# Organoids

**A brief overview of stem cell-derived organoids: how they are made and what the challenges are.**

An organoid is a 3D multicellular *in vitro* tissue construct that mimics its corresponding *in vivo* organ, such that it can be used to study aspects of that organ in the tissue culture dish. Although 3D tissue culture is decades old, the word organoid is today most commonly used to describe such constructs derived from stem cells; these could be either pluripotent (embryonic or induced) or adult stem cells from various organs. It is thought that the processes that form these tissues *in vitro* approximate natural development or tissue maintenance. The developmental potential of the starting stem cells will influence how complex the organoid can be. Most of the discussion here refers to the more complex pluripotent stem cell-derived constructs.

Early work on embryonic stem cell-derived cortical tissue and on adult stem cell-derived intestinal tissue showed the fascinating capacity for stem cell-derived constructs to organize into complex *in vivo*–like structures. Now, about a decade later, scientists have reported organoids that model (albeit incompletely) brain, retina, intestine and other organs of the gastrointestinal tract, kidney and liver, among several other organs.

## Strategies for growing organoids

Some methods for generating pluripotent stem cell-derived organoids give the cells minimal differentiation information and then leave them alone (apart from providing appropriate growth conditions and nutrients), permitting intrinsic self-organization and presumably also stochastic processes to shape the tissue. For example, one approach to generate brain organoids embeds neuroectodermal embryoid bodies from human pluripotent stem cells (hPSCs) in Matrigel, but gives no further external cues to the cells. This approach generates structures with many brain regions, including cortex, but it can also generate some mesodermal and endodermal lineages. Alternately, cells are patterned toward more specific regions of the CNS, typically based on prior knowledge of the signals controlling development, with further contribution from self-organizing processes. For example, region-specific brain organoids are generated by patterning hPSCs towards structures that consist principally of either dorsal or ventral forebrain.

For adult stem cell-derived organoids, growth conditions typically mimic signals that control tissue repair after damage or steady state tissue maintenance. Epithelial organoids derived from adult stem cells in the various organs of the gastrointestinal tract, for example, almost all need agonists of Wnt signaling (among other signaling factors , including embedding in Matrigel) to both maintain the cells and to generate an *in vivo*–like complement of cell types.

## Challenges abound

While organoids are very promising models for the study of human processes and structures, they are far from perfect. Challenges arise from the fact that these cultures are both more complex than the *in vitro* models researchers are used to, and yet they are not quite complex enough.

Though several organoid preparations contain an impressive number of cell types, work to fully characterize just how well these models recapitulate their *in vivo* counterparts is ongoing. Certainly many obvious components of tissue are lacking. Organoids typically lack vasculature and immune cells. This means that they are limited in how big they can grow without cell death, and that they cannot be used to study processes that require these components. Some types of organoids also do not fully recapitulate the structure of the organ they model. For instance, anatomical markers that researchers use to orient themselves in the *in vivo* brain can typically not be applied to brain organoids. Finally, the cells in hPSC-derived organoids are relatively immature; in most cases they match the expression profiles of fetal tissue.

On the other hand, because pluripotent stem cell-derived organoids are substantially more complex than 2D monolayer cultures of differentiating stem cells, they are likely to show commensurately greater variability. The stem cell field already grapples with the fact that different hPSC lines vary measurably in their propensity to differentiate into specific cell types. In organoids, variability could exist at many levels—between different starting cell lines or genotypes, but also between batches of organoids from the same starting material, between multiple organoids within a culture, or even between areas of a single organoid itself. Such variability will have to be minimized, or at least controlled for, if these models are to be reliably used to study cellular and molecular phenotypes *in vitro*.

## Increasing complexity and control

Several creative approaches are being taken to generate more advanced organoids. One relatively new development is the realization that constructs of greater complexity can be generated by 'fusing' organoids that have been initially patterned and grown separately. As shown for forebrain spheroids and reported now by several groups, this appears to be as simple as coculturing the desired fusion partners such that they come into physical contact. This could represent a general strategy to start with more defined organoids, but still ultimately be able to achieve complex constructs.

In a separate approach, researchers have replaced the undefined Matrigel matrix in intestinal organoid culture with defined synthetic hydrogels; they have used these to define both signaling and mechanical properties of the matrix that are important for stem cell expansion and organoid formation. Precisely defining the culture requirements for organoids will be one way to move towards less variability in these constructs. Other researchers have reported 'guided self-organization', in which fiber scaffolds generated elongated organoids that were reported to show both improved differentiation but also better reproducibility. Engineering approaches are likely to be part of continuing attempts to grow organoids that are both increasingly complex and yet better controlled.

**Natalie de Souza**

**ACKNOWLEDGMENTS**

We thank S. Pasca and M. Little for their comments on this text.