REVIEW

Organoids-on-a-chip

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Recent studies have demonstrated an array of stem cell–derived, self-organizing miniature organs, termed organoids, that replicate the key structural and functional characteristics of their in vivo counterparts. As organoid technology opens up new frontiers of research in biomedicine, there is an emerging need for innovative engineering approaches for the production, control, and analysis of organoids and their microenvironment. In this Review, we explore organ-on-a-chip technology as a platform to fulfill this need and examine how this technology may be leveraged to address major technical challenges in organoid research. We also discuss emerging opportunities and future obstacles for the development and application of organoid-on-a-chip technology.

Decades of study in developmental biology and stem cell research have advanced our ability to recapitulate the key aspects of organogenesis in vitro. Recent years have seen considerable progress toward exploiting the self-organizing properties of pluripotent or adult stem cells to generate organotypic multicellular constructs known as organoids (*1*, *2*). Thanks to their ability to emulate microarchitecture and functional characteristics of native organs, organoids are emerging as a promising approach for the modeling of development, homeostasis, and disease of various human organs (*1*–*3*).

The conventional methods of forming organoids rely on three-dimensional (3D) culture of mammalian stem cells with sequential addition of growth factors. Although this approach has been widely used because of its simplicity, there is growing recognition that the current organoid culture techniques have the potential for substantial improvement. In particular, the random configuration of traditional 3D culture makes it difficult to precisely control organoids and their local environment. Existing culture systems also have limited capacity to reproduce the complex and dynamic microenvironment of a developing organ that provides instructive cues for organogenesis (*4*, *5*). The lack of these environmental signals poses challenges to achieving more complete, in vivo–like organoid development in a reproducible manner (*3*, *6*).

To address the limitations of conventional culture techniques, researchers in stem cell and developmental biology are forming alliances with engineers and physical scientists to develop advanced in vitro technologies for organoid research. At the forefront of this undertaking is the integration of organoids with organ-on-a-chip technology.

What are organs-on-a-chip?

Organs-on-a-chip can be broadly defined as microfabricated cell culture devices designed to model the functional units of human organs in vitro (*7*–*12*). In general, the construction of any organ-on-a-chip system is guided by design principles based on a reductionist analysis of its target organ (Fig. 1). The first step is to understand the anatomy of the target organ and reduce it to the basic elements essential for physiological function. These functional units are then examined to identify key features such as different cell types, structural organization, and organ-specific biochemical and physical microenvironments. For example, the alveolar–capillary unit of the lung consists of alveolar epithelial cells (cell type 1) and pulmonary microvascular endothelial cells (cell type 2) that are closely apposed to each other and separated by a thin interstitium (structural organization) (Fig. 1A). The epithelial and endothelial layers are subjected to air and blood flow, respectively, and the multilayered interface experiences breathing-induced cyclic mechanical stretch (organ-specific microenvironment).

Next, a cell culture device is designed to replicate the identified features. The device often contains multiple, individually addressable flow-through microchambers to grow multiple cell types while controlling the culture environment in a cell type–dependent manner. If necessary, additional components are incorporated that can be actuated mechanically, chemically, electromagnetically, or optically to emulate the biochemical and mechanical environment of the target organ. Finally, the designed device is produced using microfabrication techniques such as soft lithography (*13*).

The design strategy outlined here has been successfully implemented to create an organ-on-a-chip model of the alveolar–capillary unit of the lung (*14*). This system consists of two overlapping microchannels separated by a thin, flexible, microporous membrane (Fig. 1B, left). The compartmentalized design enables coculture of alveolar epithelial cells and lung microvascular endothelial cells on either side of the membrane while the cells are exposed to their respective tissue-specific environment (i.e., air on the alveolar side and fluid flow on the vascular side) (Fig. 1B, right). To mimic the deformation of the alveolar–capillary interface during breathing, the device is also equipped with two hollow microchambers alongside the culture channels, in which cyclic vacuum application induces stretching of the cell-lined intervening membrane (Fig. 1C).

By integrating living human cells with synthetically generated yet physiologically relevant microenvironments, organs-on-a-chip can mimic integrated organ-level functions necessary for physiological homeostasis, as well as complex disease processes (*15*, *16*). Furthermore, different organ-chip models can be fluidically linked to construct "body-on-a-chip" systems capable of simulating multiorgan interactions and physiological responses at the systemic level (*17*, *18*). Although these advanced model systems are still far from achieving the functionality of real human organs, their ability to capture key aspects of human physiology and pathophysiology makes them a promising approach for complementing and reducing animal studies for preclinical assessment of drugs, medical devices, and biomaterials (*7*, *9*, *19*). Organ-on-a-chip technology also provides an attractive in vitro platform for screening adverse health effects of chemicals, environmental materials, and consumer products (*10*, *20*).

What can organs-on-a-chip do for organoids?

