Review

# Organs-on-a-chip models for biological research

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## SUMMARY

We explore the utility of bioengineered human tissues—individually or connected into physiological units—for biological research. While much smaller and simpler than their native counterparts, these tissues are complex enough to approximate distinct tissue phenotypes: molecular, structural, and functional. Unlike organoids, which form spontaneously and recapitulate development, "organs-on-a-chip" are engineered to display some specific functions of whole organs. Looking back, we discuss the key developments of this emerging technology. Thinking forward, we focus on the challenges faced to fully establish, validate, and utilize the fidelity of these models for biological research.

## INTRODUCTION

The field of tissue engineering, created in the late 1980s, was defined as "the application of the principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathologic mammalian tissues and the development of biological substitutes to restore, maintain, or improve function" (Langer and Vacanti, 1993). Interestingly, this definition of the field and its fundamental approach to enable cells to form tissues by using biomaterial scaffolds and molecular and physical regulatory signals have not changed. It is now possible to buy living skin grafts for restoration of large non-healing burns from one of the pioneers in the field, organogenesis. Tissue-engineered blood vessels will hopefully follow soon, as the clinical trials in atrioventricular shunt patients are being completed (Kirkton et al., 2019). Thus far, most success has been achieved with tissues that are either thin (skin, blood vessels, bladder) or avascular (cartilage) or have a high ability for regeneration (bone). Across the board, tissue engineering is based on providing the cells with the 3D tissue-specific environment, in striking contrast to cell monolayers. In parallel, the field of microfluidics developed rapidly with the invention of soft lithography by the Whitesides lab and the ability to prototype devices using polydimethylsiloxane (PDMS), a soft silicone-based material. Consequently, the number of reports using microfluidics increased from only a few in the early 2000s to over 16,000 per year over the last 5 years (Zhang and Radisic, 2017).

"Organs-on-a-chip" (OOC) evolved in the late 1990s, with the idea of "human-body-on-a-chip" for studies of human physiology being introduced shortly thereafter by the Shuler lab (Sin et al., 2004). OOC gained traction with the landmark "lung-on-a-chip" study by the Ingber group (Huh et al., 2010) that recreated an epithelial/endothelial barrier on a stretchable PDMS membrane mimicking the breathing motion. This simple design recapitulated the barrier function of the lung. Another example are the engineered strips of contractile cardiac muscle that were matured enough to become similar to native trabeculae (Ronaldson-Bouchard et al., 2018; Tiburcy, 2017).

While the term OOC suggests that mini-organs are grown on a chip, it is important to note that this elusive goal has not been achieved. Instead, these systems contain small tissue constructs designed to reproduce just one or a few specific functional properties of the entire organ, such as the barrier function of the skin, lung vasculature, muscle contractility, or liver metabolism (Figure 1). The simplicity of these models is a major advantage, as it allows direct assessments of the effects of genetic and environmental factors on cellular and tissue function. Clear distinction between OOC and organoids is also important (Takebe et al., 2017) (Figure 1).

Organoids naturally form into multicellular structures that provide faithful models for studying early development and some diseases (Clevers, 2016), in contrast to OOCs that utilize bioengineering tools to assemble matured tissue constructs that display distinct organ functions.

Approaches from tissue engineering are harnessed in OOCs, which utilize cell culture on scaffolds, physical signals (fluid-dynamic, mechanical, electrical), and microfabrication of culture spaces and channels (Figure 1). Through these features, OOCs provide improved consistency of tissue structure and phenotypes for studies of organ-level functions, while often incorporating only a few cell types found in the native organ. Examples of OOCs include heart muscle (Mathur et al., 2015; Ronaldson-Bouchard et al., 2018; Zhao et al., 2019; Tiburcy, 2017), liver ((Kostrzewski et al., 2019; Schepers et al., 2016) alveolar unit of the lung (Huh et al., 2010), brain (Harberts et al., 2020) and blood-brain barrier (Vatine et al., 2019), kidney glomerulus and proximal tubule (Zhou et al., 2016; Homan et al., 2016), neuromuscular junction (Afshar Bakooshli et al., 2019), vascular network (Zhang et al., 2016), skin (Abaci et al., 2018), retina (Achberger et al., 2019), pancreas (Bauer et al., 2017), gut (Kim et al., 2016), bone marrow (Chou et al., 2020) placenta (Blundell et al.,

