DOI: 10.1111/nyas.14874

CONCISE REPORT

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Organoids as tools for fundamental discovery and translation—a Keystone Symposia report

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Funding information
Michigan-Cambridge Collaboration Initiative; University of Michigan Mcubed Fund; 21st Century Jobs Trust Fund; State of Michigan, Grant/Award Number: CASE-315037; National Science Foundation, Grant/Award Numbers: I-Corps 2112458, CBET 1901718, CMMI 1917304; National Institutes of Health, Grant/Award Numbers: R21 HD100931, R21 NS113518

Abstract

Complex three-dimensional *in vitro* organ-like models, or organoids, offer a unique biological tool with distinct advantages over two-dimensional cell culture systems, which can be too simplistic, and animal models, which can be too complex and may fail to recapitulate human physiology and pathology. Significant progress has been made in driving stem cells to differentiate into different organoid types, though several challenges remain. For example, many organoid models suffer from high heterogeneity, and it can be difficult to fully incorporate the complexity of *in vivo* tissue and organ development to faithfully reproduce human biology. Successfully addressing such limitations would increase the viability of organoids as models for drug development and preclinical testing. On April 3–6, 2022, experts in organoid development and biology convened at the Keystone Symposium “Organoids as Tools for Fundamental Discovery and Translation” to discuss recent advances and insights from this relatively new model system into human development and disease.

KEYWORDS
development, differentiation, inflammatory bowel disease, kidney disease, microfluidics, organoids, single-cell sequencing

INTRODUCTION

Organoids are three-dimensional *in vitro* organ-like models generated from pluripotent stem cells (PSCs) or primary donor tissues. Protocols have been developed to drive the differentiation of PSCs toward multiple different organoid systems that recapitulate the structural, molecular, and functional characteristics of *in vivo* tissues and organs, including those associated with the gastrointestinal tract, central nervous system, liver, kidney, and skeletal muscle. These organs-in-a-dish hold immense potential as a model for human development and disease. They offer more complexity than traditional two-dimensional cell culture systems while being easier to control, manipulate, and quantify than *in vivo* models. Regarding development, organoids are more accessible than developing embryos. Human organoids may also recapitulate human biology more faithfully than animal models. That said, it can be difficult to reproducibly generate organoids and to incorporate the complexity so important for the proper function of native tissues and organs. Organoids often lack characteristic cellular organization or important organ-supportive tissues, for example, immune, vasculature, lymphatic system, stroma, and innervation tissues.

On April 3–6, 2022, experts in organoid development met for the Keystone Symposium “Organoids as Tools for Fundamental Discovery and Translation” to discuss recent advances and insights using organoid systems to study human development and disease. This was a joint meeting held concurrently with “Engineering Multi-Cellular Living Systems” (for a report from speakers for this meeting, see DOI: 10.1111/nyas.14896).

Speakers discussed efforts to benchmark organoid systems against their *in vivo* counterparts, to control organoid development via bioengineering approaches, and to increase organoid complexity and enhance their organization and functions by incorporating multiple cells and tissue types. They also highlighted insights organoids have provided on human development and disease, including identifying possible drug targets for various diseases.

ENGINEERING EPITHELIAL ORGANOIDS

Matthias P. Lutolf from Roche Institute for Translational Bioengineering and Ecole Polytechnique Fédérale de Lausanne presented work on using advanced bioengineering to generate organoids that more closely resemble *in vivo* structures, with a focus on intestinal epithelial organoids. Pioneering work in Hans Clevers’s lab at the Hubrecht Institute showed that the crypt–villus structure of the mouse intestinal epithelium could be recapitulated *in vitro* from even a single stem cell. However, the resulting organoids were heterogeneous in size, shape, and cellular composition,[1] which is a common problem with most conventional organoids. Using bioengineering approaches, Lutolf’s group showed that confining these organoids in hydrogel microcavities can reproducibly generate organoids of a predefined shape and size; the organoids also show the characteristic cell type patterning seen in the intestinal epithelium. Lutolf explained that the geometry of the crypt creates differential cell crowding and cell shape that lead to differences in YAP and NOTCH signaling and the spatially controlled emergence of

Paneth cells, the signaling source for the crypt domain.[2] Lutolf's group is now using this principle to create macroscopic tissues that faithfully recapitulate features of the native tissue. The group has developed crypt–villus substrates that mimic the intestine, generating tissues that incorporate both cell patterning and the macroscopic crypt–villus structures of the intestine. They are using these organoids to study tissue-dynamic processes like cell shedding that occurs at the tip of the villus.

Lutolf also described work to generate tubular perfusable mouse organoids on biomicrofluidic chips. The system allows dead cells to be continually removed, creating a homeostatic culture condition in which organoids can be maintained for months. Fluid can be delivered to the apical side of the lumen to model processes like drug delivery, bacteria colonization, or viral infection. These "mini-gut" tubes possess several qualities of natural tissue that are not often seen in classical organoids, including rare cell types and the capacity to regenerate after injury.[3] Lutolf's group is now working on building reliable human epithelial organoids of the intestine, bladder, respiratory tract, and other tissues to model disease as well as increasing the complexity of the organoids by incorporating other tissue compartments, like vasculature and immune cells. Lutolf also presented unpublished data on using mouse colon organoids to better understand tumorigenesis and the potential for this tumor-on-a-chip to delineate the spatiotemporal dynamics involved in tumorigenesis as well as the impact of genetic and environmental factors.

EMBRYOIDS AND GASTRULOIDS FOR EARLY DEVELOPMENT

Understanding postimplantation with synthetic human embryo-like structures

While preimplantation embryonic development has been relatively well characterized, it is more difficult to study postimplantation development because implanted embryos are difficult to observe or manipulate *in vivo*.

