Features

Don't Avoid the Organoid:
Promising Advances in the Stem Cell Field

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In Search of a New System

In order to better understand biological systems and develop therapeutics for disease, researchers use a variety of model systems such as two-dimensional cell cultures and model organisms. Although it is easy to manipulate and investigate specific cell types – most relevantly, human cells types – in cell cultures, two-dimensional cell cultures remain limited in their ability to recapitulate the heterogeneous cell interactions found in living organisms (*in vivo*). This inter-cellular communication is vital to understanding our biology, as our organs are made up of many different cell types that communicate with each other throughout development and everyday function. Researchers use model organisms (such as the fruit fly, chicken, or mouse) in order to better study this aspect of cell-to-cell communication. Unfortunately, these model systems are limited by their differences to human physiology and are challenging to perform large-scale screens with.

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What if cell culture could be three-dimensional and demonstrate the cell-to-cell interactions that occur within organs? This question is not novel, as researchers in as early as 19061 have sought to recapitulate organogenesis to treat patients in need of new organs. They attempted organogenesis through *ex vivo* (outside the body) transplants of organs by taking thin slices of organs and growing them in culture; however, growing *ex vivo* transplants does not work for all organs and is generally restricted to certain tissue types such as the kidney[2]. It was not until recently that researchers succeeded in generating robust, three-dimensional organ-like structures. They were termed organoids and are defined as three-dimensional organ buds that develop from self-organizing progenitors (such as stem cells) and recapitulate the tissue architecture and functionality of true organs[3,4,5,6]. So far, research has shown that organoids hold tremendous potential to study development, test therapeutics, and yield exciting insight about stem cells.

From One Cell, Many

One of the main types of organoids, the stem cell organoid, develops from a single cell, called the stem cell, that proliferates and is pluripotent, which means that it is able to differentiate into various cell types with specialized functions. Somatic cells (such as skin cells) are induced to a pluripotent state by genetic reprogramming. After induced pluripotency, the cells are grown in media with appropriate signaling molecules that normally regulate development, such as Wnt, Bone Morphogenetic Proteins (BMPs), and Fibroblast Growth Factor (FGF) molecules[3]. These different factors nudge developing cells to

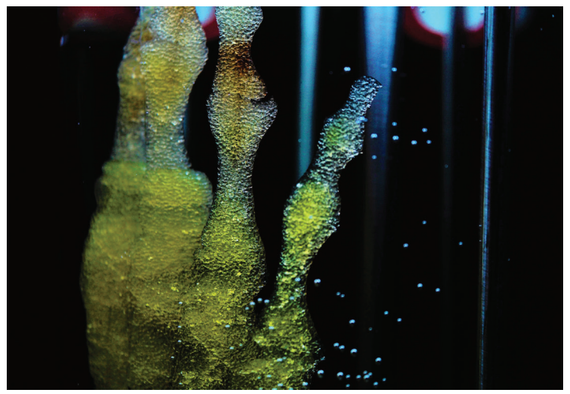
adopt specific fates, self-aggregate, and pattern into an organoid[1].

Stem cells must be in the same physical and chemical environment as the organ would be *in vivo* in order to develop properly; this kind of environment is called the niche and can be mimicked through culturing in specific media and hydrogels (a scaffold full of hydrophilic polymer chains). Matrigel, a commonly used hydrogel secreted from a mouse sarcoma line, facilitates organoid development because it is rich in important extracellular proteins such as laminin and glycoproteins[7]. These proteins signal the developing organoid to maintain its stem cell-like state and allow it to grow to a size sufficient for self-organization[7].

The optic cup organoid, derived from human embryonic stem cells (hESCs), is an excellent example of stem cells' intrinsic ability to organize into the correct structure[8]. With guided Wnt signaling, an aggregate of hESCs are able to form into eye-like structures such as the retinal epithelium, and eventually give rise to other optic cup structures such as the neural retina (which contains six different cell types: photoreceptor cells, bipolar cells, and ganglion cells to name a few) or retinal pigment epithelium (cells that support the neural retina)[8]. The developing optic cup has an apically convex curvature[8] just like the true eye. Once we have this structure, we can then use the organoid as a model system to study further biological questions.

Putting Organoids to Work

Organoids are incredibly exciting in the field of stem cell biology. Since 2008, many different research groups have started to develop more robust protocols for generating organoids for the gut, the brain, the kidney, and other organs[3]. The way human-derived organoids are generated provides novel insight about tissue patterning, the blueprint and timing of molecular patterning factors, and the importance of the niche. For example, in 2016 Clevers and colleagues found that intestinal Lgr5 stem cells – the cells that continuously regenerate gut cells – were dependent on signaling from Paneth cells, which sit nearby these stem cells after initial embryonic development[9]. They used organoids to investigate the importance of Paneth cells



Organoids have tremendous potential to act as a new model system for diseases

and other signals in vitro, a task which would have been difficult to manipulate within mice and impossible to recapitulate in two-dimensional cell culture.

What really captures the enthusiasm of the stem cell field is the organoids' tremendous potential to act as a new model system for diseases. More representative of human physiology, organoids can model diseases that were difficult to study in vivo or in two-dimensional culture. For example, microcephaly, a neurodevelopmental disorder in which the brain is much smaller than usual, was difficult to recapitulate in mice[10]. Some microcephaly patients had mutations in the gene encoding CDK5RAP2, a protein kinase that is involved with regulating neuronal differentiation[10]. Unfortunately, knocking out that gene in mice did not lead to the microcephaly phenotype [10]. In 2013, Lancaster and colleagues found that knocking down this gene (reducing the amount of protein the gene produces) in the brain organoid model resulted in very few neuronal progenitors, a phenotype which decreases the brain size similar to microcephaly. Using human-derived organoids for modeling purposes can help us develop more effective drug screens and disease therapies as has already been established for liver and kidney toxicity models and cancer lines[10].

The Blood, Sweat, and Tears of Organoid Generation

Although organoids hold tremendous potential, researchers in this field currently face challenges in making organoids robust systems for modeling. Many of these organoids fail to develop the structure and physiology characteristic of adult organs because the "blueprint" of molecular signaling for development is still incomplete[10]. Additionally, researchers culturing these organoids must contend with long wait times. For example, generating brain organoids may involve a wait time of 9 months between stem cell culturing and full development of the organoid[11].

As of right now, cross-talk between different organ systems can only be efficiently modeled through in vivo systems; organoids lack the vascularization and innervation that is often important for signaling[3]. Although some groups have generated vascularized tissue such as tissue of the liver by co-culturing the organ-bud with endothelial cells[12], vascularization is still restricted to certain kinds of tissues and is not as robust as it would be in the physiological system.

Perhaps the most pressing concern that organoids face is the heterogeneity within each population. Even organoids cultured within the same system can vary widely in shape and cell type composition[13], which limits their ability to be used for large screenings and disease modeling. One group found that genomic imprinting caused some variability in gene expression between different hESC-derived lines[14]. A reliable drug screen requires that the model being tested is homogenous so that there is no additional variability introduced. Therefore, the variability in organoids evokes concern that they are still unfit to use in drug testing. These limitations are not trivial, but this technology is still in development. The process of organoid generation will be even more refined as our understanding of them increases, and in the coming years organoids will prove a strong alternative to classical models.

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