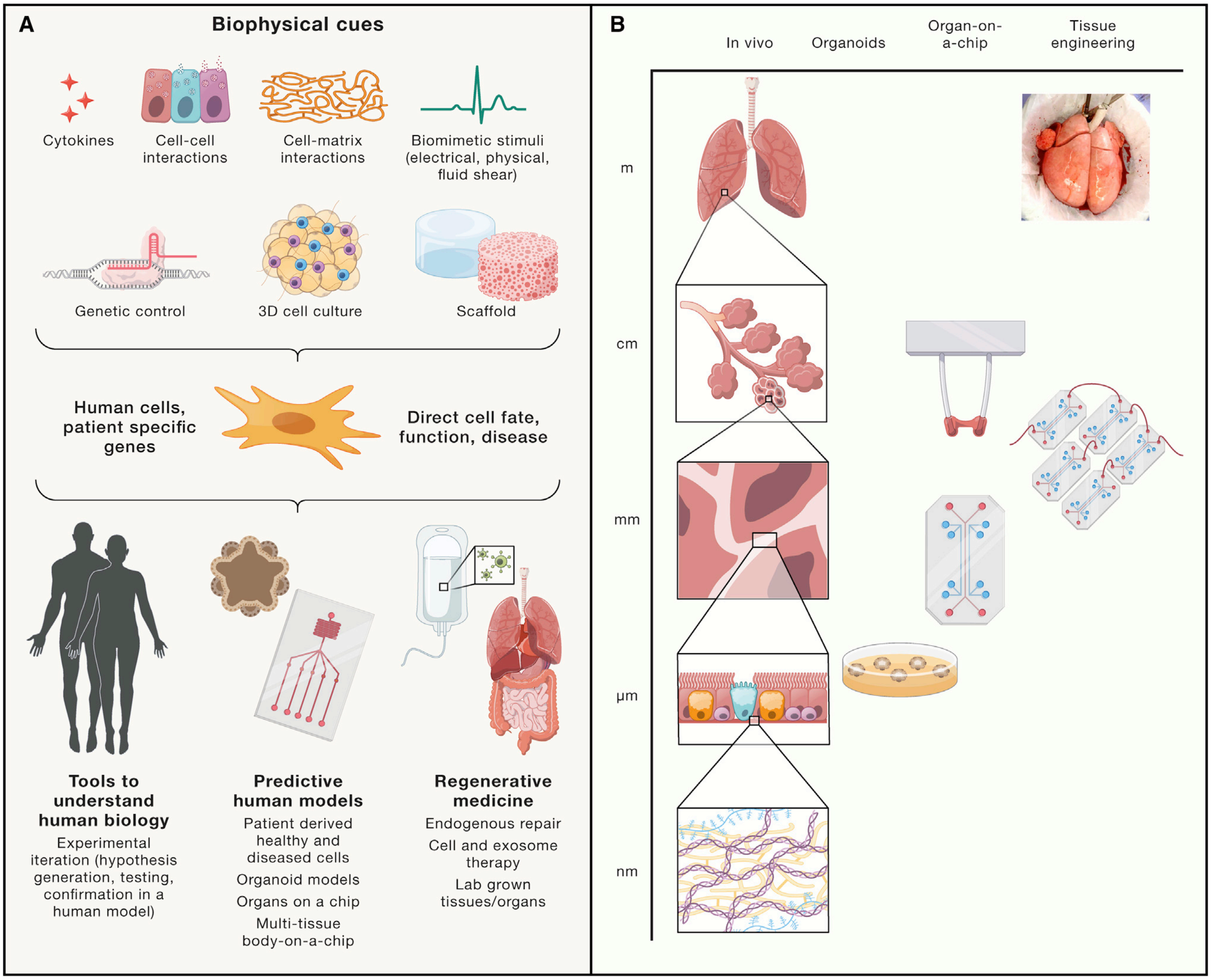



**Figure 1. Cell microenvironment in organ-on-a-chip (OOC) engineering**
(A) OOCs rely on the use of cells, biomaterials, and culture systems (bioreactors) to re-create environments to reproduce key functional properties of the tissue or organ of interest.
(B) Living-cell-made structures in OOC devices are on the order of micrometers to millimeters in size, whereas regenerative engineering re-creates structures on the order of millimeters to centimeters.

2016), and tumors (Lai et al., 2020), all of which are being used to study tissue maturation, regeneration, and disease.

By coupling multiple OOCs together through vascular perfusion of a shared blood substitute or supernatant exchange, one can study organ-organ interactions and systemic diseases such as cancer (Jeon et al., 2015; Oyirifi et al., 2019; Xu et al., 2016), inflammation (Lin et al., 2019; Trapecar et al., 2020), or infection (Grassart et al., 2019).

We discuss the tissue engineering paradigm and its application to human OOCs, from minimally functional single-tissue units to multiple tissues connected into physiological units. Our focus is on the utility of OOCs in biological research, where living cells can be investigated in the native-like contexts of development, physiology, or disease Figure 2. We also discuss some of the challenges and prospects in this rapidly evolving field providing human tissue constructs that approximate the molecular makeup, form, and function of their native counterparts for quantitative biological studies.

## TISSUE ENGINEERING PARADIGM

Here, we summarize some of the key developments, lessons learned, and tools we now have available for OOC studies. More detail can be found in several excellent reviews (Low et al., 2020; Marx et al., 2016; Takebe et al., 2017).

Initially, tissue engineering focused on creating tissues from primary cells sourced from young animals with high regenerative ability (Tissue Engineering 1.0). The original paradigm involved cell seeding into biomaterial scaffolds and either direct implantation or bioreactor cultivation to reach a certain level of functional