Jianping Fu from the University of Michigan discussed his work using human PSCs (hPSC) to model peri-implantation human development. In collaboration with Deb Gumucio at the University of Michigan, Fu's group showed that when grown in 3D culture, a subset of hPSC colonies organize and develop into 3D structures that mimic early postimplantation human developmental events, including tissue morphogenesis, lineage diversification, and tissue–tissue interaction. Some colonies even undergo symmetry breaking and gastrulation-related events. The gastrulating cells upregulated canonical markers for gastrulation. Fu's group used this system to identify the molecular pathways involved in these early developmental events and cell fate decisions, identifying BMP activity as a key pathway driving amniogenesis from pluripotent hPSCs.[4,5]

Fu's group has also developed a microfluidic model to investigate amnion development in early human development. They showed that hPSCs loaded into this microfluidic system display synchronous development and undergo lumenogenesis, forming an epiblast-like sac. Exogenous signals can be introduced into the microfluidic device to establish asymmetric chemical stimulation. Fu showed, for example, that asymmetric BMP stimulation induced embryonic-like sac formation—amnion-like cells developed at the pole directly exposed to BMP4stimulation, while the opposite pole remained pluripotent. Continuous development of the embryonic-like sac led to gastrulation-related events at the pluripotent pole. They also looked at the emergence of primordial germ cells in the model, as there has been some controversy in the literature over where they develop. In addition, expression of BRACHYURY, a key gene marking the onset of gastrulation, at the junction of the amniotic ectoderm and epiblast compartments suggested an important role for tissue–tissue interactions. Coculture of amnion and epiblast cells revealed that the amnion induces gastrulation via noncanonical WNT secretion. Fu's group is currently investigating other molecular players and pathways involved.[6] His group is also characterizing their embryoids using single-cell sequencing methods and comparing the embryoids to both human and nonhuman primate embryos.

OCTOPUS: Engineering organoid cultures to enhance organogenesis

Sunghee Estelle Park from Dongeun Huh's lab at the University of Pennsylvania presented work on a new platform called organoid culture-based three-dimensional organogenesis platform with unrestricted supply of soluble signals (OCTOPUS). Huh's lab focuses on developing microengineered cell culture devices to grow human organs on a chip.[7] They have previously established models of human muscle,[8] eye,[9] and placenta.[10] More recently, the OCTOPUS platform was designed to increase the lifespan and maturity of organoids beyond what can be achieved with traditional Matrigel drop culture methods. In the OCTOPUS platform, stem cells are equally distributed between eight culture chambers, though different chamber designs and sizes are available. Nutrients diffuse freely throughout the culture chambers, obviating the necrotic core that drop-based cultures develop. Park showed that intestinal enteroids grown in the OCTOPUS system were highly viable after 14 days of culture and could grow up to 2–3 mm in size. By comparison, Matrigel-drop enteroids showed a significant decrease in viability at 14 days. Single-cell RNAseq showed that the cellular diversity of OCTOPUS-grown enteroids more closely resembled *in vivo* tissue than Matrigel-grown enteroids. Park also described how they are using OCTOPUS-developed enteroids to model disease, specifically inflammatory bowel disease (IBD). Enteroids grown from intestinal stem cells from IBD patients are smaller than those grown from normal cells, and they have markers of decreased cell proliferation, increased apoptosis, and impaired tight junction formation. Single-cell RNAseq of the IBD enteroids confirmed the upregulation of several IBD genes. Coculture of human enteroids with blood vessels produced vascularized organoids that enabled Park to assess vascular abnormalities in IBD and their ability to recruit immune cells. These data provide proof of concept that OCTOPUS can serve as *in vitro* platform to engineer

