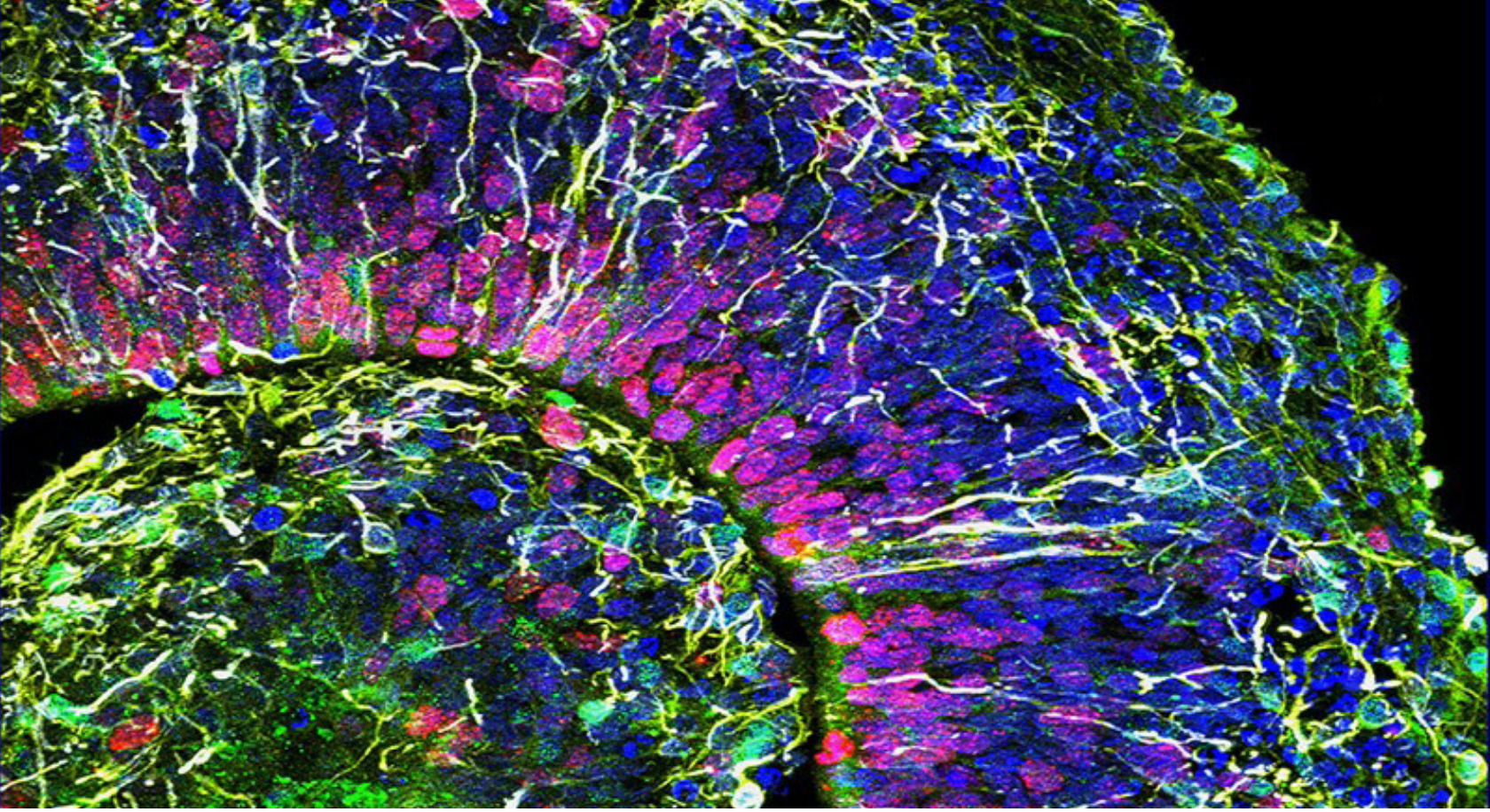

ASSEMBLOIDS: THE MODEL OF THE FUTURE

BY ANNA CASTELLO

For city planners, maps and models come in all shapes and sizes. Wastewater maps, for example, project cities onto flat planes, allowing planners to trace how water flows from drain to ocean. 3D models, in contrast, grant architects the power to visualize how sounds and shadows are shaped by elevation. And through the usage of software like ArcGIS, city planners can explore how all the moving parts of a city work together cohesively.

Models of the human body, however, aren't as effective. Most current models of human systems don't capture the full extent of the human body's interconnectedness. But a new type of cell culture system, called an assembloid, might offer the next step for scientists trying to understand how the human body works.