The key to answering this question is to understand that organs-on-a-chip and organoids represent two fundamentally different yet complementary approaches toward the same goal of recapitulating the complexity of human organs in vitro. Organ-on-a-chip technology relies on our knowledge of human organs to engineer man-made constructs in which cells and their microenvironment are precisely controlled. In contrast, organoids follow intrinsic developmental programs and develop from self-organizing stem cells to reproduce the key structural and functional properties of their in vivo counterparts. Researchers are now exploring the possibility of synergistically combining the best features of each approach (*21*) to develop a more powerful in vitro technology. Here, we review recent studies inspired by this idea and examine how organ-on-a-chip technology can contribute to addressing major technical challenges in organoid research.

Challenge one: Microenvironmental control of organoids

A common strategy for constructing organoid models is to culture pluripotent or adult stem cells in a 3D environment. Although conventional 3D culture techniques provide a simple and effective means to produce organoids in a routine laboratory setting, their simplicity and ease of use often come at the expense of precise control. In this section, we elaborate on this problem and introduce emerging solutions provided by organ-on-a-chip technology.

Control of the biochemical microenvironment

Organoid development requires properly timed activation of morphogenetic signaling pathways to induce cell-fate specification and the physical

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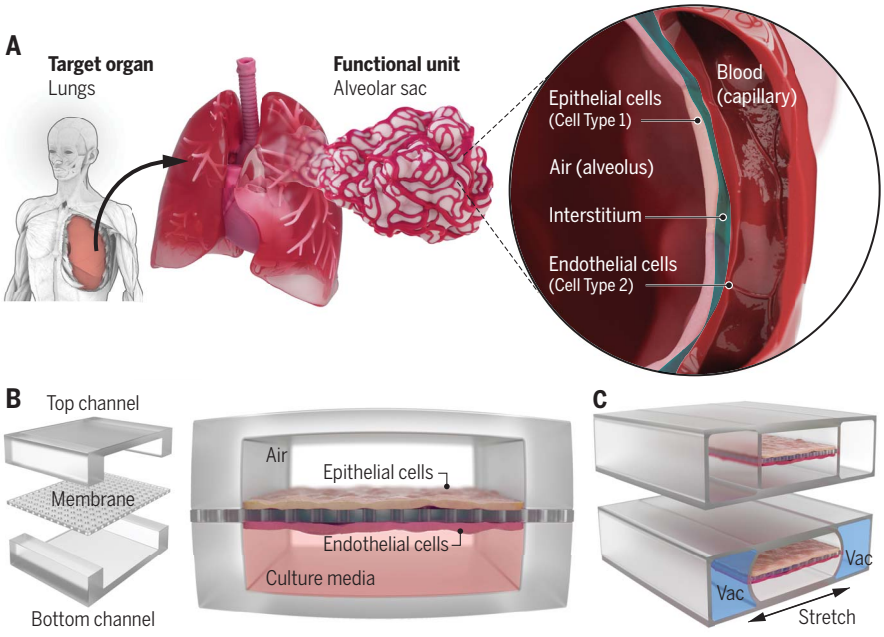

Fig. 1. Organ-on-a-chip design principles. (**A**) Reductionist analysis of a target organ (lung) identifies alveoli as the functional unit composed of epithelial and endothelial cells separated by a thin interstitium. (**B**) An analogous model is constructed from three layers to bring these two cell types into physiological proximity. (**C**) To mimic breathing-induced mechanical activity, the cells are cyclically stretched by applying vacuum (vac) to the side chambers. [Illustration: BIOLines Lab]

segregation of different cell types that together guide the process of self-organization (*22*). In conventional organoid culture, this is accomplished by applying exogenous morphogens at defined time points. During culture, diffusion of morphogens and cell-secreted soluble factors in organoids produces biochemical gradients in the local microenvironment of stem cells. These gradients, however, are not readily controllable owing to their spontaneous nature and often fail to simulate graded morphogen distributions critical for tissue patterning during organogenesis in vivo (*23*).

Researchers are beginning to harness the power of microengineering techniques to address this problem. A representative example can be found in a microfluidic system developed for in vitro modeling of neural tube development (*24*). This device contains a pair of microchannels that serve as the source and sink of soluble factors to generate stable morphogen gradients via diffusion across embryonic stem cell (ESC)–laden hydrogel constructs in a central culture chamber (Fig. 2A). The microengineered platform was used to mimic opposing gradients of sonic hedgehog (Shh) signaling molecules and bone morphogenetic protein (BMP) along the dorsoventral axis of neural tube, which induce neural tube patterning during spinal cord development. This study demonstrated spatially directed self-organization and differentiation of ESCs into motor neurons to generate in vivo–like tissue patterns. Microfluidic control of morphogen gradients also made it possible to examine the key determinants and temporal dynamics of Shh-induced ESC differentiation, revealing the existence of optimal morphogen concentrations for motor neuron differentiation. A similar method of generating gradients was employed in the development of a human brain organoid-on-a-chip to investigate adverse effects of nicotine on cortical development (*25*).