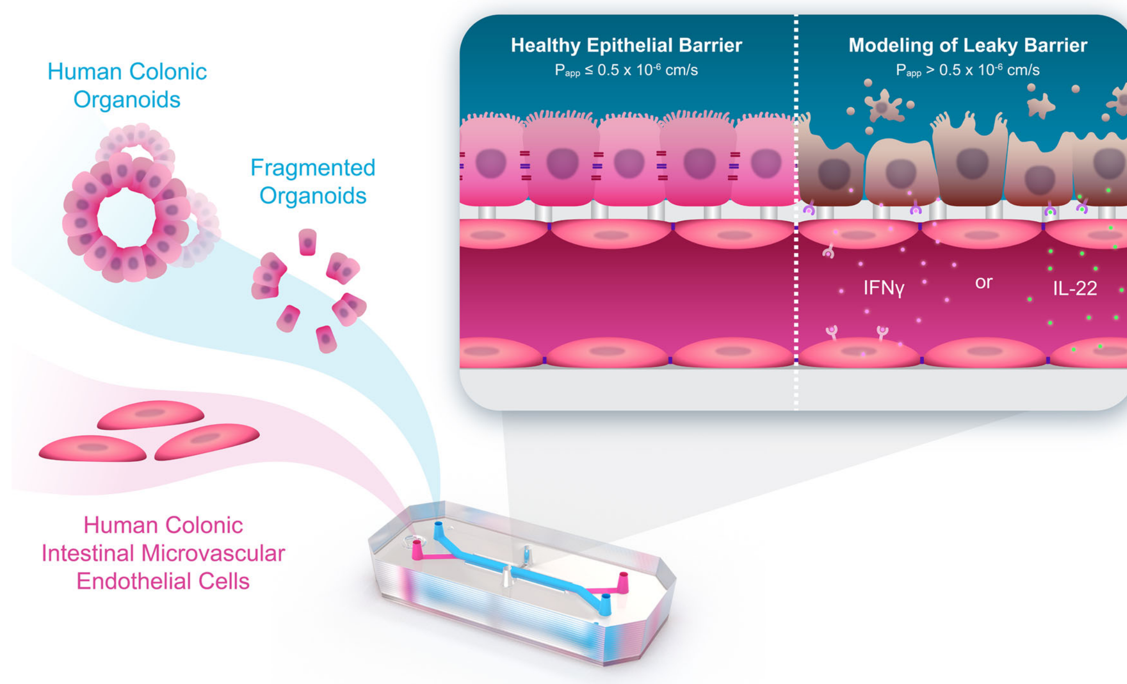

FIGURE 1 Colon Intestine-Chip, seeded with human colon crypt–derived epithelial and primary microvascular endothelial cells, used to investigate leaky gut. This *ex vivo* platform recapitulates the effects of proinflammatory cytokines in the intestinal epithelial barrier and identified novel mechanisms of action. From Apostolou *et al.* (2021) DOI: https://doi.org/10.1016/j.jcmgh.2021.07.004. By CC BY-NC-ND 4.0. license.

vascularized organoids and expand the capabilities of conventional organoid culture systems.

HIGH CONTENT SCREENING WITH ORGANOIDS

Developing an organ-on-a-chip for drug discovery

Athanasia Apostolou from Emulate Inc. described the company's work to generate *ex vivo* experimental models that better predict human physiology and could improve the effectiveness of the drug discovery pipeline. The Human Emulation System® developed by Emulate is a complete organ-on-a-chip system that enables an automated fluidic culture and incorporates *in vivo* relevant mechanical forces. Apostolou described the use of the Colon Intestine-Chip to model mechanisms that elicit the collapse of the intestinal epithelial barrier integrity in pathophysiological conditions, such as IBD. Emulate has developed Intestine-Chips from several cell sources, including Caco2 cells, human tissue biopsies, and iPSC-derived organoids.[11–13] Apostolou showed that the transcriptome profiles of both Duodenum- and Colon-Chips grown in their system are more similar to human tissue than to organoids grown in suspension culture.[12,14] During her talk, Apostolou focused on the phenotypic and functional validation of the Colon Intestine-Chip, developed from human biopsy-derived colonoids cocultured with endothelium cells. She showed that coculture of endothelial and epithelial cells recreated human colon physiology more closely than do classical organoids, with enhanced formation of a tight junction network and epithelial polarity (Figure 1). Applying mechanical force to the Colon-Chips altered transcription, increasing the expression of genes involved in ion, lipoprotein, and water transportation.[15] Apostolou also showed how the Colon Intestine-Chip responds to factors known to play a role in IBD while providing additional insights into their effects on the colon. The addition of IFN-γ, a cytokine that disrupts the epithelial barrier,[16] resulted in a time-dependent collapse of the epithelial barrier, compromised epithelial morphology, disruption of tight junctions, and restructuring of the actin cytoskeleton. The Colon Intestine-Chip also recapitulated the polarized secretion of cytokines as well as interindividual variability observed in the clinic. The addition of IL-22, which has been implicated in the pathogenesis of IBD and is generally thought to support the epithelial barrier,[17–19] showed, as anticipated, that IL-22 induced STAT3 activation in a concentration- and time-dependent manner. However, unexpectedly, IL-22 compromised epithelial morphology and induced apoptosis.[15] These data show the importance of using more *in vivo*-like and species-specific experimental models to understand human diseases.

A multiorganoid platform to study SARS-CoV-2 infection and drug screening

While the lung is a major target for SARS-CoV-2, the virus has effects on multiple organs. **Shuibing Chen** from Weill Cornell Medical College discussed work using organoids to better understand SARS-CoV-2 infection and to screen for drugs that inhibit infection. Chen and colleagues are leveraging multiple cells and organoid models derived from hPSCs, including macrophages, cardiomyocytes, endothelial cells,

alveolar organoids, airway organoids, small intestine organoids, colon organoids, pancreatic endocrine organoids, liver organoids, and neurons. Chen showed that a SARS-CoV-2 pseudovirus was able to infect various cell types known to be susceptible to infection, such as colon, lung, and liver, as well as additional cell types, including cardiomyocytes, pancreatic endocrine cells, and dopaminergic neurons.[20] Single-cell RNAseq of infected cells showed that infection affects different cell types differently. For example, infection induces pacemaker cells to undergo ferroptosis; cardiomyocytes and alveolar and airway organoids show signs of apoptosis; while pancreatic endocrine cells undergo transdifferentiation.[21–23]

In addition, Chen's group, in collaboration with David Ho at Columbia University and Ben tenOever at New York University, has used these organoid models to screen for compounds that inhibit SARS-CoV-2 infection. In a high-throughput chemical screen in which alveolar organoids were infected with a SARS-CoV-2 pseudovirus, three small molecules were identified to block viral entry. Chen showed that these molecules also inhibited live SARS-CoV-2 infection in a humanized mouse model in which human alveolar organoids are xenografted into mice. This system provides an *in vivo* model with which to evaluate drug activity on human cells.[21] In a separate drug screen using airway organoids and authentic SARS-CoV-2 virus, the compound GW6471 was shown to block viral replication. Mechanistic studies indicate that GW6471 blocks the HIF1α pathway, which inhibits downstream fatty acid synthesis. Small molecules that block fatty acid synthesis inhibited viral replication in the organoid model. These data demonstrate the importance of the HIF1α–glycolysis axis in mediating SARS-CoV-2 infection in the human airway.[22]

