

May 20, 2021

# From Cells to Organs – the Organoid Stepping-Stone

## Introduction

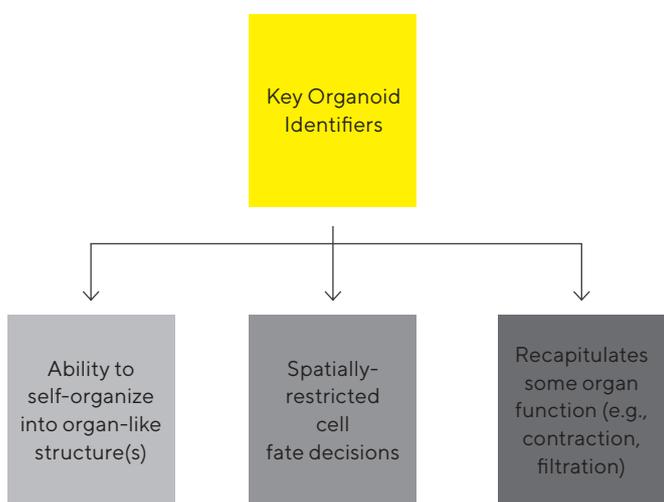
In recent years, biomedical research has pushed the limits of 2D cell culture to develop more advanced models for studying developmental biology and disease-modelling and also to identify more efficient tissue-replacement therapies. Of particular interest in these areas is tailored 3D cell culture approaches that give rise to organ-like structures known as “organoids”. In vitro organoid formation was first observed in epithelial cells that were embedded in extracellular matrix hydrogels. Anchored in the hydrogels, the cells self-organized into tissue structures composed of a heterogeneous cell population<sup>1,2</sup>. Further analysis revealed that the tissue structures and cell types present resembled what was typically seen with in vivo organ structures (e.g., kidney epithelial cells formed tubules). Pluripotent stem cells (PSCs), with their unique ability to differentiate and specialize into all the cell types of the body (referred to as pluripotency), have been singled out as the most promising starting cell type source for building organoids.

PSCs are characterized by the ability to asymmetrically divide into an identical daughter cell (known as self-renewal) or a more specialized progenitor cell that can further differentiate into a specialized tissue cell. Pluripotent stem cells include embryonic stem cells (ESCs) that are derived from the inner cell mass of a day-5 blastocyst and induced pluripotent stem cells (iPSCs) that arise from somatic cells genetically reprogrammed to a pluripotent stem cell state. With appropriate signal stimuli in 3D in vitro culture systems, pluripotent stem cells can readily develop into organoids.

Organoids are typically characterized by 3 main features (Fig. 1);<sup>3</sup>. The first is the ability of organoid cells to organize and self-assemble to produce a tissue organization highly similar to what is observed in vivo. This phenomenon is proposed to be driven by the unique combination of cellular adhesion proteins present on the surface of different cells. Here, cells with similar adhesive properties will sort out into thermodynamically stable domains<sup>4</sup>.

The second consistently observed organoid feature is spatially restricted cell fate decisions that encourage overall tissue organization.

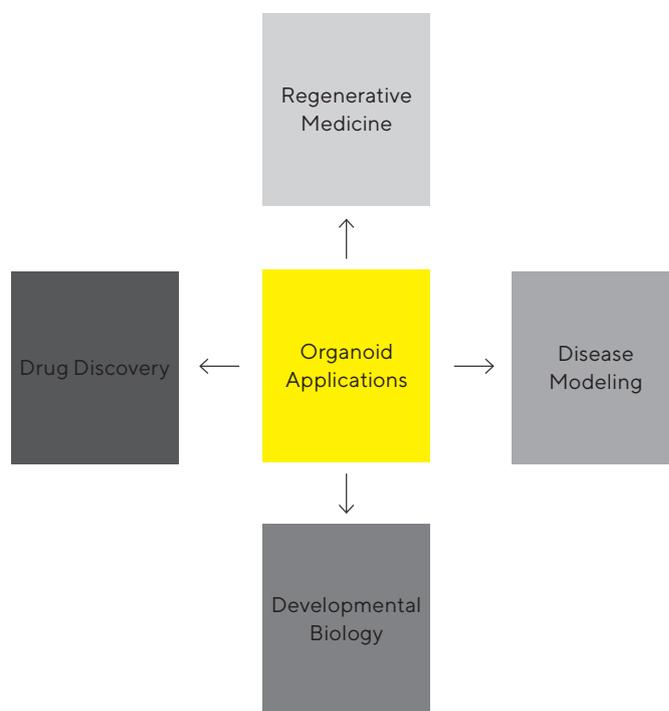
Tissue stratification within an organoid is driven by both the asymmetric division of PSCs and the migration of differentiated daughter cells to specific locations within the tissue. The final characteristic of organoids is the capacity to recapitulate some organ-specific function(s) in vitro, such as contraction for cardiac organoids or filtration for kidney organoids.



