# ORGANOIDS: THE FUTURE OF MEDICINE

BY JONATHAN KUO

On Friday, December 13, 1799, the end of the century brought with it the end of the first president of the United States: George Washington would die in less than 48 hours. His treatment? Molasses, vinegar, butter, sage tea, calomel, various natural salves, and the removal of nearly 2.5 liters of his blood, or what amounted to about half his total blood volume.[1,2] While such a treatment regimen may seem crude by modern medicinal standards, such was the norm since the times of Ancient Greece, when Hippocrates first theorized that the imbalance of four bodily fluids, called "humors," causes disease. Draining an excess humor soon became a common medical treatment, leading to the phenomenon of bloodletting and one basis of nearly 2,000 years of medicine.[3]

▶ ***Image: Cerebral organoids expressing green fluorescent protein.*** *(Dr. Abed Mansour, Gage Lab, The Salk Institute).*

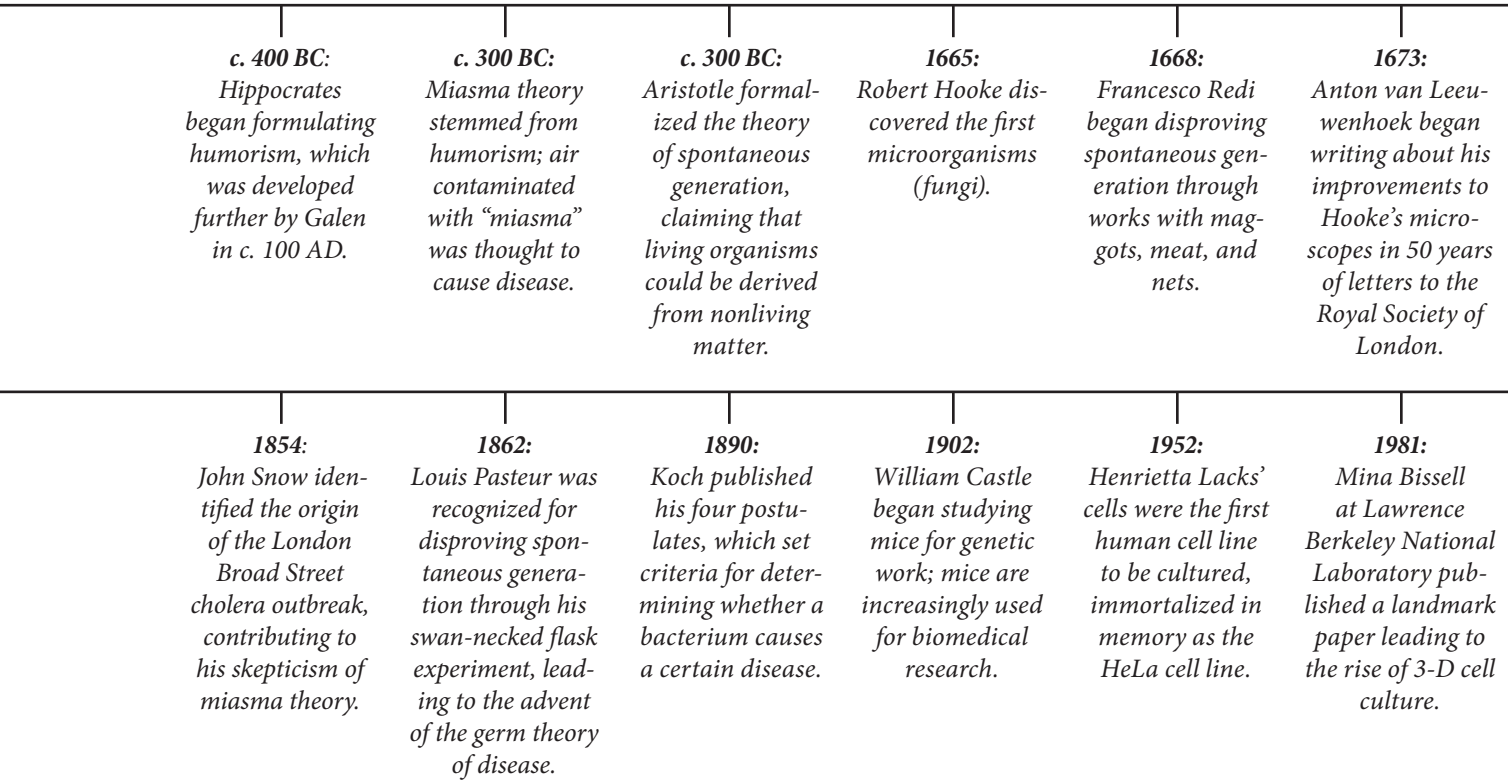


***Figure 1: A brief timeline of the history of disease and disease modeling.***

But as humanity's understanding of the world around us—and within us—expanded throughout the centuries, so too did our understanding of disease. Through advancements in microscopy, scientists uncovered a world of microbial organisms concealed within the environment, a world largely invisible to the naked eye. With the discovery of this microbial world, humorism and other theories were replaced by the germ theory of disease. Understanding microorganisms, however, solved only part of the problem. Efforts during the past century sought to reveal how these disease-causing agents interacted with and infected humans, leading to the development of cell culture and animal modeling. Yet the fight did not stop there. As humanity began to make progress against the foes of the microbial worlds, other public health problems emerged. Today, rather than succumbing to microorganisms, people die more frequently from genetic diseases, cancer, and lifestyle disorders, in part due to increased human life expectancy. Fortunately, by serving as miniaturized models of human organs, organoids may prove to be the gold standard for the future of studying disease.

## DISEASES IN THE LABORATORY

Currently, diseases are roughly studied in two contexts: cell culture and animal models. In cell culture, diseases are modeled by infecting or mutating cells and growing them in Petri dishes. Infection is used when the disease is caused by some external pathogen, like a virus, while mutation is used when the disease is caused by mistakes in the genetic code, like cancer.[4] Animal models operate under a similar basis, but animals such as mice or rats are grown in cages rather than Petri dishes.[5] Treatments, such as novel pharmaceutical drugs, can then be administered to the diseased cells or animals. Theoretically, if treatments are effective in these contexts, they are more likely to be effective in humans, generating a treatment development pipeline from cells to animals to clinical trials to clinical application.