Chen's group has also used these organoid models to understand immune-mediated host damage during SARS-CoV-2 infection. In a coculture of cardiomyocytes and macrophages, SARS-CoV-2 infection resulted in an increase in reactive oxygen species and apoptotic cells, which was not seen without macrophages. Additional data and mechanistic studies support a model in which SARS-CoV-2 infection of cardiomyocytes induces the secretion of the cytokine CCL-2, which recruits monocytes to the site of infection. The monocytes differentiate into macrophages that secrete proinflammatory cytokines IL-6 and TNFα, which can damage cardiomyocytes. A high-throughput drug screen showed that a JAK inhibitor that blocks downstream events in TNFα signaling can block macrophage-mediated cardiac damage.[24,25]

A mini-kidney organoid to model genetic disease

Cheng Jack Song from Andrew McMahon's group at the University of Southern California presented work on developing mini-kidney organoids to model the genetic disease autosomal dominant polycystic kidney disease (ADPKD). While various protocols have been developed to generate kidney organoids, creating a unified, quantifiable system has been challenging.[26–31] Song has developed a protocol to create more unified mini-kidney organoids from hPSCs.[32] Single-cell RNAseq shows that these mini organoids differentiate like other kidney organoids and human fetal kidneys. Knocking out the genes responsible for ADPKD in the mini organoids recapitulates the cyst formation indicative of the disease. Song has used this model to screen for small molecules that prevent cyst formation. During the talk, he focused on two compounds: celastrol, which had previously been shown to inhibit cyst formation in *in vitro* and in *vivo models*, and QNZ, a novel compound that potently blocks cyst formation in a dose-dependent manner. While high amounts of QNZ negatively impact nephrogenesis, it appears that there may be a therapeutic window in which cyst formation is blocked without kidney toxicity. Song is using the model to understand the mechanism of ADPKD and other kidney diseases.

INCREASING COMPLEXITY IN ORGANOIDS BY LEVERAGING DEVELOPMENT

Increasing organoid complexity is a key challenge that, as it is overcome, will improve the utility of these systems to model disease. Several speakers spoke about their work to leverage insights into human development to increase organoid complexity, primarily by understanding and increasing cell diversity.

Using organoids to identify and characterize new cell types

Jason R. Spence from the University of Michigan described unpublished work on characterizing a newly identified secretory cell in the fetal human lung. Spence's group is broadly interested in translational embryology, that is, leveraging insights from developmental biology to differentiate PSCs toward certain lineages. Spence's group has observed that iPSCs sometimes give rise to previously unknown cell types, underscoring the need for a comprehensive single-cell reference atlas of developing human tissues. To address this gap, Spence collaborated with J. Gray Camp at the University of Basel and Barbara Treutlein at ETH Zurich to characterize the lung epithelial cell types present in the developing human lung over time and space[33] and to generate an endoderm atlas of multiple organ systems for benchmarking organoid models.[34] The scRNAseq data of lung epithelial cells revealed a previously unidentified secretory progenitor cell type, fetal airway secretory (FAS) cells. Fairly abundant within the fetal lung, FAS cells are enriched in the middle airway at 15–18 weeks post conception and display a unique molecular profile.[33] Spence showed unpublished data using organoid models to understand the function of FAS cells. This work helps to demonstrate how organoids can be used to identify new cell types that are relevant *in vivo*.

Understanding symmetry breaking in intestinal organoids

Prisca Liberali from the Friedrich Miescher Institute discussed efforts to understand how symmetry breaking is achieved during intestinal

organoid development. Liberali's group is broadly interested in how genetically identical cells coordinate across short- and long-range scales to generate multicellular systems and structures. Liberali's group is using mouse intestinal organoids as a model system. Regarding symmetry breaking, Liberali put forth a model in which genetically identical pluripotent cells within a population must inherently acquire meta-stable, transient cellular states that "interpret" and respond to their environment differently. Some cells, therefore, have a higher probability to irreversibly differentiate, leading to symmetry breaking.[35,36] Multiplexed time-course imaging of mouse intestinal organoids showed that organoid formation is very plastic—both Lgr5$^+$ cells (representing the stem cell population) and Lgr5$^-$ cells form organoids via similar development pathways. In addition, some organoids develop without breaking symmetry—these consist entirely of enterocytes and do not contain the Paneth cells found in the intestinal crypt. Liberali found that organoid formation depends on transient activation of the transcription factor Yap, and that cell-to-cell variability in Yap1 expression is required for symmetry breaking.[37]

Newer work on the function of Yap1 showed that tissue geometry can affect Yap1 signaling. Specifically, symmetry breaking occurs at areas of higher curvature and higher polarity. Single-cell RNAseq conducted at different timepoints during the early stages of symmetry breaking revealed decreased Stretch genes, increased ERK and WNT target genes, and glycolytic activity. These data indicate that symmetry breaking requires the emergence of cell states that differ in their mechanical and polarization state, signaling state, and metabolic profile. Liberali put forth a model in which symmetry breaking occurs during a select time window, between 16 and 32 cells, when the cell population contains the proper distribution of these cell states.[2]

The impact of tissue architecture on brain organoid development

Madeline Lancaster from the Medical Research Council Laboratory of Molecular Biology showed how her lab is using organoids to understand human brain development. Using brain organoids from humans and apes, Lancaster's group elucidated the mechanisms that drive brain size. Compared with ape brain organoids, human brain organoids display delayed neuroepithelial transitions that result in slower elongation of neuroepithelial cells, which correlates with a short cell cycle length. In other words, in human brain organoids, neuroepithelial cells divide more, giving rise to more neurons and thus bigger brains. Because these events happen early in development, they impact all the cortical neuron layers evenly, which is consistent with morphological differences between human and ape brains.[38]