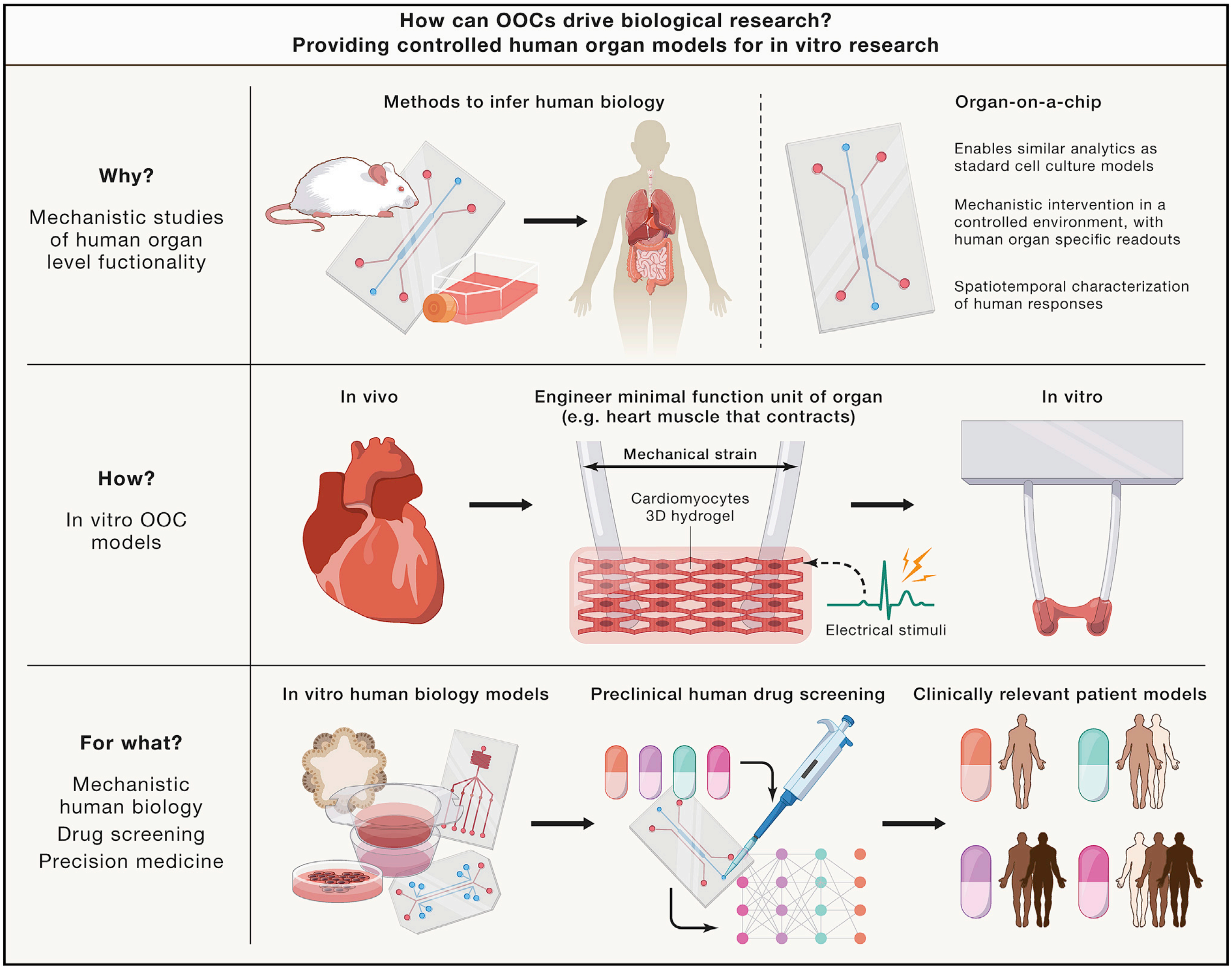



**Figure 2. OOC devices drive biological research**
OOC devices uniquely enable studies of human-organ-level functionality, using standard methods validated in 2D and animal studies, through engineered systems aimed at reproducing a single organ or multiple organs. They are already used in human disease modeling, drug screening, and precision medicine.

competence. In the second phase (Tissue Engineering 2.0), the concept of isomorphous tissue regeneration was introduced, meaning that the scaffolds were designed to degrade at the rate of new tissue formation. With the advent of human induced pluripotent stem cells (iPSCs), tissue engineering entered its third phase (Tissue Engineering 3.0). The patient-specific cells and scaffolds are being harnessed to create individualized approaches to organ regeneration and restore the anatomy and function of the original tissue. In parallel, the individualized approaches were extended into the *in vitro* modeling of biological processes using OOC. Adoption of methodologies developed for regenerative engineering, and advances in microfabrication and soft lithography, facilitated the rapid development of OOCs.

## Cells

As the actual "tissue engineers," cells are indispensable for tissue formation, either being supplied exogenously or mobilized *in vivo*. The right cell phenotypes are needed for regulating cell function and remodeling the cellular environment. One of the key findings of tissue engineering was that the co-culture of different cell types that comprise native tissues truly improves the tissue outcomes (Aleman et al., 2019; Alimperti et al., 2017). The supporting cells (fibroblasts, pericytes, and vasculature) in the stromal environment largely determine tissue functionality, through molecular and physical signaling and deposition of extracellular matrix (ECM). Also, the stroma can dramatically change in the context of disease, particularly in systemic diseases such as fibrosis and cancer.