**Figure 1:** Organoids are defined by 3 key characteristics: self-organization, spatially-restricted cell fate decisions, and ability to perform at least one function observed in in vivo organ tissue.

## Current Applications: The Promise of Organoids

The advent of 3D culture approaches and organoids has altered the landscape of biomedical and developmental biology research (Fig. 2). Because traditional 2D monolayer cell culture approaches have a narrow capacity to recreate cell-cell and cellmatrix interactions, 2D cultures are limited in their ability to recapitulate organ tissue structure and behavior. These limitations are overcome by 3D cell culture methods that allow for in vitro recreation of the cellular environment and cellular interactions observed in vivo. 3D Organoid culture techniques include seeding cells onto presynthesized scaffolds in culture medium or spheroid formation with PSCs either by centrifugation or gravity. Each approach creates a mini autonomous environment similar to the tissue niche observed in vivo. In these microenvironments, cells can continue to differentiate and organize themselves into an organ-like structure in vitro. The resulting organoids allow researchers to answer questions and identify solutions to problems that have long been too technically challenging or time-consuming to answer using traditional 2D culture and/or animal models.



**Figure 2:** The proposed uses for organoids in research and biomedical applications span several different areas.

## Drug Discovery & Cytotoxicity Testing

Organoids in early drug discovery and cytotoxicity studies offers the opportunity to enhance study efficiency in a number of ways. First and foremost, human cell-derived organoids provide an opportunity to reduce the number of animals required for such studies. Moreover, liver metabolic processes have been well-documented to demonstrate inter-species variation that could lead to false-positives or false-negatives in animal-based evaluations<sup>5-6</sup>. Human organoids in place of animals during early evaluations could allow better biological relevance and swifter identification of drug functionality or toxicity for humans. This, in turn, can encourage higher drug screening throughput as promising drug candidates are identified earlier in the development process and unlikely drug candidates are dropped sooner. Human liver organoids in particular show great promise for drug discovery endeavors as they recapture the unique metabolic profile of the human liver<sup>7</sup>—the critical organ involved in drug metabolism. Other organoids, however, are also being pursued for drug discovery purposes. For instance, lung organoids have been successfully generated from patient-specific iPSCs using high-throughput 3D culture system approaches<sup>8</sup>. By combining scalable culture techniques and 3D cultures, Wilkinson et al. produced lung organoids that mimicked the architecture and cellular composition of the distal lung and could possibly be used to both quickly screen targeted lung disease therapies.

## Regenerative Medicine

Organoid-based approaches have been proposed for organ tissue transplants as a way to overcome the current challenge of finding appropriate donor matches and requiring lifelong anti-rejection medications to accommodate tissue transplants. A biopsy of cells from a patient could theoretically be genetically reprogrammed to produce patient-specific iPSCs that are then cultured to make organoid tissue for transplantation. Using patients' own cells to grow healthy, replacement tissue circumvents current immunomodulation and transplant rejection issues. Early studies in mouse models suggest the feasibility of this approach in repairing injured tissues<sup>9-10</sup>. Organoid tissue repair approaches have also been recommended for use in conjunction with the correction of genetic defects<sup>11-12</sup>. Here, genome-editing technology (such as CRISPR/Cas9) would be used to correct gene-related organ issues on patient iPSCs *ex vivo*. The gene-corrected cells would then be induced to form organoid tissue for subsequent transplantation.

## Disease Modeling

Patient-derived iPSCs also offer an avenue for building organoid models of tissue diseases, genetic disorders (e.g., retinitis pigmentosa), infectious diseases, degenerative diseases (e.g., fibrosis, cystic kidney disease), and cancer<sup>13-16</sup>. In this way, organoids may be constructed from iPSCs derived from patients with the disease or disorder under study. Numerous groups have developed tailored organoid generation protocols, aimed to produce specific disease models from dopaminergic neuron tissue for studying neurodegenerative disease<sup>17</sup> to renal carcinoma cancer models<sup>16</sup>. Disease-specific organoids can then be used to create disease-specific research models to uncover a better understanding of disease pathologies. Disease-specific organoid models could be used as an additional approach to evaluate potential treatments and therapies *in vitro* during the drug discovery process.