Other studies Lancaster discussed focus on the impact of sex steroids on neurogenesis. Lancaster showed that androgens increase the proliferation of radial glial progenitors and increase the neurogenetic potential of excitatory neurons, but not inhibitory progenitors. These findings may have implications for neurodevelopmental conditions like autism spectrum disorder (ASD), which is more prevalent in males and shows differences in the balance and maturity of excitatory and inhibitory neurons.[39]

Lancaster also presented unpublished work on the impact of tissue architecture on brain organoid development. Previous work suggests that fate determination in neurogenesis is not dependent on tissue architecture and that developmental programs are at least partially hard-wired.[40,41] More recent studies using scRNAseq showed that brain organoids more closely recapitulate the *in vivo* transcriptome profile than do cells grown in 2D culture, suggesting a role for tissue architecture and geometry.[42] Other studies are mixed on whether organoids are more similar to *in vivo* models than 2D culture.[43,44] Lancaster's group is working to reconcile these contradictory findings to better understand how tissue architecture affects fate determination and temporal development during brain development.

Generating epicardioids to understand heart development and disease

Anna B. Meier from the Technical University of Munich presented work on creating human heart organoid models that incorporate both the epicardium and myocardium. The heart has proven relatively recalcitrant to the development of self-organizing organoids. It is still not possible to generate organoids that spontaneously form all three layers of the heart—the epicardium, myocardium, and endocardium. Researchers have developed protocols to create cardiogenic gastruloids that imitate gastrulating embryos,[45] multilineage organoids that form more mature tissues,[46,47] and cardioids that contain the myocardium and endocardium.[48] Despite this progress, incorporating the epicardium into these models has been difficult. Meier noted that the epicardium plays an important role in the developing embryo—where epicardial cells give rise to multiple cardiac lineages—and promotes myocardial development and repair. In species that can regenerate the adult heart, like zebrafish, the epicardium plays an important role in regenerating the myocardium.[49] Understanding the cross-talk between the epicardium and myocardium could, therefore, have significant implications in mitigating the effects of heart disease. Meier presented unpublished work on developing self-organizing organoids of the myocardium and epicardium, with the aim of using these epicardioids to understand how epicardial cells give rise to different cardiac cell lineages during development, the crosstalk between the myocardium and epicardium, and how to model heart disease.

An organoid model to study alveologenesis

Nicole Min Qian Pek from Mingxia Gu's lab at the University of Cincinnati presented work on using blood vessel organoids to understand how the pulmonary vasculature influences alveologenesis. Disrupted alveologenesis underlies a group of congenital lung diseases, including alveolar capillary dysplasia (ACD). ACD is associated with malposition of the pulmonary vein; most patients die during the first few weeks

of life due to respiratory failure. Mutations in the gene *FOXF1* and the surrounding chromosome have been associated with ACD.[50,51] Pek described unpublished work being done in collaboration with Darrell Kotton at Boston University and Robbert Rottier at Erasmus University Medical Center to generate blood vessel organoids generated from ACD patient iPSCs that contain three unique mutations in *FOXF1*. They plan to use these models to understand the impact of *FOXF1* mutation on vascular development and its role in alveolar/capillary defects in patients with ACD.

IMPROVEMENTS IN ORGANOID MATURATION

Adding complexity via codevelopment and separate development

James M. Wells from Cincinnati Children's Hospital Medical Center discussed two approaches his lab is taking to introduce more complexity into organoids—by codeveloping different cell types within an organoid and by combining separately generated progenitor populations. Wells is ultimately interested in using organoids to understand more complex organ-related functions and crosstalk. In the first example, which is unpublished, Wells focused on work to characterize the cell types of a PSC-derived human colonic organoid model developed in his lab after observing a population of codeveloping immune cells.[52] In the second example, Wells showed how they have generated functional gastric organoids by combining the three progenitor cell populations[53]—the endoderm, which forms the epithelium, glands, and endocrine cells; the mesoderm, which forms vascular, smooth muscle, and immune cells; and the ectoderm, which forms the enteric nervous system. In this process, hPSCs are separately differentiated into ectoderm, endoderm, and mesoderm. Using previously established protocols, endoderm cells are differentiated into foregut epithelium, and ectoderm cells are differentiated into neural crest stem cells.[54,55] Wells's collaborators at Cincinnati Children's devised a new protocol to differentiate mesoderm cells into splanchnic mesenchyme via signaling pathways identified by Han *et al.*, who developed a single-cell atlas of the developing mouse foregut.[56] Combining these three cell types led to the self-assembly of gastric organoids with the expected cell diversity, morphology, and function.[53]