Another key factor determining the biological fidelity of engineered tissues in all areas of application is the maturity of their molecular, structural, and functional phenotypes. One can enhance tissue maturation by (1) *developmental engineering*, using developmental cues and extended culture times (Keung et al., 2014; Musah et al., 2017); (2) *biomimetic engineering*, by replicating the in vivo environment (e.g., physical stimuli, 3D culture, multiple cell types) (Guilak et al., 2009; Ronaldson-Bouchard et al., 2018; Zhao et al., 2019); and (3) *bioactivation*, by activating certain pathways (via endogenous signaling, environmental stimuli, and transcriptomic factors). While the first two approaches are well established, bioactivation is just emerging. Matured engineered tissues can also help advance our understanding of epigenetic regulation of diseases.

### Scaffolds

Most cells require a scaffold to serve as a structural and logistic template for attachment, tissue formation, and remodeling. In general, the scaffold is designed to mimic the composition, structure, and biomechanics of the native tissue matrix. The role of the scaffold is only temporary, and it should biodegrade at the rate allowing deposition and remodeling of new ECM. Tissue engineering has largely driven the evolution of permanent scaffolding materials (such as nylon) into those biodegrading at a given rate, and inert scaffolds (polyesters) into those with bioactive properties (chemically modified materials with ligands and conductive polymers). Preparations of native tissue matrix from decellularized tissues are becoming increasingly common, including mineralized bone matrix as a scaffold for bone tissue (Chramiec et al., 2020; Jusoh et al., 2015) or fibrin for the formation of muscle (Ronaldson-Bouchard et al., 2019).

The field is now starting to explore adaptive-responsive biomaterials that can sense and actuate cells or respond to the local environment to drive functional restoration of complex tissue structures. This new class of scaffold materials will allow a shift from predetermined scaffold properties to scaffolds that can change in response to cellular and environmental signals, or on demand.

### Bioreactors

Certain conditions and duration of culture are needed for the collections of cells to organize into functional tissue units. Approaches that rely on mimicking the native organ environment have become increasingly successful, including the use of medium flow to mimic blood perfusion and electrical and mechanical stimuli to drive the maturation of muscle, neural tissues or bone (Alimperti et al., 2017; Blundell et al., 2016; de Graaf et al., 2019; Ronaldson-Bouchard et al., 2018; Vatine et al., 2019; Zhao et al., 2019). Culture systems (bioreactors) are designed to control environmental conditions; exchange oxygen, nutrients, and metabolites; and provide the molecular and physical regulatory factors.

The requirements for bioreactor design and operation can be different for OOCs than for engineering large grafts for regenerative medicine, and they vary from one tissue type to another. In general, clinically sized tissue grafts cannot be kept alive without vascular perfusion, as a hypoxic core rapidly forms once the oxygen diffusion length exceeds $\sim$100–200 μm. The only exception are thin tissues, such as skin or bladder. However, vascularization is also important for achieving biological fidelity of cultured tissues, as it supports paracrine interactions between the cells. Small sizes of tissues in OOCs allow control of microenvironmental cues across short diffusional distances, thereby maintaining tissue viability and function. Dynamic mechanical stresses can be imposed through organ-specific pressures, mechanical stress, and pulsatile blood flow, along with the provision of spatiotemporal regulation of biochemical signals and concentration gradients. It is interesting that unmet challenges in one area (regenerative medicine) propelled another area (OOC) into a fast-track application of tissue engineering.

Increased complexity may be needed to mature tissues and create high-fidelity biological models of health and disease. More complex questions require more complex models, while straightforward models (i.e., those designed to analyze the effects of a single point gene mutation) can be well served by rather simple approaches that avoid confounding effects and facilitate interpreting the results. As complexity increases, so does the physiological relevance of the developed model (Oyirifi et al., 2019). However, increasing complexity reduces the level of user control and increases biological variability, which can complicate interpretation of the results. To determine the necessary level of complexity, it can be useful to first determine the context of use for the model and then work backward to define which variables need to be recapitulated to answer a specific question in a most straightforward way.

