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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

COMMENTARY

Taming the Wild West of Organoids, Enteroids, and Mini-Guts

The complex nature of an organ, made up of multiple tissue and cells types, poses an incredible challenge to biologists wishing to interrogate homeostasis or disease. Because of this complexity, it often is difficult to ascertain the cause-and-effect relationship of an experimental manipulation in these systems. For example, in the intestine, a perturbation to the epithelium may elicit an immune response; however, it is difficult to determine if this response is a direct result of the epithelial perturbation, or a consequence of a secondary effect, such as translocation of bacteria across the epithelium. The desire to assign cause-and-effect within complex systems has therefore made simple, reductionist, and easily controlled cell culture models appealing, allowing for exquisite experimental precision coupled with the ability for straightforward data collection and live cell imaging. For decades, cell culture models have been a workhorse of discovery, have led to many novel biological insights, have increased our understanding of disease, and have been used in drug development pipelines. However, despite the beautiful simplicity of cell culture models, historically, these come with several caveats: they are often transformed, or are derived from diseased tissue (ie, cancer), and, as such, often do not mimic a cell or tissue type at homeostasis and in a healthy state.

The bridge between these 2 extremes—the complex in vivo environment vs simple transformed cell lines—is the organoid.[1] Organoid, which means *organ-like*, are 3-dimensional structures derived from primary tissue, and grown in an artificial niche that supports a self-renewing population of organ-specific stem/progenitor cells. By definition, organoids possess some (but not all) of the form and function of the native tissue from which they derived. The first examples showing the ability to culture gastrointestinal organoids were shown nearly a decade ago.[2,3] These landmark studies showed the ability to isolate and culture murine intestinal tissue as 3-dimensional cultures possessing diverse cell types, with the ability to propagate and expand for many generations in vitro. Subsequent studies have shown that gastrointestinal organoid cultures could also be derived from human pluripotent stem cells,[4–6] and organoids could be grown from multiple mouse and human organ/tissue types and from developing or mature tissue.[7]

Given the rapidity with which organoid technologies have been developed, and the diverse array of organoid systems to consider, there has been a lot of hype, a lot of hope, and even some confusion surrounding these systems.[8] Especially for those that are new or unfamiliar to fields of research in which multiple organoid systems exist, one of the simplest reasons for confusion surrounding organoid is nomenclature. Indeed, organoid is a rather broad, catch-all term that can represent epithelium-only systems, epithelium plus nonepithelial components (mesenchyme/stroma, immune cells, neurons, and so forth),[9,10] and organoids can be derived from different sources (ie, patients, human pluripotent stem cells). Perhaps making matters more confusing was a proposal for standardized nomenclature[11] that has not been universally adopted. This has contributed to the same experimental organoid system being called by multiple names. For example, patient-derived intestinal epithelium grown in isolation (epithelium-only organoids) have been referred to as *intestinal organoids*, *enteroids*, *enterospheres*, and *mini-guts*. Moreover, attempts to affix "-oid" to different cultured human tissue has led to creative names such as *esophageoid*, *gastroid*, *enteroid*, *colonoid*, *pancreatoid*, *hepatoid*, and *cholangeoid*, to name a few, and has caused many an ear to bleed. However, overcoming challenges with nomenclature is straightforward because one simply needs to provide a clear definition for the system being used.

Another area in which organoid technologies have suffered is reproducibility.[8] There is significant variation in the process of culturing organoids from laboratory-to-laboratory, and, in the case of human patient–derived organoids, the incredible genetic heterogeneity across the population likely is reflected in organoids; yet, there has been no attempt to standardize how experiments are performed, and there is no well-accepted reference sample that has been universally adopted by the field. Experimental design is therefore without a well-accepted standard for an appropriate number of biological replicates, leaving every laboratory to set their own standard. This lack of standardization is partly owing to a lack of data, and partly owing to practicality; how many independent biological specimens are required to accurately capture the heterogeneity within the population, and how many times can/should an experiment be reproduced before the time and cost of performing additional replicates is diminished? Further confounding use of organoids is how cultures may change over time, especially because early cultures may possess short-lived populations that are present after isolation, but that are lost over time in culture. This could also significantly influence how experiments should be designed and performed.