A suspension-based culture system for human intestinal organoids

Meghan M. Capeling from Jason Spence's lab at the University of Michigan presented work on developing a suspension-based culture system for intestinal organoids. Typically, hPSC-derived human intestinal organoids are embedded in a Matrigel matrix; these organoids often suffer from limited reproducibility, are expensive, and are generally immature—they do not recapitulate the full cell diversity seen *in vivo* and must be transplanted into mice to give rise to more mature intestinal tissue.[57,58] Alternative, Matrigel-free culture systems could offer greater experimental control and better recapitulate the diversity seen *in vivo*.[59,60] Capeling showed that human intestinal organoids can be grown in suspension culture on a similar timescale to Matrigel-based organoids as a cheaper, simpler, and more scalable alternative without the poorly defined composition of Matrigel. Since the organoids contain both an epithelium and mesenchyme, they form their own niche without any external cues from Matrigel. Suspension-grown organoids contained an epithelium comparable to Matrigel-grown organoids with the expected epithelial cell types, proper polarization, and mature intestinal cell types like enterocytes and goblet cells. One key difference between the two organoid types was the mesenchymal layer. In Matrigel-grown organoids, the mesenchyme is disorganized and lacks the serosal mesothelium, the outermost layer, which contributes to mesenchyme and vascular smooth muscle development and is implicated in adhesions that form after abdominal surgery. In contrast, the mesenchyme in suspension-grown organoids was better organized, more closely resembled the circular architecture of human tissue, and formed a defined outer serosal mesothelium. An scRNAseq analysis revealed molecular similarities between suspension-grown intestinal organoid serosa and human fetal serosa. Capeling is using these organoids as a model to understand how the serosa forms, which has been previously difficult to address. Using small molecule inhibitors that target different signaling pathways involved in intestinal development, she has identified Hedgehog and WNT signaling as important regulators of serosa development.[61]

Understanding cortical cell diversity

Ana Uzquiano from Paola Arlotta's lab at Harvard University presented work using human cortical organoids to understand how cell diversity emerged during cortical development. Uzquiano has used the cortical organoids to build a single-cell resolution map of human cortical development; the map consists of both transcriptional and chromatin accessibility information of more than 600,000 cells from 83 organoids representing eight timepoints from 23 days to 6 months of development. Uzquiano showed that organoids reproducibly recapitulated the processes of cell diversification of the developing human cortex;[62] data defining the lineage relationships and longitudinal molecular trajectories of cortical cell types during development in organoids; and that cell diversification in the organoids correlated with human fetal datasets.[62–64] Uzquiano showed that the identity of the majority of cortical cell types present in this cortical organoid model is not affected by diverging metabolic states, which affects only two cell types, one of which does not have a fetal counterpart ("unspecified projection neurons"). These two cell types localize to the inner region of the organoid and display an altered metabolic profile characterized by upregulation of glycolysis and hypoxia-related pathways. With the exception of these cell types, cell identity was not affected by the metabolic state. Finally, by inferring the molecular trajectories associated with human cortical lineages and comparing this information to data from the developing mouse cortex,[65] Uzquiano identified novel regulators of neurogenesis and cell identity acquisition in humans.[66]

ORGANOIDS FOR DISEASE MODELING

Integrating stem cell and organ chip technologies to model and understand human kidney disease

Samira Musah from Duke University presented work on developing stem cell-derived organ chips as *in vitro* platforms to model human diseases and for therapeutic drug development. Mush described models that incorporate molecular and mechanical forces, depending on the organ of interest, to mimic the *in vivo* environment as faithfully as possible.[66,67] She stressed that while they are very early in terms of being able to model organs, they believe that *in vitro* platforms will be able to model disease in a patient-specific manner.

Musah's group has developed several methodologies to promote pluripotency *in vitro* using synthetic matrices,[68,69] as well as to drive cell fate commitment to different lineages, including neuronal cells,[70] endothelial cells,[70] and kidney epithelial cells.[72–74] During her talk, Musah focused on their efforts in developing a glomerulus-on-a-chip. The filtering unit of the kidney, the glomerulus is often the site of damage in patients with kidney disease and drug-related kidney toxicity. The absence of physiologically relevant models has made it difficult to identify biomarkers and drug targets for kidney disease; consequently, there are still no targeted therapies in this area despite its prevalence.

Musah's group developed a novel method to direct the differentiation of kidney podocytes from human iPSCs. They incorporated these cells into a microfluidic device that recapitulates the structure of the glomerulus. Musah showed that this glomerulus-on-a-chip mimics the tissue–tissue structure of the glomerulus, with iPS-derived podocytes and a glomerular endothelium. The system can filter out small molecules while retaining larger proteins—similar to the kidney—and is susceptible to genetic and exogenous signals that mimic disease. The system can also mimic different stages of development by introducing mechanical strain, that is, adding strain resulted in tissue that more closely resembles the tissue–tissue interface of the intact glomerulus, while lack of strain produced a tissue structure that mimics earlier stages of development.[72] Musah showed how her group has used this platform to demonstrate that SARS-CoV-2 can directly infect podocytes,[75] as well as to identify possible therapeutic targets for kidney disease.[76] In addition to these efforts, her group is working to use the platform for drug screening.

Developing organoids to investigate mechanisms of liver regeneration and disease

Meritxell Huch from the Max Planck Institute of Molecular Cell Biology and Genetics presented work on liver organoids derived from adult tissue. For the past 10 years, the Huch lab has been developing organoids from healthy and diseased, human and mouse, and adult and embryonic tissues for a range of organs, including stomach,[77] liver,[78] and pancreas.[79] The work of the Huch lab has shown how adult liver organoids can be used to investigate mechanisms of liver regeneration and cancer at different biological scales, from molecules to cells and tissues. In the first part of her talk, Huch focused on molecular mechanisms of cellular plasticity during regeneration and cancer. The group had shown that liver organoids recapitulate many aspects of liver regeneration in a dish.[80] In her talk, Huch showed that *in vivo* and in mouse models, both liver organoid formation and liver regeneration require transient, genome-wide transcriptional and epigenetic reprogramming to switch on regenerative programs. These transcriptional and epigenetic changes enable the cellular plasticity required to license liver differentiated cells for organoid formation and *in vivo* regeneration. Huch also showed preliminary data on how the lab is transferring this knowledge to study cellular plasticity in cancer using patient-derived liver cancer organoids the lab had previously established in 2017.[81]