Recent studies have also demonstrated the combination of the source–sink approach with micropatterned culture scaffolds to generate physiological biochemical gradients in stem cell and organoid cultures. This strategy has been described in an intestine-on-a-chip system created by culturing self-renewing epithelial cells isolated from human enteroids on a microfabricated array of collagen pillars and microwells in a modified Transwell insert that mimicked the intestinal villi and crypts (Fig. 2B) (*26*). This device was used to recapitulate opposing gradients of Wnt and BMP signaling in the native biochemical niche of the intestinal epithelium. Notably, the morphogen gradients induced in vivo–like tissue compartmentalization in which stem cells and differentiated epithelial cells were confined to the crypts and the villi, respectively. This platform was modified in a separate study to model colonic crypts and reveal suppressed stem cell activity due to physiological gradients of proinflammatory cytokines and bacterial metabolites along the crypt–villus axis (*27*).

Control of nutrient supply

Organoids in 3D culture rely solely on passive diffusion to receive nutrients and oxygen and to remove waste products. As organoids grow larger, however, diffusive transport becomes insufficient to meet their increasing metabolic needs, eventually failing to support their growth and maturation (*28*). This problem in turn raises the question of how embryos in the body cope with the high metabolic demands of developing organs. Research in developmental biology has established that embryogenesis is tightly coupled with vascular development and that functional vasculature capable of delivering adequate blood supply is a requirement for later stages of organogenesis (*29*). On the basis of this observation, vascularization of organoids is emerging as a promising strategy to address the problem of limited nutrient supply and life span in traditional organoid models.

The ability of organs-on-a-chip to mimic perfusable blood vessels (*30*–*34*) may play an instrumental role in this effort, as illustrated by a tumor organoid-on-a-chip developed by Shirure *et al.* (*35*). The model was created in a microfluidic device consisting of three interconnected chambers that supported vasculogenic self-assembly of endothelial cells into a 3D network of perfusable blood vessels and their angiogenic growth toward organoid-like constructs derived from breast cancer patients (Fig. 2C). Although this process differs from blood vessel formation during embryogenesis, the microfluidic platform permitted vascularization of tumoroids and their perfusion under physiological flow conditions that recapitulated transport characteristics of the tumor microenvironment in vivo. The ability of this microdevice to support long-term culture was demonstrated by the maintenance of vascularized tumoroids for 22 days. By showing significantly reduced tumor growth after vascular perfusion with paclitaxel, this study also suggested the potential use of the model for preclinical screening of patient-specific responses to chemotherapy.

Although this study shows the feasibility of vascularizing and perfusing 3D multicellular constructs in microdevices, the same approach has not been demonstrated in the culture of pluripotent stem cell (PSC)–derived organoids. Vascularizing these types of organoids is potentially problematic, as specialized media required for stem cell differentiation may interfere with vascular self-assembly and remodeling. As an alternative strategy, it is possible to create miniaturized bioreactors that exploit forced convection and mixing of media to enhance nutrient supply for production and prolonged maintenance of organoids. This approach has been leveraged in brain and pancreatic organoid models (*36*–*38*).

Control of the biophysical microenvironment

A developing embryo experiences various types of mechanical forces, ranging from single-cell–generated traction forces to fluid shear stress and solid mechanical forces that arise from coordinated mechanical activity of large groups of cells (e.g., heart contraction, fetal breathing movement) (*39*). These forces act in concert with soluble morphogens and extracellular matrix (ECM) signals to regulate organ development and maturation