Cells respond to the entire context of their environment, *in vivo* and *in vitro*, including cytokines, surrounding cells, ECM, and physical forces (Figure 1). Cell fate is a result of the combined effects of environmental factors, which can lead both to the favorable (differentiation and self-renewal) and unfavorable (apoptosis and de-differentiation) outcomes. The purpose of tissue engineering is to enable the cell to conduct its biological function by providing an appropriate environment for the formation, maintenance or maturation of a specific tissue. In turn, we may also create a specific pathological environment if the goal is to establish a model of injury or disease to evaluate therapeutic modalities. These considerations are the scientific premise for biomimetic principles of tissue engineering. Some of the universal requirements are to include multiple cell types, scaffolds with appropriate ECM signaling and physical signals, and achieve some level of maturation. More sophisticated models incorporate provisions for vascular perfusion, tissue connectiveness, systemic factors (immune cells, cytokines), and, for some tissues (such as the heart and bone), the structural and mechanical anisotropy. Incorporation of inducible cell reporters further allows longitudinal dynamic studies of the dynamics and progression of disease, injury, and healing.

## SINGLE-OOC MODELS

The scientific premise for engineering single-OOC devices is that appropriate function will follow the appropriate form, because the creation of a cell-matrix structure emulating that in a native organ will guide the cells to assume an appropriate function. Based on the function they are modeling, OOC approaches can be broadly classified into *barrier function devices* and *parenchymal function devices*. In terms of hardware design, two general trends emerge: (1) closed devices, with sealed channels and pump-driven flow; and (2) open devices that resemble well plates and are perfused by gravity-driven flow or use rocking platforms. Each design has advantages and disadvantages (Figure 3).