In the second part of her talk, Huch elaborated on how a novel organoid coculture system her lab has developed has enabled the study of cellular mechanisms of liver regeneration. She focused on a mesenchymal population that resides near the ductal epithelium. She first showed that the numbers between both populations dynamically change during the damage-repair response *in vivo*. To investigate whether dynamic changes in these cellular interactions could regulate the repair response, the group developed a coculture system of ductal epithelial and portal mesenchymal cells by microencapsulating both cell types to facilitate cellular interactions. Huch showed that the tissue architecture and direct cell–cell interactions between both populations can be recapitulated *in vitro* in this novel coculture system (Figure 2).[82] Interestingly, this organoid coculture system was crucial to underscore a very interesting paradox (that would otherwise had remained unnoticed) that mesenchymal cell contact inhibits epithelial cell proliferation while paracrine signaling promotes it. Notably, nonphysiological numbers of mesenchymal cells resulted in the loss of ductal epithelial integrity and collapse of the epithelial organoid structure. This effect could not be rescued by supplementing the cultures with growth factors, indicating that cell contact inhibition is dominant over growth factor presence. These organoid-based observations could potentially reconcile the dichotomy between a homeostatic/proquiescent niche and a proliferating/proregenerative niche, suggesting the number of cellular interactions, not the total number of cells, is the critical parameter during tissue regeneration and, consequently, during organoid formation. Overall, Huch provided an overview of how adult tissue-derived organoid cultures represent excellent *in vitro* models to gain a mechanistic understanding of basic biological principles of tissue regeneration and cancer across different biological scales.

Developing a model of the neuromuscular system

Mina Gouti from the Max Delbruck Center for Molecular Medicine presented work on developing human neuromuscular organoids (NMOs) from PSCs. Insights from developmental biology show that the

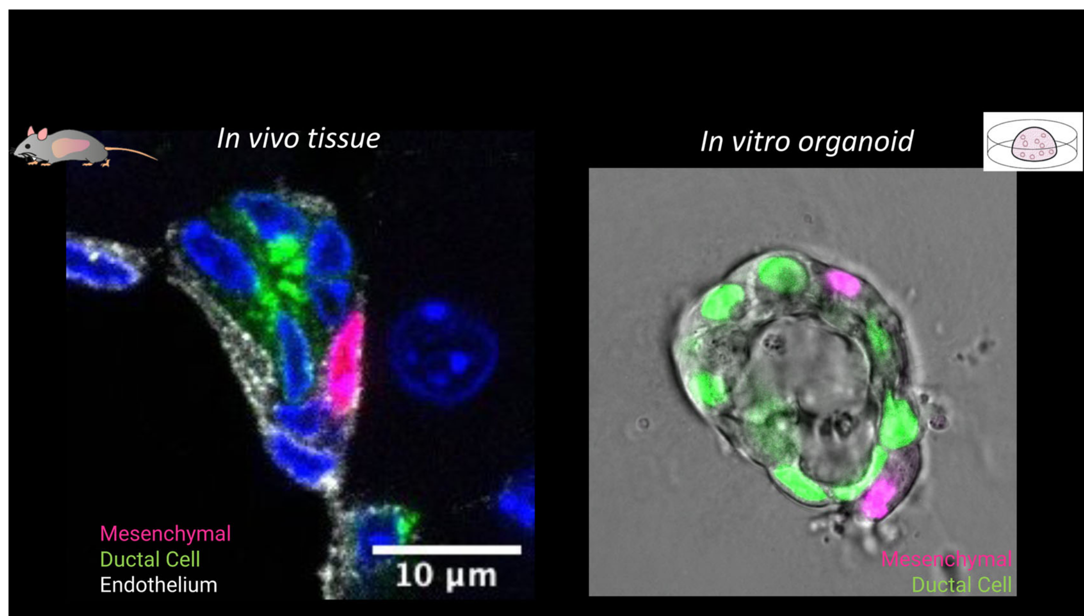

FIGURE 2 Tissue architecture and direct cell–cell interactions between ductal epithelial and portal mesenchymal cells can be recapitulated *in vitro* in a novel coculture system.

spinal cord motor neurons that enervate skeletal muscle express different types of *HOX* genes based on where they are found in the body. Gouti's work has been instrumental in defining the developmental origins of the posterior and anterior neuromuscular systems. In brief, the anterior and posterior regions have distinct developmental origins. Anterior neuroprogenitors generate the brain and hindbrain, while neuromesodermal progenitors (NMPs) generate spinal neurons and the posterior somites that generate the musculoskeletal system.[83–85] Therefore, to study the development of the neuromuscular system, it is necessary to understand the development and differentiation of NMPs.

Gouti has previously developed a protocol to generate mouse and human NMPs from PSCs *in vitro*.[83] Gouti's group has pioneered the generation of human NMOs from NMPs, which are the building blocks of the posterior neuromuscular system. When grown in a nonadherent cell culture system, NMPs generate both spinal cord and muscle tissues that self-organize into two distinct regions within the organoid (Figure 3).[86,87] Single-cell sequencing of the NMOs revealed two primary differentiation trajectories—a neural lineage and skeletal muscle lineage. After approximately 2 months of culture, the organoids consist of skeletal muscle cells surrounded by spinal cord neurons and contain other mature cell types and features (such as glia, interneurons, myelinated axons, and terminal Schwann cells) and functional neuromuscular junctions.[87] Gouti's group has successfully cultured these NMOs for over 2 years and is now using them to model different neuromuscular diseases. For example, the addition of autoantibodies derived from patients with myasthenia gravis, an autoimmune disease in which autoantibodies destroy acetylcholine receptors, reduced the number of neuromuscular junctions and affected contractions in the organoid model.[87] Her group is also using these NMOs to model ALS and SMA. Gouti showed preliminary data on the effect of mutations responsible for SMA on NMO function and structure. Ultimately, Gouti plans to use these organoids as a platform for drug screening for these diseases.