As with any new technology, the initial phase of the organoid era seems to have begun to settle down and has now transitioned to a stage in which the technology becomes a standard in the field. This allows researchers to shift focus away from tool development and toward implementation—what exciting biology does the tool allow researchers to address in a way not previously possible? There are many examples now showing how organoids can be leveraged to study patient-specific diseases, and efforts to prospectively cryopreserve fresh tissue for organoid generation may be an important consideration for future biobanking efforts to maximize access to important human

samples. Use of organoids also has raised questions of how modifications to the tissue culture environment may be implemented to improve utility and rigorous use of these systems. For example, access to the apical surface of 3-dimensional organoids is accomplished via microinjection, which can be cumbersome, and many studies have begun to transition 3-dimensional organoids to a 2-dimensional environment to generate polarized monolayers that allow more straightforward access to the apical epithelial surface, and that can be used to interrogate transepithelial migration and barrier function. Another exciting emerging trend is the cross-over between organoid technology and quantitative and engineering approaches to both control and measure experimental outcomes in organoid systems.[12] It is difficult to know exactly how organoids will be used moving forward, but given the ability to add and remove complexity in a modular nature, and flexibility to adapt organoids for use with different engineered systems, suggests that creative implementation of this tool may be the most significant limitation in the future.

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References

1. Hill DR, Spence JR. Gastrointestinal organoids: understanding the molecular basis of the host-microbe interface. Cell Mol Gastroenterol Hepatol 2017;3:138–149.
2. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459:262–265.
3. Ootani A, Li X, Sangiorgi E, Ho QT, Ueno H, Toda S, Sugihara H, Fujimoto K, Weissman IL, Capecchi MR, Kuo CJ. Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. Nat Med 2009;15:701–706.
4. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, Hoskins EE, Kalinichenko VV, Wells SI, Zorn AM, Shroyer NF, Wells JM. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 2011;470:105–109.
5. Tsai Y-H, Hill DR, Kumar N, Huang S, Chin AM, Dye BR, Nagy MS, Verzi MP, Spence JR. LGR4 and LGR5 function redundantly during human endoderm differentiation. Cell Mol Gastroenterol Hepatol 2016;2:648–662.e8.
6. Tsai Y-H, Nattiv R, Dedhia PH, Nagy MS, Chin AM, Thomson M, Klein OD, Spence JR. In vitro patterning of pluripotent stem cell-derived intestine recapitulates in vivo human development. Development 2016;144:1045–1055.
7. Finkbeiner SR, Hill DR, Altheim CH, Dedhia PH, Taylor MJ, Tsai YH, Chin AM, Mahe MM, Watson CL, Freeman JJ, Nattiv R, Thomson M, Klein OD, Shroyer NF, Helmrath MA, Teitelbaum DH, Dempsey PJ, Spence JR. Transcriptome-wide analysis reveals hallmarks of human intestine development and maturation in vitro and in vivo. Stem Cell Reports 2015;4:1140–1155.
8. Huch M, Knoblich JA, Lutolf MP, Martinez-Arias A. The hope and the hype of organoid research. Development 2017;144:938–941.
9. Finkbeiner SR, Freeman JJ, Wieck MM, El-Nachef W, Altheim CH, Tsai YH, Huang S, Dyal R, White ES, Grikscheit TC, Teitelbaum DH, Spence JR. Generation of tissue-engineered small intestine using embryonic stem cell-derived human intestinal organoids. Biol Open 2015;4:1462–1472.
10. Schlieve CR, Fowler KL, Thornton M, Huang S, Hajjali I, Hou X, Grubbs B, Spence JR, Grikscheit TC. Neural crest cell implantation restores enteric nervous system function and alters the gastrointestinal transcriptome in human tissue-engineered small intestine. Stem Cell Reports 2017;9:883–896.
11. Stelzner M, Helmrath MA, Dunn JCY, Henning SJ, Houchen CW, Kuo C, Lynch J, Li L, Magness ST, Martin MG, Wong MH, Yu J; NIH Intestinal Stem Cell Consortium. A nomenclature for intestinal in vitro cultures. Am J Physiol Gastrointest Liver Physiol 2012;302:G1359–G1363.
12. Cruz-Acuña R, Quirós M, Farkas AE, Dedhia PH, Huang S, Siuda D, García-Hernández V, Miller AJ, Spence JR, Nusrat A, García AJ. Synthetic hydrogels for human intestinal organoid generation and colonic wound repair. Nat Cell Biol 2017;19:1326–1335.

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