ORGANOIDS AND ASSEMBLOIDS

Assembloids are largely based on a relatively recent cell culture system called organoids. Organoids are miniaturized models of organs or organ parts that are used to study biological processes. They are made in the laboratory from human induced pluripotent stem cells (hiPSCs), a special cell type that has been genetically reprogrammed to have the ability to differentiate into any cell type in the adult human body.[1] This differentiation is achieved through the forced expression of specific sets of transcription factors.[2] With the right environments and growth factors, hiPSCs can self-assemble into an organoid (Fig. 1) consisting of various cell types needed

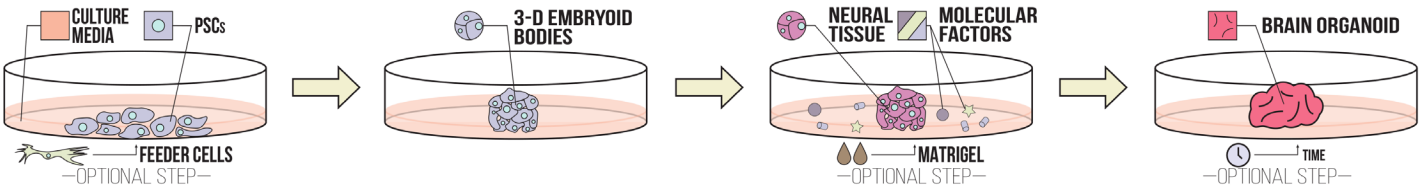

Figure 1: General brain organoid culture protocol. *Human pluripotent stem cells (PSCs) are cultured in appropriate culture media. After addition of certain enzymes that break the physical linkages between the PSCs and the plate they are growing on, the PSCs aggregate into 3-D spheres called embryoid bodies (EBs). The EBs then differentiate into neural tissue after specific molecular factors are added.*[22]

to mimic the approximate structure and function of the target organ.[3,4,5,6] These self-organizing collections of cells have been used to model many diseases such as Alzheimer's and Zika fever.[7,8,9,10]

While organoids have contributed to incredible advancements in medicine, they fail to represent the body's interconnectedness—they only permit the study of a disease or a drug on a single organ type. However, many diseases and drugs affect more than one of the body's operations. Organoids do not allow researchers to see how multiple organs and tissues work together as they would *in vivo*.

While they aren't perfect, assembloids offer one solution to this problem. The principle underlying their creation is relatively simple: place two organoids next to one another and they can fuse, integrating multiple cell types in one 3-D model. While these models will likely prove useful in many branches of biology, so far they have mostly been used in a couple specific contexts: studying the progression of disease, and studying drug interactions that involve multiple organ systems. The cortico-striatal assembloid in particular has rapidly gained traction as a useful model for disease development, at least for neuroscientists.[11,12]

CORTICO-STRIATAL ASSEMBLOID

Cortico-striatal assembloids link the cortex and the striatum, two closely-related regions of the brain. Anatomically, these regions interface most closely in the basal ganglia, a portion of the brain responsible for motion.[13] Normally the cortex and the striatum work together to initiate voluntary movement and inhibit opposing movement, resulting in a smooth and controlled motor response.[14] The cortex can be thought of as the mayor of a model city, thinking of new plans to coordinate the workforce (in the body's case, the muscles). These plans are then communicated to the striatum, or the project developer, who clearly relays the instructions to the muscles.

But sometimes, this communication can be muddled. In cities, poor coordination causes police officers to knock on the wrong doors and construction crews to demolish the wrong buildings. In the body, poor coordination can have equally serious consequences: Parkinson's, for example, causes patients to experience tremors and perform rigid movements as a result of muddled synaptic communication.[15]

Dysfunctions of the cortico-striatal circuits appear to be linked to not only the inability to form fluid voluntary muscle contractions, but also to the presence of neuropsychiatric diseases such as autism spectrum disorder, schizophrenia, and obsessive-compulsive disorder.[16,17,18,19] Assembloids could help us understand these conditions better by providing us a model for communication between the cortex and striatum.[12] Researchers from Stanford University have been able to create such a model by connecting two individual region-specific brain organoids that model the human cerebral cortex and striatum to form an interconnected cortico-striatal assembloid.[12] The formation of this assembloid was closely monitored through imaging and testing of the synaptic connections which allowed for greater insight into how this circuit is assembled during development and how and when functional defects during development arise. Because assembloids involve live cells, they can be imaged and experimented on at any time during their formation and maturation. This provides us with a better understanding of the miniscule changes that occur during development than we can garner from studying human fetuses.