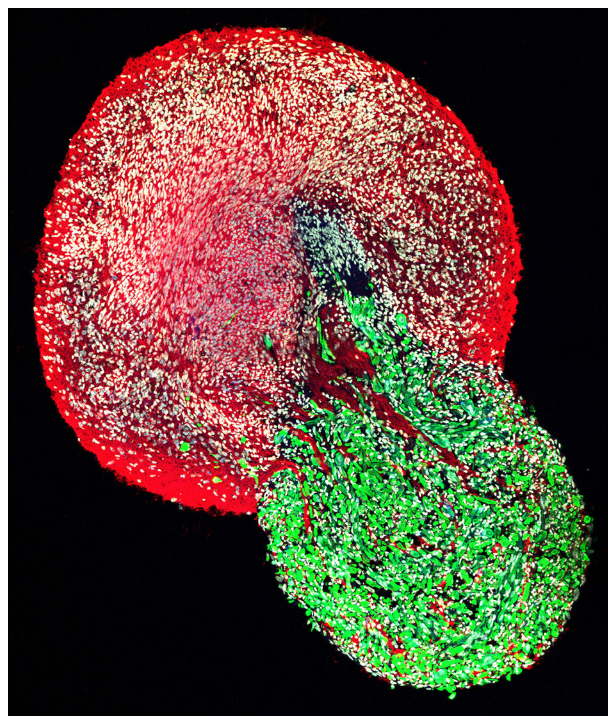

FIGURE 3 Neuromesodermal progenitors generate both spinal cord and muscle tissues that self-organize into two distinct regions within the organoid.

Tumor–immune cell organoids

Pleun Hombrink from Hubrecht Organoid Technology (HUB) presented the company's approach to developing personalized organoid cocultures for use in drug development for immunotherapies. Immunotherapy has revolutionized the field of oncology by leveraging the body's T cells to kill cancer cells. Several modalities are either currently in use or under investigation, including monoclonal antibodies that regulate T cell activity, cell-based therapies, cancer vaccines, oncolytic viruses, and bispecific antibodies. HUB has created several organoid biobanks to serve as preclinical models for drug development.

During his talk, Hombrink focused on patient-derived colorectal cancer organoids. The platform consists of colon organoids from patient tumors and healthy colon tissue and patient-derived tumor-infiltrating lymphocytes (TILs). Hombrink showed that they could isolate and expand TILs in a scalable manner and enrich tumor-reactive T cells. They plan to use this organoid–TILs coculture model to investigate personalized tumor-specific immune responses and as a screening platform for immunotherapies.

Increasing connectivity in brain organoids

Several speakers throughout the meeting showed how brain organoids can recapitulate much of the cell diversity of brain tissue, but several macroscopic features are still lacking. During development, the regions of the brain develop simultaneously forming macrocircuit connectivities that link different brain regions. In organoid models, however, different brain regions develop consecutively. Organoids thus lack macrocircuit connectivity and activity-dependent maturation.

Giorgia Quadrato from the University of Southern California presented work on modeling SYNGAP1-related disorders with human cortical organoids. SYNGAP1 is a top ASD risk gene and one of the most abundant proteins found at the postsynaptic density of excitatory synapses. Cortical organoids haploinsufficient for *SYNGAP1* revealed novel information about the expression pattern and functionality of this gene initially thought to be expressed only in neurons underscoring the importance of dissecting the role of genes associated with ASD in distinct cell types and across developmental stages. Quadrato also described the establishment of a method for generating functional 3D human cerebellar organoids that can reproducibly generate the cellular diversity of the human cerebellum within and across multiple cell lines.

Jean-Paul Urenda, a PhD student in Giorgia Quadrato's lab, presented work on improving the physiological relevance of human brain organoids, with a focus on the human visual system. Quadrato's lab is investigating the use of microfluidic devices to connect different brain structures grown independently. Previous work has shown that brain organoids connected via hydrogels can form long-range axonal fascicle and that these organoids show improved maturation than fused organoids.[88–90] Urenda showed unpublished work on an effort to optimize connectivity and culture conditions for different brain organoid structures.

ACKNOWLEDGMENTS

The work in Jianping Fu's lab is supported by the Michigan-Cambridge Collaboration Initiative, the University of Michigan Mcubed Fund, the 21st Century Jobs Trust Fund received through the Michigan Strategic Fund from the State of Michigan (grant CASE-315037), the National Science Foundation (I-Corps 2112458, CBET 1901718, and CMMI 1917304), and the National Institutes of Health (R21 NS113518 and R21 HD100931).

COMPETING INTERESTS

The authors declare no competing interests.

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How to cite this article: Cable, J., Lutolf, M. P., Fu, J., Park, S. E., Apostolou, A., Chen, S., Song, C. J., Spence, J. R., Liberali, P., Lancaster, M., Meier, A. B., Pek, N. M. Q., Wells, J. M., Capeling, M. M., Uzquiano, A., Musah, S., Huch, M., Gouti, M., Hombrink, P., … Urenda, J.-P. (2022). Organoids as tools for fundamental discovery and translation—a Keystone Symposia report. *Ann NY Acad Sci.*, *1518*, 196–208.
https://doi.org/10.1111/nyas.14874