## Developmental Biology

Early studies of organoid development and function found that human PSC-derived organoids undergo molecular processes similar to *in vivo* organ development<sup>18-19</sup>. As with drug discovery endeavors, using human organoids in developmental biology studies offers a more biologically-relevant and timely alternative to animal models. Moreover, human organoids offer biological models that can help to answer developmental questions that are difficult to address with traditional animal models alone.

## Summary

Human PSC-derived organoids have a promising future in many research and biomedical endeavors—from drug discovery to developmental biology. Organoid formation capitalizes on PSC capacity to specialize into numerous cell types that comprise all the tissues of the body. With appropriate chemical, spatial, and physical stimuli, PSCs can be readily directed to form particular organoid types in 3D in vitro culture systems. The promise of PSCs in organoid formation, however, is critically rooted in PSC pluripotency and maintenance quality. Poor starting quality can negatively impact PSC differentiation capacity and the success of organoid formation. PSC quality is best maintained with a proven, high-quality serum-free and xeno-free PSC maintenance medium, such as NutriStem® (Fig. 3). High quality organoid research starts with quality cultures.

| Product                   | Cat.#     | Size  |
|---------------------------|-----------|-------|
| NutriStem® hPSC XF Medium | 05-100-1A | 500mL |



**Figure 3:** high-quality, reliable pluripotency maintenance medium like NutriStem® hPSC XF will ensure that appropriate PSC quality is preserved.

## Citations

1. Montesano, R., Schaller, G. & Orci, L. Induction of epithelial tubular morphogenesis in vitro by fibroblast-derived soluble factors. *Cell* 66, 697–711 (1991).
2. Hagios, C., Lochter, A. & Bissell, M. J. Tissue architecture: the ultimate regulator of epithelial function? *Philos Trans R Soc Lond B Biol Sci* 353, 857–870 (1998).
3. Lancaster, M. A. & Knoblich, J. A. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 345, 1247–1255 (2014).
4. Steinberg, M. S. in *Cellular Membranes in Development* (ed. Locke, M.) 321–366 (Academic Press, 1964).
5. Murphy, S. D. Liver Metabolism and Toxicity of Thiophosphate Insecticides in Mammalian, Avian and Piscine Species. *Proceedings of the Society for Experimental Biology and Medicine* 123, 392–398 (1966).
6. Dingell, J. V., Sulser, F. & Gillette, J. R. Species Differences in the Metabolism of Imipramine and Desmethyylimipramine (dmi). *J Pharmacol Exp Ther* 143, 14–22 (1964).
7. Takebe, T. et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 499, 481–484 (2013).
8. Wilkinson, D. C. et al. Development of a Three-Dimensional Bioengineering Technology to Generate Lung Tissue for Personalized Disease Modeling. *Stem Cells Transl Med* 6, 622–633 (2017).
9. Yui, S. et al. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5+ stem cell. *Nat. Med.* 18, 618–623 (2012).
10. Fordham, R. P. et al. Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury. *Cell Stem Cell* 13, 734–744 (2013).
11. Deng, W.-L. et al. Gene Correction Reverses Ciliopathy and Photoreceptor Loss in iPSC-Derived Retinal Organoids from Retinitis Pigmentosa Patients. *Stem Cell Reports* 10, 1267–1281 (2018).
12. Driehuis, E. & Clevers, H. CRISPR/Cas 9 genome editing and its applications in organoids. *Am. J. Physiol. Gastrointest. Liver Physiol.* 312, G257–G265 (2017).
13. Onuma, K. et al. Genetic reconstitution of tumorigenesis in primary intestinal cells. *Proc Natl Acad Sci U S A* 110, 11127–11132 (2013).
14. Dekkers, J. F. et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* 19, 939–945 (2013).
15. Bigorgne, A. E. et al. TTC7A mutations disrupt intestinal epithelial apicobasal polarity. *J. Clin. Invest.* 124, 328–337 (2014).
16. Maliszewska-Olejniczak, K., Brodaczevska, K. K., Bielecka, Z. F. & Czarnecka, A. M. Three-Dimensional Cell Culture Model Utilization in Renal Carcinoma Cancer Stem Cell Research. in *Epithelial Cell Culture* 47–66 (Humana Press, New York, NY, 2018). doi:10.1007/978-1-4939-8600-2\_6
17. Tieng, V. et al. Engineering of midbrain organoids containing long-lived dopaminergic neurons. *Stem Cells Dev.* 23, 1535–1547 (2014).
18. Lévy, E., Delvin, E., Ménard, D. & Beaulieu, J.-F. Functional development of human fetal gastrointestinal tract. *Methods Mol. Biol.* 550, 205–224 (2009).
19. Nakano, T. et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* 10, 771–785 (2012).

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