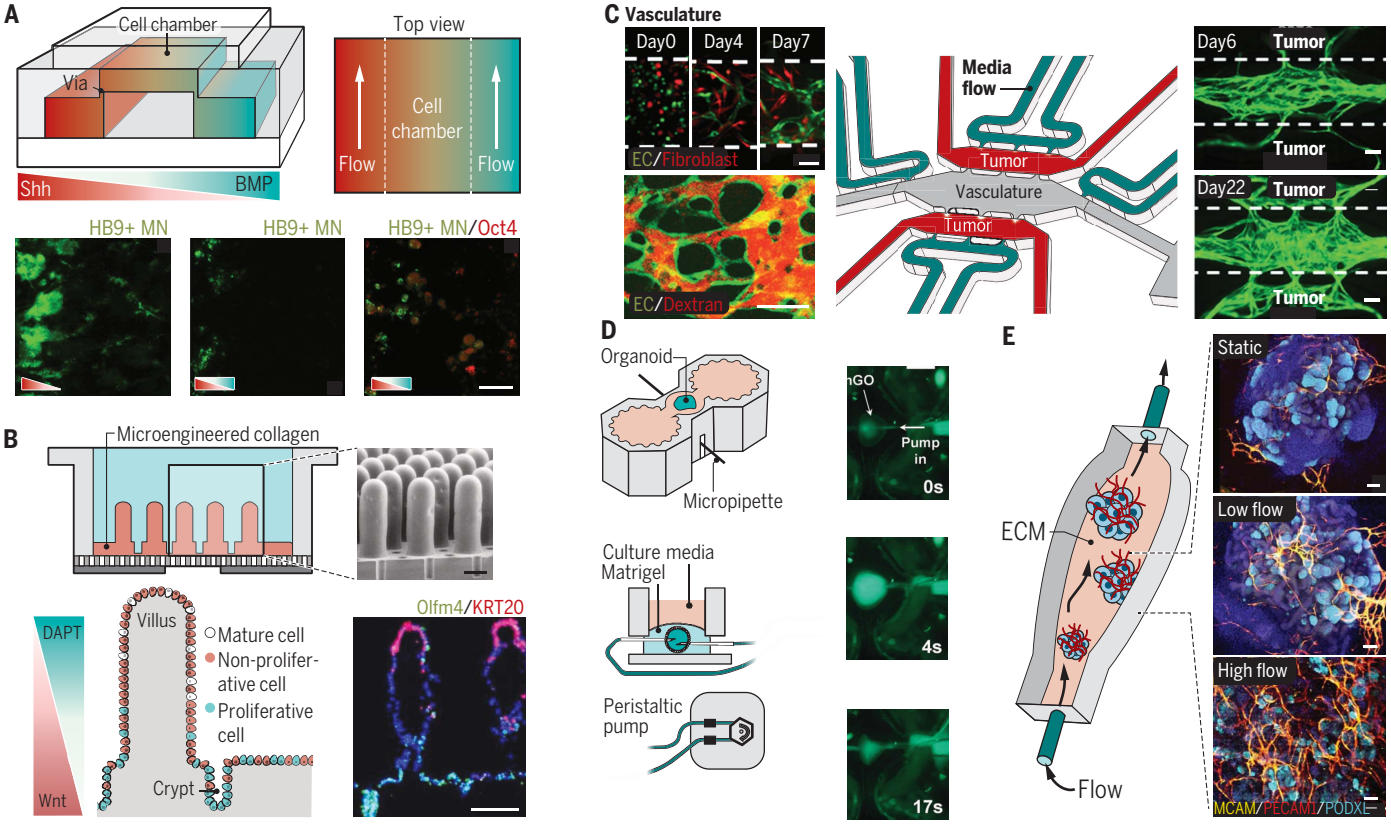

Fig. 2. Controlling the microenvironment of organoids-on-a-chip. (**A**) Physiological morphogen gradients are generated by diffusion between the source and sink microchannels. Purmorphamine (PM; an Shh agonist) gradients promote self-organization and differentiation of *HB9::GFP* transgenic reporter ESCs in the culture chamber into motor neurons (MN; green). Opposing gradients of PM and BMP induce spatial localization of motor neurons and pluripotent ESCs (Oct4; red). Scale bar, 200 μm. [Adapted from (*24*) with permission] (**B**) An intestine-on-a-chip can generate opposing gradients of Wnt and a γ-secretase inhibitor (DAPT) along the crypt–villus axis, which induces compartmentalization of proliferative (Olfm4) and nonproliferative (KRT20) cells. Scale bar, 100 μm. [Adapted from (*26*) with permission from Elsevier] (**C**) In a tumor organoid-on-a-chip, blood vessels are formed in the central chamber by coculturing endothelial cells (ECs) with fibroblasts in a hydrogel. Vascular perfusability is demonstrated by the flow of fluorescent dextran. The vessels in the central chamber grow into the tumor chambers to vascularize tumoroids. Scale bars, 100 μm. [Adapted from (*35*) with permission of Royal Society of Chemistry] (**D**) Luminal flow in the stomach is simulated in a stomach organoid-on-a-chip by cannulating human gastric organoids (hGO) in Matrigel to deliver fluid. The flow also induces cyclic deformation of the organoids, mimicking gastric motility. Scale bars, 2 mm. [Adapted from (*41*) with permission of Royal Society of Chemistry] (**E**) Kidney organoids are cultured under flow in a 3D printed device. Fluid shear stress enhances vascularization and maturation of kidney organoids, as demonstrated by increased vascular density and robust expression of vascular markers (PECAM1 and MCAM) and PODXL$^+$ cells. Scale bars, 100 μm. [Adapted from (*42*) with permission from Springer Nature]

(*40*). The lack of this biomechanical control is increasingly recognized as a limitation to developing fully mature organoids in culture and constructing physiologically relevant organoid models.

To address this problem, recent studies have demonstrated mechanically actuatable microengineered platforms that can generate and apply in vivo–like mechanical forces to organoids. For example, Lee *et al.* developed a stomach-on-a-chip model in which stomach organoids prepared from human PSCs were cultured in Matrigel and cannulated with a pair of micropipettes for fluidic access to the inner compartment (Fig. 2D) (*41*). With the use of a peristaltic pump connected to the pipettes, this platform generated fluid flow through the organoids to mimic luminal flow and rhythmic contraction of the stomach in vivo.

These types of mechanically active culture systems can also be used to generate physiological biomechanical cues that promote structural and functional maturation of developing organoids. The proof-of-concept of this approach was demonstrated by the recent report of human kidney organoids-on-a-chip, which were engineered in a 3D printed millimeter-scale chamber to examine the effect of fluid flow on vascularization and maturation of human PSC–derived kidney organoids (Fig. 2E) (*42*). During nephrogenesis, application of continuous flow resulted in the expansion of endothelial progenitors within the organoids and the formation of perfusable blood vessels in a shear stress–dependent manner. Notably, this response was accompanied by the production of more-mature tubular structures. Shear stress also induced prominent vascularization of glomerular compartments and increased formation and maturation of podocyte foot processes, revealing that flow-generated microenvironmental cues contribute to structural and functional development of kidney organoids in vitro. The beneficial effects of physiological fluid flow on the maturation of organoids have also been described in microengineered organoid models of the pancreas (*43*) and the intestine (*44*). These studies exemplify how organoids and organs-on-a-chip can be synergistically combined to achieve a level of cellular maturity not attainable when either technology is used alone.

