of "consciousness in a dish." Indeed, perhaps the more interesting philosophical implication of these organoids is the extent to which these seemingly bland and undifferentiated (albeit totipotent) ES cells can self-assemble into such a complex emergent structure.

As tissue engineering further improves the structure and reproducibility of these organoids, they will likely find their strongest application in the modeling of diseases (*7*). Combining new stem cell technologies with genome-editing tools, such as TALEN and CRISPR-Cas9 (*8*), will allow genetic modeling of many neurological and neuropsychiatric disorders. This may allow rapid screening of disease phenotypes, pathogenic mechanisms, and drug effects. Functional studies of human-specific genetic changes using human cerebral organoids may also be possible, providing insight into similar genes that act differently in humans and other mammals throughout evolution. Human pluripotent ES cells and cerebral organoids promise to advance our understanding of neuroscience and stem cell biology … and quickly.


**Reference and Notes**

1. J. H. Lui, D. V. Hansen, A. R. Kriegstein, *Cell* **146**, 18 (2011).
2. M. A. Lancaster *et al*., *Nature* **501**, 373 (2013).
3. Y. Sasai, *Nature* **493**, 318 (2013).
4. J. A. Siegenthaler *et al*., *Cell* **139**, 597 (2009).
5. S. B. Lizarraga *et al*., *Development* **137**, 1907 (2010).
6. J. A. Barrera *et al*., *Dev. Cell* **18**, 913 (2010).
7. Y. Sasai, *Cell Stem Cell* **12**, 520 (2013).
8. T. Gaj, C. A. Gersbach, C. F. Barbas 3rd, *Trends Biotechnol.* **31**, 397 (2013).



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MATERIALS SCIENCE

# Structure and Motion of a 2D Glass

The structure and dynamics of a two-dimensional silica film provide fundamental insights into amorphous materials.

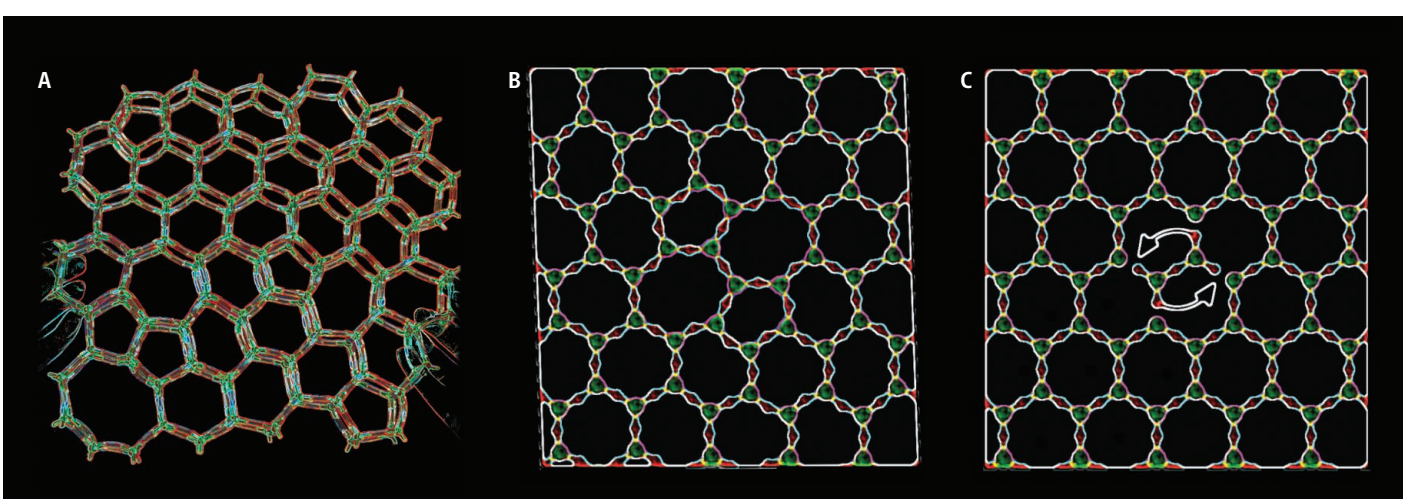

**Markus Heyde**

Fritz Haber Institute of the Max Planck Society, Faradayweg 4-6, 14195 Berlin, Germany. E-mail: heyde@fhi-berlin.mpg.de


Silicon oxide (silica) glass plays a key role in many modern technologies, from semiconductor devices and optical fibers to supporting materials in heterogeneous catalysis and novel durable glasses. Yet little is known about the atomic structure of amorphous materials. Recent studies of two-layer glass structures have started to shed light on the structure of amorphous silica (*1*, *2*). On page 224 of this issue, Huang *et al*. provide direct evidence for dynamic rearrangements of such two-dimensional (2D) silica films under a probing electron beam (*3*).

Diffraction methods are widely used to determine the structures of crystals and their surfaces. However, diffraction is of limited value for analyzing amorphous materials, which have no long-range order and periodicity. Zallen once wrote that "the atomic structure of an amorphous solid is one of its key mysteries, and structural information must be won with great effort" (*4*).

Transmission electron microscopy (TEM) and scanning tunneling microscopy (STM) methods have the potential to overcome these difficulties. In TEM, increased resolution as a result of aberration correction has brought a renaissance to the field (*5*). Scanning probe microscopes are now also capable of true atomic resolution. Molecular motions or even chemical reactions can be followed with both techniques (*6*–*8*). It has been proposed that noncrystalline materials can also be characterized with these methods (*9*, *10*), but this has not yet been realized.




CREDIT: FIGURE PANELS B AND C FROM (1)

**Follow the motion.** Silica consists of silicon and bridging oxygen atoms. In the 2D structure, crystalline silica has only six-membered rings. Zachariasen proposed more than 80 years ago that amorphous silica forms a network of rings of different sizes (*12*); recent studies of 2D silica bilayers have verified this model (**A**) (*1*, *2*). Huang *et al*. now provide experimental evidence for a transformation from amorphous (**B**) to crystalline structures (**C**) in such bilayers.