Basic imaging, however, isn't enough to determine whether these assembloids are executing their main function: talking to each other through synaptic connections. To do that, researchers need to use a technique called retrograde viral tracing (Fig. 2). When a cortico-striatal assembloid is properly connected, retrograde viral tracing shows that cortical neurons extend into the striatum and form synaptic connections with medium spiny neurons, major components of the striatum.[12,20]

This same technique of making assembloids can be used with cells derived from clinical patients. This has been used to study Phelan-McDermid syndrome, for example, which is characterized by a wide variety of defects throughout the body, ranging from the brain to the heart and the gastrointestinal system. Using individualized assembloids created from the stem cells of patients, scientists have been able to observe hyperactivity in medium spiny neurons, a cellular hallmark of Phelan-McDermid syndrome that

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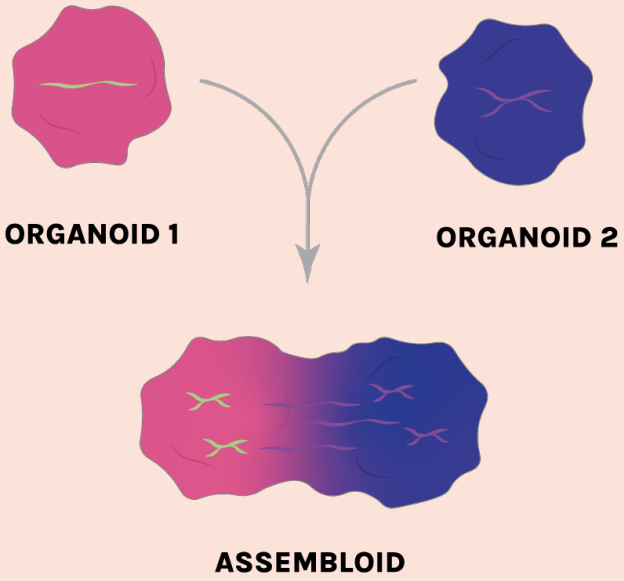

Figure 2: Retrograde viral tracing .

Retrograde viral tracing is a technique that employs viruses that encode visualization markers, such as fluorescent proteins, to visualize neural connections. Virus is taken up at the axonal terminal and travels to the cell body (retrograde transport).

The technique is used here to visualize neuronal projections from cells in the cortical (hSC) organoid into cells in the striatal (hStrS) organoid. hCS cells were infected with the virus AAV-DIO-mCherry, which expresses the red fluorescent protein mCherry only in the presence of the protein Cre. hStrS cells were infected with the virus rabies-ΔG-Cre-eGFP, which expresses both the protein Cre and the green fluorescent protein eGFP. When hCS cells project to hStrS cells, the projections take up rabies-ΔG-Cre-eGFP from the hStrS cell, introducing Cre into hCS cells and inciting expression of mCherry. Thus, only connections between hCS and hStrS cells will be stained with both eGFP and mCherry.[12,21]

"This means there is, in theory, no cap on the types of organoids that can be assembled together."

can be observed in the neurons of mice and human suffering from the disease.[12] The nascent success of the cortico-striatal assembloid demonstrates that connected brain region-specific organoids can be used to better understand the mechanism of neuropsychological diseases and to accelerate the search for novel treatments.

WHAT IS NEXT?

While it seems relatively easy to connect various neurons together, as in the cortico-striatal assembloid, connecting vastly different cell types to make an assembloid that incorporates several body systems is more difficult. But recently, researchers have been able to create a cortico-motor assembloid connecting cortical, spinal, and muscle organoids.[11] In this assembloid, the decision-making cortex, akin to a mayor, relays information to the spinal cord—the city's communication office— which then connects with the workforce to incentivize action, quantified by the presence of a twitch in the muscle. Such a model could provide the means to study potential cures for amyotrophic lateral sclerosis (ALS), among other diseases.

In contrast with the cortico-striatal assembloid which is only composed of neurons, the cortico-motor assembloid proves that different tissue types can be combined to form a model that better encapsulates the body's complex interactions. This means there is, in theory, no cap on the types of organoids that can be assembled together to then be used to understand many different diseases in a level that organoids individually never could.

Importantly, assembloids could also become standards for personalized medicine, a type of medicine that tailors treatments to a specific individual's genetic information and environmental conditions. Assembloids created directly from a patient's own cells could help researchers identify what genetic, cellular, and molecular issues a patient is facing and provide a platform to test how the individual might react to certain treatments—a useful feature, considering that the wrong medication might have deadly side effects. Assembloids provide a means to understand any patient, eliminating some of the guesswork of medicine and helping researchers and doctors give the best possible care to every patient.

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