Challenge two: Modeling tissue–tissue and multiorgan interactions

From a systems perspective, the complexity of the human body originates from dynamic interactions between its components within and across different levels of organization. The ability to emulate these interactions is essential for mimicking the integrated behavior of complex physiological systems in vitro. Organoids have the inherent ability to approximate the repertoire of cell types that

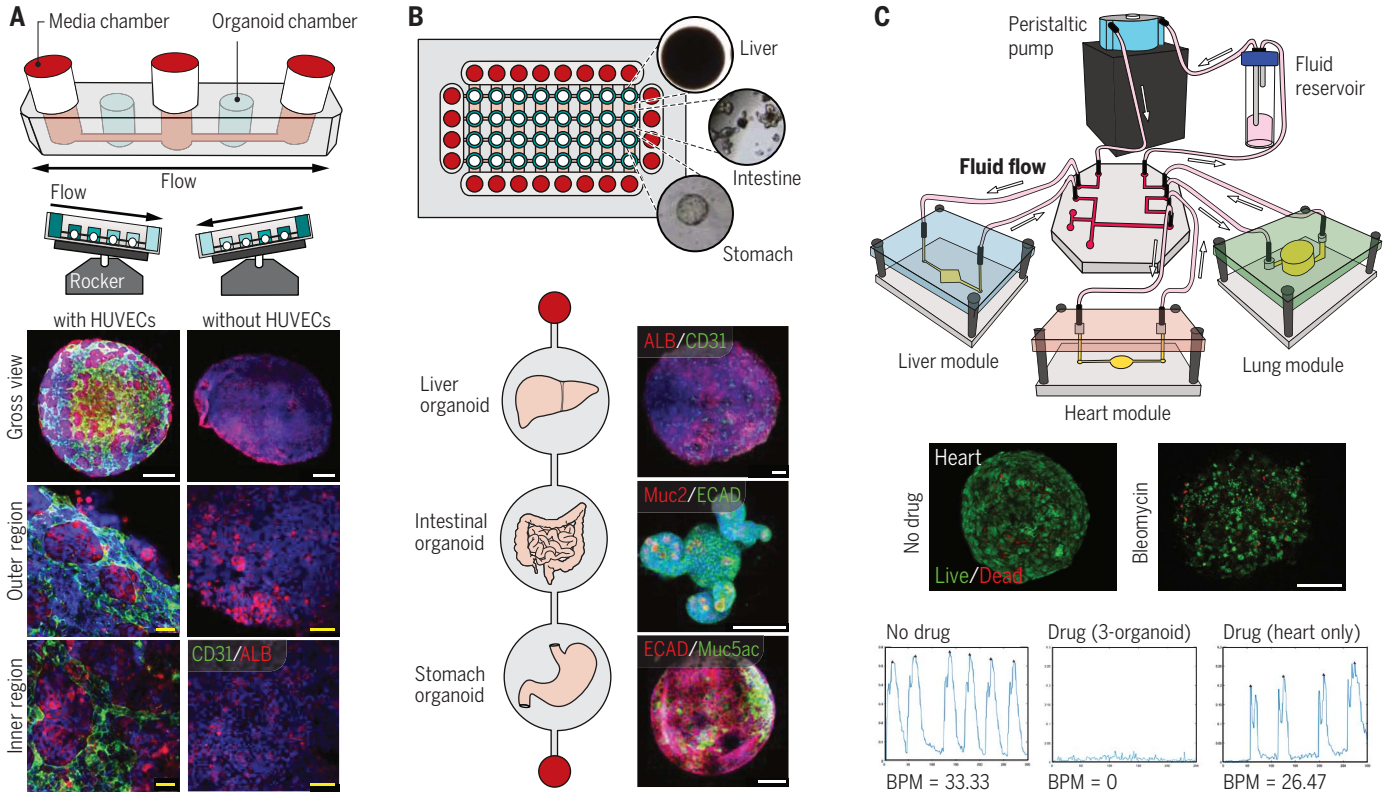

Fig. 3. Modeling tissue–tissue and organ–organ interactions in organoids-on-a-chip. (**A**) A vascularized liver organoid model is established in a rocker-actuated device containing serially connected media and culture chambers. Liver organoids grown with human umbilical vein endothelial cells (HUVECs) show increased albumin expression (ALB; red). Scale bars, 500 μm (white), 50 μm (yellow). [Adapted from (*45*) with permission from John Wiley and Sons] (**B**) A microfluidic array is used to demonstrate a multiorganoid model. Scale bars, 200 μm. [Adapted from (*45*) with permission from John Wiley and Sons] (**C**) A microengineered heart-lung-liver model is created by fluidically linking three culture modules for drug testing. Bleomycin treatment results in a loss of beating in heart organoids in the three-organ model (middle graph), but this response is absent in the heart-only model (right graph). Scale bar, 100 μm. BPM, beats per minute. [Adapted from (*46*) (CC BY 4.0)]

constitute the native organs and to recapitulate complex cellular cross-talk. Modeling biological interactions at higher levels of organization, however, remains a major challenge in current organoid models. This section will examine how organoid-on-a-chip technology may provide solutions to this problem.