Practically, this isn't quite the case. Both cellular and animal models suffer from several issues that prevent them from accurately forecasting success in clinical trials. Experimental cell lines are different from human cells because replicating cells introduces errors in the genome, so modified cell lines that replicate continuously are more similar to cancer cells than human cells. Cell cultures are also particularly simplistic compared to the human body because cultured cells usually consist of only one cell type (such as kidney cells) and exist in 2-D.[4] On the other hand, while animals simulate more complex conditions, animals and humans exist on different time scales of life and differ in factors such as their physical size, metabolic rate, and diet, factors that introduce variation scientists are unable to control.[6,7] In short, both cell cultures and animal models do not accurately model the conditions of the human body, so it isn't surprising that treatments affect them in different ways than they affect humans. Organoids, however, may be an improvement over existing non-human models by resolving limitations in both types of models.

## HOW TO GROW A BRAIN ORGANOID

Organoids have been defined as "containing several cell types that develop from stem cells or organ progenitors and self-organize through cell sorting and spatially restricted lineage commitment, similar to the process in vivo."[8] Put simply, organoids are grown from stem cells, and have three key features: (a) they contain multiple cell types specific to the organ they originate from, (b) they can replicate a function of an organ, such as neural activity, and (c) they are structurally and spatially similar to an organ.[8,9] But how exactly are organoids grown and applied in research? Tracing the development of a brain organoid from its "birth" may provide insight into this question.

A typical brain organoid, like any other organoid, is first grown from stem cells, specifically pluripotent stem cells (PSCs). These stem cells have the potential to grow into multiple different types of cells, hence the name "pluripotent." These stem cells are seeded into a liquid medium and develop into 3-D spherical embryoid bodies, which are then transported to a firm matrix made of gel. This matrix, which is similar to the human body's extracellular matrix, contains many factors that initiate signalling pathways within the embryoid bodies, causing further development and specialization of the PSCs. Finally, physical agitation of the embryoid bodies causes them to form cavities that resemble ventricles, the neural cavities that contain cerebrospinal fluid (CSF). This culture can be maintained for several months, during which it begins to resemble a brain even more closely: after 6 months, functional synapses can be seen, while after more than 9 months, higher-level organization of features such as active neuronal networks can be observed. Notably, however, whether these networks are similar to those of humans is still uncertain, and further research needs to be done to compare the two.[10,11,12,13]