Modeling tissue–tissue interactions

In virtually every organ, the interactions between different tissue types play a fundamental role in organ development, homeostasis, and disease. Efforts to advance organoid technology now aim to generate additional tissue types absent in traditional organoid cultures to more closely recapitulate the collection of specialized tissues in native organs and their dynamic interactions. This has been suggested as a promising approach to promote the maturation of organoids and improve their capacity to model complex physiological responses (*28*).

To this end, organs-on-a-chip provide a platform to engineer more-controllable and more-conducive environments for coculture of different cell and tissue types in organoid systems. Such capabilities have recently been demonstrated by a vascularized liver organoid-on-a-chip (*45*). In this work, a multicompartment microdevice was created to grow embryonic fibroblast-derived induced hepatic cells with human endothelial cells in an ECM hydrogel (Fig. 3A). The 3D constructs were also continuously perfused with media by using a laboratory rocker to mimic blood circulation in vivo. Coculture under this flow condition for 21 days produced vascularized liver organoids with robust staining for albumin, which was not observed in static culture. Notably, this study revealed that the interaction of hepatic tissues with the vasculature within the organoids increased the expression of hepatocyte-specific markers and improved hepatic functions. The enhanced maturity of coculture organoids also permitted more-sensitive detection of drug-induced liver toxicities.

Modeling multiorgan interactions

Efforts to establish a holistic understanding of human physiology have been challenged by the difficulty of modeling the human body as a system of interconnected and interdependent organs. Although organoids provide a powerful platform for modeling individual organs, their capabilities for recapitulating physiological interactions between different organs have yet to be investigated. Building upon advances in body-on-a-chip technology (*17*), researchers are beginning to microengineer in vitro platforms for coculture of different types of organoids to simulate multiorgan interactions.

Jin *et al.* developed a multiorgan model using a microfluidic array to coculture stem cell–derived liver, intestinal, and stomach organoids (Fig. 3B) (*45*). The organoids were maintained in different compartments but were allowed to communicate via rocker-induced media flow between culture chambers. The key demonstration of this study was to simulate bile acid homeostasis regulated by the interaction between the liver and the intestine in vivo. In response to exogenously applied bile acids, the model showed reduced expression of a bile acid synthesis enzyme (CYP7A1) in the liver organoids due to paracrine factors produced by the intestinal organoid, demonstrating the physiological interorgan cross-talk.

These types of multiorgan systems are believed to play an instrumental role in developing predictive preclinical models for biopharmaceutical applications. Skardal *et al.* explored this emerging opportunity in their microengineered heart-lung-liver model (*46*). This system was constructed

by combining 3D printed liver and heart organoids with microengineered lung tissues in a modular fashion and perfusing them with a common media in a closed loop (Fig. 3C). Notably, this model revealed previously unknown cardiotoxicity of a chemotherapeutic drug (bleomycin) due to the cytokine-mediated cross-talk between the lung and the heart tissues. Although further validation is necessary, this study illustrates the potential of microengineered multiorganoid systems as an advanced in vitro platform for preclinical drug screening.

Challenge three: Reducing variability

In contrast to the highly reproducible process of organogenesis in vivo, organoids develop with substantial variability in size, structural organization, functional capacity, and gene expression. This interorganoid variability has been identified as a major issue that limits the potential of organoid technology, especially for applications in disease modeling, drug screening, and transplantation (*6*, *28*). As discussed above, microengineered organoid systems can recapitulate the native niche of stem cells in developing embryos, providing a means to generate in vivo–like instructive cues and reproduce the tightly regulated programs of organogenesis to reduce stochasticity and variability. Recent advances in organ-on-a-chip technology also suggest other approaches to tackling this problem that rely on advanced instrumentation of microengineered culture devices.

Automated control of organoid culture

The precision and repeatability of mechanical automation provide an avenue to decrease the variability caused by inconsistent manual manipulation during tedious laboratory procedures such as organoid culture. The fragility, small size, and dynamic culture requirements of organoids, however, impose difficulties on our ability to directly leverage bulky laboratory instruments for automated culture. Microengineered systems are uniquely suited to address this problem by enabling precise and dynamic handling of fluids and tissues at the biological length scales of organoids while providing a platform amenable to automation.

For example, an entirely automated digital microfluidic platform demonstrated electrowetting-based control of hepatic organoids in a microdevice patterned with an array of electrodes (*47*) (Fig. 4A). By activating the electrodes in a defined sequence, this system allowed fluid droplets to move, merge, and split in a programmed manner. Using this platform, a study was conducted to demonstrate automated culture of organoid-like 3D hepatic constructs in media droplets and monitoring of their liver-specific function. The programmed actuation of the system also enabled the analysis of acetaminophen-induced hepatotoxicity without manual intervention. This type of integrated automation is key to achieving reproducibility in procedures that require precisely timed or continuous accurate manipulation over extended periods.

High-throughput manipulation and analysis of organoids

Another benefit of using microengineered systems for organoid culture is the opportunity to substantially increase the density at which organoids can be cultured and analyzed. This capability is potentially advantageous for reducing variability, as it offers the promise of enabling selection, manipulation, and screening of organoids in a high-throughput manner. A microfluidic platform created by Jin *et al.* provides an early demonstration of this approach (*48*). This system contained a high-density array of microfabricated pillars to immobilize many intestinal organoids and conduct optical measurement of their swelling as a result of osmotic changes and cholera toxin (Fig. 4B). Notably, the mechanical traps provided a means to physically screen out enteroids with improper size and capture the ones appropriate for analysis. Because the size of the trapping pillars is readily adjustable during microfabrication, this approach is tunable for size-based selection and also compatible with integration into more complex systems to enrich the homogeneity of organoid populations. Similar strategies have been demonstrated in microengineered culture of brain and liver organoids for drug testing applications (*49*, *50*).

Integration of biosensing

Efforts to address variability may be greatly facilitated by incorporating biosensing elements into culture platforms to permit continuous screening of organoids. A good example can be found in a multiorgan-on-a-chip device integrated with label-free biosensors for long-term monitoring of cardiac and liver organoids and primary hepatic spheroids (*51*). This system featured a universal electrochemical immunobiosensing platform capable of detecting up to eight different targets with high sensitivity and wide dynamic ranges (Fig. 4C). The multiplexed sensing capability was demonstrated by continuous and simultaneous triplet analysis of albumin, glutathione *S*-transferase α, and creatine kinase MB during drug treatment. Although the system was leveraged to measure drug-induced toxicities in this study, its capacity to perform continuous biosensing of secreted products from organoids would be highly valuable for screening-based

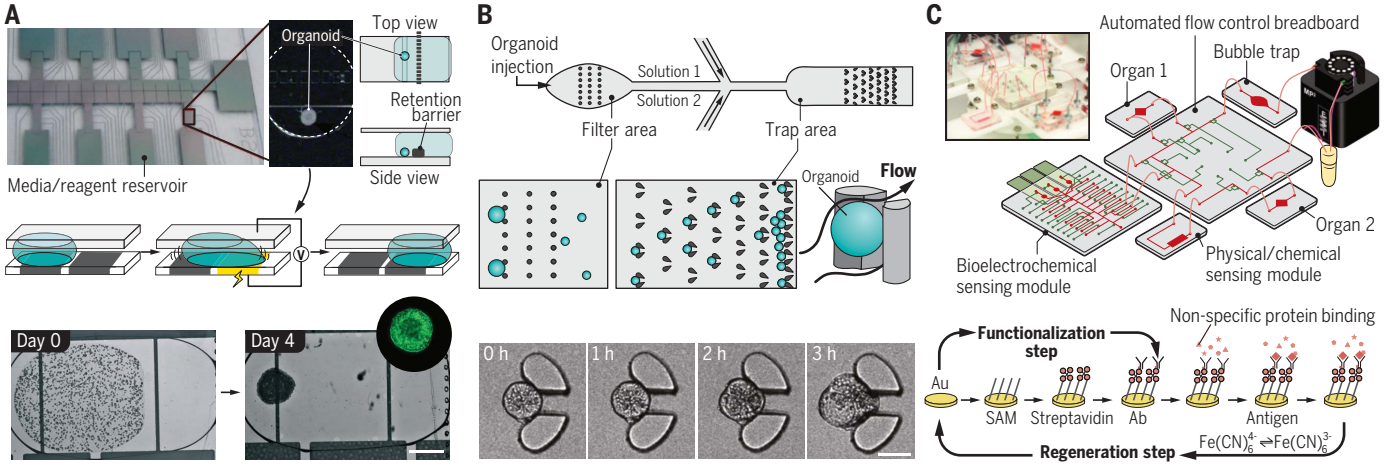

Fig. 4. Advanced organoid culture systems toward reduced variability. (**A**) In an electrowetting device, externally applied electric field renders the surface over the energized electrode (yellow) hydrophilic, inducing the motion of a liquid droplet toward the energized electrode. This principle is used to automate culture of liver organoids. Organoids grown in droplets are retained by microfabricated structures and undergo contraction over time. Scale bar, 100 μm. [Adapted from (*47*) with permission of Royal Society of Chemistry] (**B**) A microfluidic high-density pillar array allows for size-based filtering (upstream) and capturing of organoids (downstream) for analysis of swelling due to cholera toxin. Scale bar, 100 μm. [Adapted from (*48*) with permission of AIP Publishing] (**C**) A sensor-integrated multiorgan platform enables in situ monitoring of organoids. Gold microelectrodes act as immunobiosensors to detect specific antigens that use changes in interfacial electron-transfer kinetics of the probe, owing to their binding to surface-bound antibodies (Ab). SAM, self-assembled monolayer. [Adapted from (*51*)]

optimization of organoid culture conditions to minimize variability.