Cultured brain organoids can model a wide variety of different categories of diseases. The causes of some neurological diseases are easy to identify, like Sandhoff disease, which is caused by a mutation in the HEXB gene leading to a buildup of fats in the central nervous system, eventually leading to death. Scientists from the NIH and the University of Massachusetts have grown two sets of brain organoids; one with the HEXB mutation, the other without. They found that the HEXB-mutated organoids showed a buildup of fats similar to that in Sandhoff disease and were able to monitor how the disease modulated the development and differentiation of the stem cells.[14] Other diseases, such as Alzheimer's Disease (AD), have causes that are more complex. Some features of AD are known, such as a characteristic buildup of amyloid plaques and neurofibrillary tangles. Scientists who have grown brain organoids afflicted with AD have also observed a similar buildup of amyloid plaques and neurofibrillary tangles, demonstrating the potential utility of these organoids in accurately testing therapies against AD.[15] And, of course, cancers, which can affect most organs in the body and are notoriously difficult to treat, have been modeled by organoids. By inducing mutations in stem cells using technologies like CRISPR-Cas9,[16] scientists have created brain organoids with features that resemble cancers like glioblastomas.[17] These models of disease are significantly cheaper to produce, easier to organize, and less labor-intensive than animal models. Perhaps most importantly, these models more accurately resemble humans compared to cell culture and animal models, demonstrating their superiority to current models of disease.[18] And as advanced microscopy methods and big data image analytics develop further, permitting better visualization of organoids, organoids will play a major role in general live tissue 4-D cell biology of the future.[19]

Organoid technology, however, isn't quite the perfect model of diseases and treatments yet. Tissues in the human body are surrounded by an extensive vascular network consisting of arteries, veins, capillaries, and lymphatic vessels that help feed nutrients to cells and remove waste products. The complexity of current vascularization networks created for organoids pale in comparison, and the size of organoids are limited in part because they can not be vascularized well enough.[20] Body tissues are also bathed in extracellular fluids that create particular biochemical microenvironments for

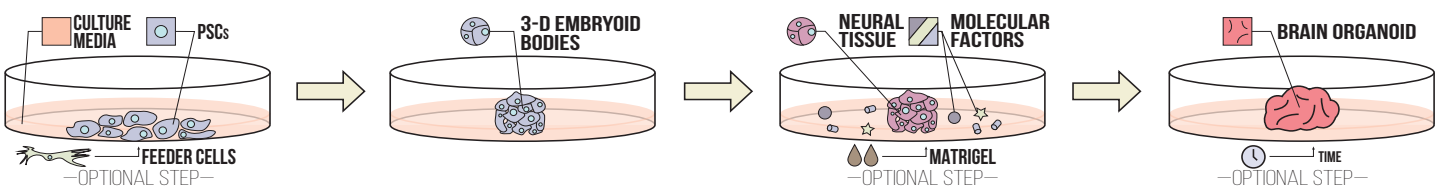


***Figure 2: General brain organoid culture protocol.*** *Growing brain organoids can be generalized to about four steps. First, human pluripotent stem cells (PSCs) are cultured in appropriate culture media. These cells can be cultured with feeder cells that provide the stem cells with additional nutrients, although feeder-free media such as mTeSR1 have been developed. After some time and the addition of certain enzymes that break the physical linkages between the PSCs and the plate they are growing on, the PSCs aggregate into 3-D spheres called embryoid bodies (EBs). Next, the embryoid bodies begin differentiating into neural tissue after specific molecular factors are added. Any remaining steps serve to further refine the structure of the differentiated EBs. For instance, growing the EBs in Matrigel, a gelatinous matrix produced by mouse cancer cells, promotes self-organization of the organoids into layers similar to those in the human brain. Additionally, growing the organoids in bioreactors that periodically agitate the organoids allow more nutrients to perfuse throughout the organoids, increasing the size limits observed in other types of cultures. The protocol described in this paper is only one of many, many ways to culture a brain organoid.*

"By inducing mutations in stem cells using technologies like CRISPR-Cas9, scientists have created brain organoids with features that resemble cancers like glioblastoma."

tissue types, but organoids sometimes lack these environments. The brain, for instance, is surrounded by a bath of CSF, but organoids that create CSF have not been yet been described in literature. And finally, certain developmental cues that guide organ development in humans have not been replicated—such as the growth of tissues in specific directions on specific axes—so organoids do not quite perfectly resemble organ superstructure.[21]

Interestingly enough, organoids can be derived from patient cells. That is, cells can be collected from a patient suffering from a genetic condition and grown into organoids, allowing testing of medicine without significant risk to the patient.[13,14] Organoids could therefore possibly be used in a future of personalized medicine, when treatment regimens are not only created based on specific patient phenotypes, but are also tested and modulated before ever being used on patients. Such applications would reduce some of the inherent risks of medicine—rather than hoping that treatments will be effective for patients, why not just grow some organoids and test on those instead?

***Acknowledgements:*** I would like to acknowledge postdoctoral fellows Dr. Johannes Schöneberg (UC Berkeley) and Dr. Aparna Bhaduri (UCSF) for their detailed feedback and fruitful discussions about organoids and language during the writing process.

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