Outlook

Despite our best efforts, there is still a large gap between the current state-of-the-art of organoid technology and the reality of how organs develop and function in the body. Through the cross-fertilization of organoids with organs-on-a-chip, researchers are now seeking to fill this gap, with the ultimate goal of mirroring the complex inner workings of human organs in easily accessible and controllable model systems. Looking into the future of this very young field, we identify an array of opportunities.

Among the most promising applications of organoids-on-a-chip is drug discovery. Organoids or organs-on-a-chip alone have limited capacity to meet the broad range of needs that arise in the drug discovery process. The similarities of organoids to actual organs make them more attractive for target identification and validation early in the pipeline, whereas organs-on-a-chip as more-reproducible and more-controllable engineered constructs are better suited for efficacy and safety screening (*9*). By combining the strength of the two technologies, organoids-on-a-chip may serve as more versatile and predictive preclinical models that are broadly applicable to conventional and emerging drug discovery processes.

Organoids-on-a-chip may also play an instrumental role in creating patient- and population-specific disease models for personalized medicine. Microengineered devices provide a means to generate disease-specific culture environment that reconstitutes altered properties and biological interactions of diseased tissues (*7*, *8*, *10*, *15*). Such capabilities can be leveraged for maturation of patient-derived organoids to enhance the expression of in vivo–like disease phenotypes, which remains a major hurdle in the application of organoids for personalized medicine (*28*). Organoids-on-a-chip may also enable the development of personalized disease models using patient-derived tissue specimens as organoids. As evidenced by tumor organoids grown directly from patient biopsies, these types of organoids often display unpredictable growth patterns and substantial heterogeneity (*52*), which are difficult to handle using traditional in vitro techniques. The advantages of microengineered organoid systems may contribute to resolving some of these issues.

Regenerative medicine is another important area of opportunity. Based on the potential of organoids as unlimited source of tissue with regenerative capacity, studies have shown the feasibility of transplanting in vitro expanded organoids into animals to repair damaged organs (*53*). Clinical translation of this approach, however, remains a distant goal, owing to the low efficiency and safety concerns of transplantation (*28*). Organoids-on-a-chip may help address this problem by providing a platform for both high-content and high-throughput analysis to identify optimal conditions for in vitro expansion of organoids. Another exciting possibility is to engineer advanced anatomical and physiological features (e.g., vasculature, nerves) into organoids during in vitro expansion, with the goal of enhancing their engraftment and sustained function in vivo. Using microfabricated devices to produce such organoids on a clinically relevant scale is certainly a challenge, but with increasing efforts to develop high-throughput culture systems, this idea may open up a new frontier of regenerative medicine.

Although many opportunities lie ahead, organoids-on-a-chip as a nascent technology also face challenges. Typically, organoid-on-a-chip models are designed and constructed in a predetermined manner and thus have limited ability to recapitulate dynamic structural, environmental, and functional changes that occur during organogenesis. Addressing this limitation will require efforts to understand the spatiotemporal dynamics of organ development and to devise advanced engineering techniques to reproduce the evolution of developing organs in microengineered culture of organoids. The formation of organoids often requires hydrogels composed of Matrigel or similar basement membrane extracts that provide 3D structural support and enable proper morphogenesis (*54*, *55*). These materials, however, suffer from poorly defined compositions and batch-to-batch variability, which may be problematic for achieving a high degree of environmental controllability. Ongoing efforts to engineer new types of biomaterials with well-defined and tunable properties for organoid culture may hold the key to addressing this problem (*3*, *56*, *57*).

On a related note, the dominance of polydimethylsiloxane, which is known to absorb small molecules (*58*), in the fabrication of microdevices is a major concern for pharmaceutical applications. To tackle this issue, studies are under way to develop alternative materials and surface engineering techniques (*59*). For integrated multiorgan models, further investigations are needed to identify optimal culture conditions and media compositions for coculture of various cell types. Finally, three dimensionality and structural complexity of engineered organoid constructs will likely present distinctive challenges to imaging and analysis. As demonstrated by the recent report of brain organoids (*60*), this problem may be addressed by implementing device designs that enable precise positioning and confinement of organoids to make them more amenable for microscopic imaging. Tissue-clearing methods (*61*) and advanced 3D imaging techniques (*62*) may also be potential solutions.

The question of what organs-on-a-chip can do for organoids is shaping a new field of inquiry and a wave of innovation that will continue to evolve with inputs from a wide range of disciplines in science and engineering. Combining the strongest qualities of today's two most advanced in vitro technologies will require a vigorous exchange of ideas, perspectives, expertise, and resources between physical and biological sciences. With these efforts under way, the best of organoids is yet to come!

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ACKNOWLEDGMENTS

We thank G. Al, G. S. Worthen, and A. Paris for their input. **Funding:** This work was supported by the National Institutes of Health (NIH) (1DP2HL127720-01, 1UG3TR002198-01, and 1UC4DK104196-01), the National Science Foundation (CMMI:15-48571), the Paul G. Allen Family Foundation, the Alternatives Research and Development Foundation, and the University of Pennsylvania. D.H. is a recipient of the NIH Director's New Innovator Award and the Cancer Research Institute Technology Impact Award. **Competing interests:** D.H. holds equity in Emulate Inc. and consults for the company.

10.1126/science.aaw7894