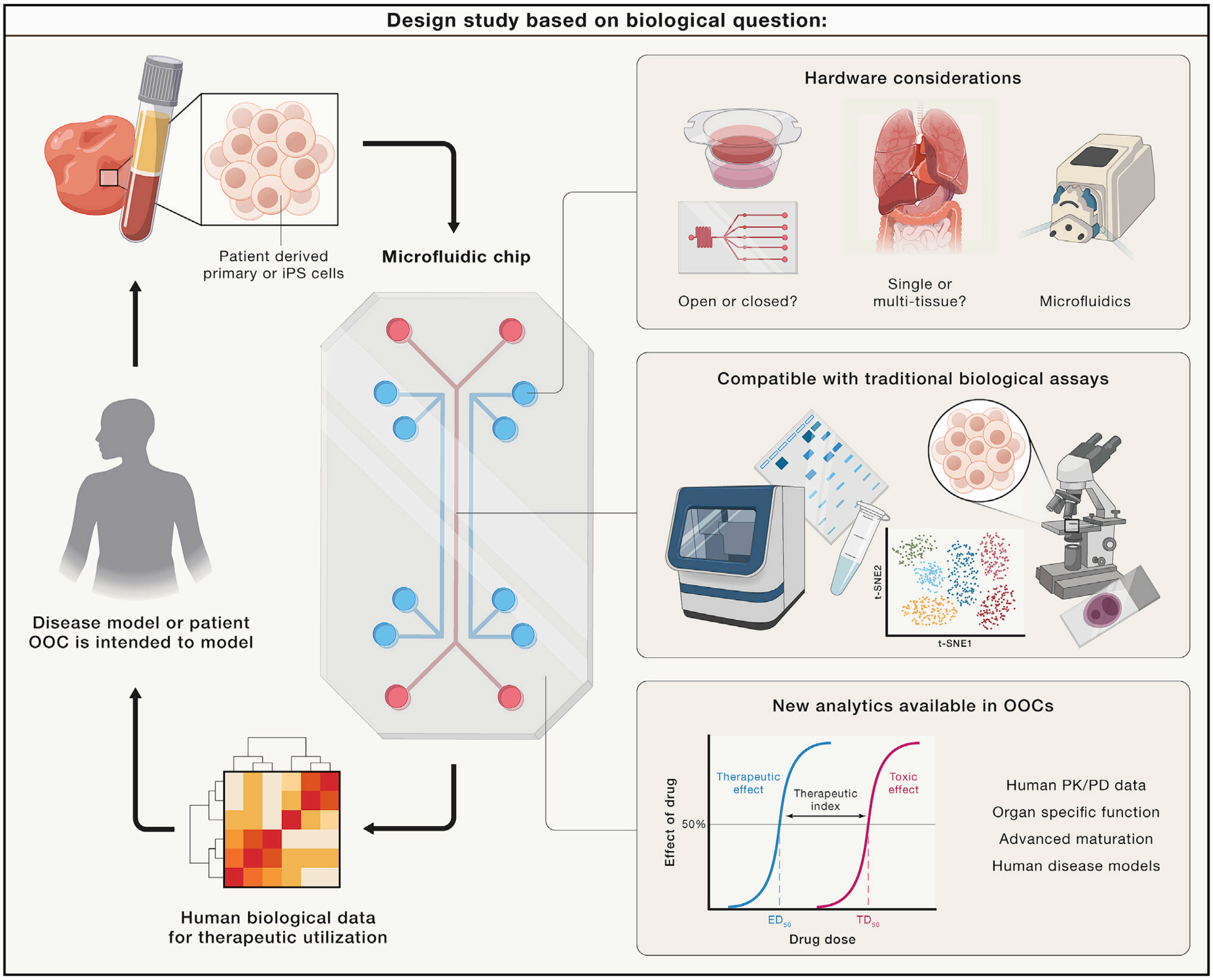



**Figure 3. Biological question drives design considerations**
The configuration of OOCs can be selected depending on the biological question studied, among the open or closed, single- or multi-organ OOCs, with gravity- or pump-driven flow. OOCs are compatible with traditional assays such as immunofluorescence, -omics, or the use of ion or membrane and calcium dyes. They enable cell maturation, not routinely possible in 2D culture, and the tissue-specific readouts such as contraction force, barrier function, and impulse propagation.

Whereas pump-driven flow is highly controllable and allows complex flow patterns, device operation, fluid and cell sampling can be more difficult. In contrast, open devices allow facile tissue and media retrieval at the expense of less precise environmental control.

Recent years have seen an explosive growth of companies commercializing OOC devices (~30 companies in 7 years) (Zhang and Radisic, 2017) fueled by their promise to transform drug discovery (Esch et al., 2015). The one-size-fits all paradigm in biological and medical research can now be overcome by using OOCs made using donor-specific genetically defined cells. This approach opens a whole range of possibilities for individualized studies and parsing out the effects of genetic and environmental factors (Figure 3). An overview of single-OOC models is shown in Figure 4, with comprehensive details provided in a recent review (Zhang et al., 2018).

## STUDIES OF TISSUE DEVELOPMENT AND FUNCTION

### Barrier function

Barrier function was the first one to be established using an OOC approach in a lung-on-a-chip device (Huh et al., 2010) (Figure 4A) that can recapitulate many epithelial barriers. For example, by cultivating podocytes on one side of the membrane and endothelial cells on the other side, it is possible to establish a barrier that selectively filters species according to molecular weight, thus mimicking the function of kidney glomerulus (Figure 4B). While large molecules (albumin and antibodies such as immunoglobulin G [IgG]) remain in the endothelial compartment, small molecules (inulin) can cross the barrier (Zhou et al., 2016). The barrier function weakens at the higher flow rate/pressure, thus exhibiting hallmarks of hypertensive nephropathy. Also, the curvature affects gene expression in endothelial cells and stability of

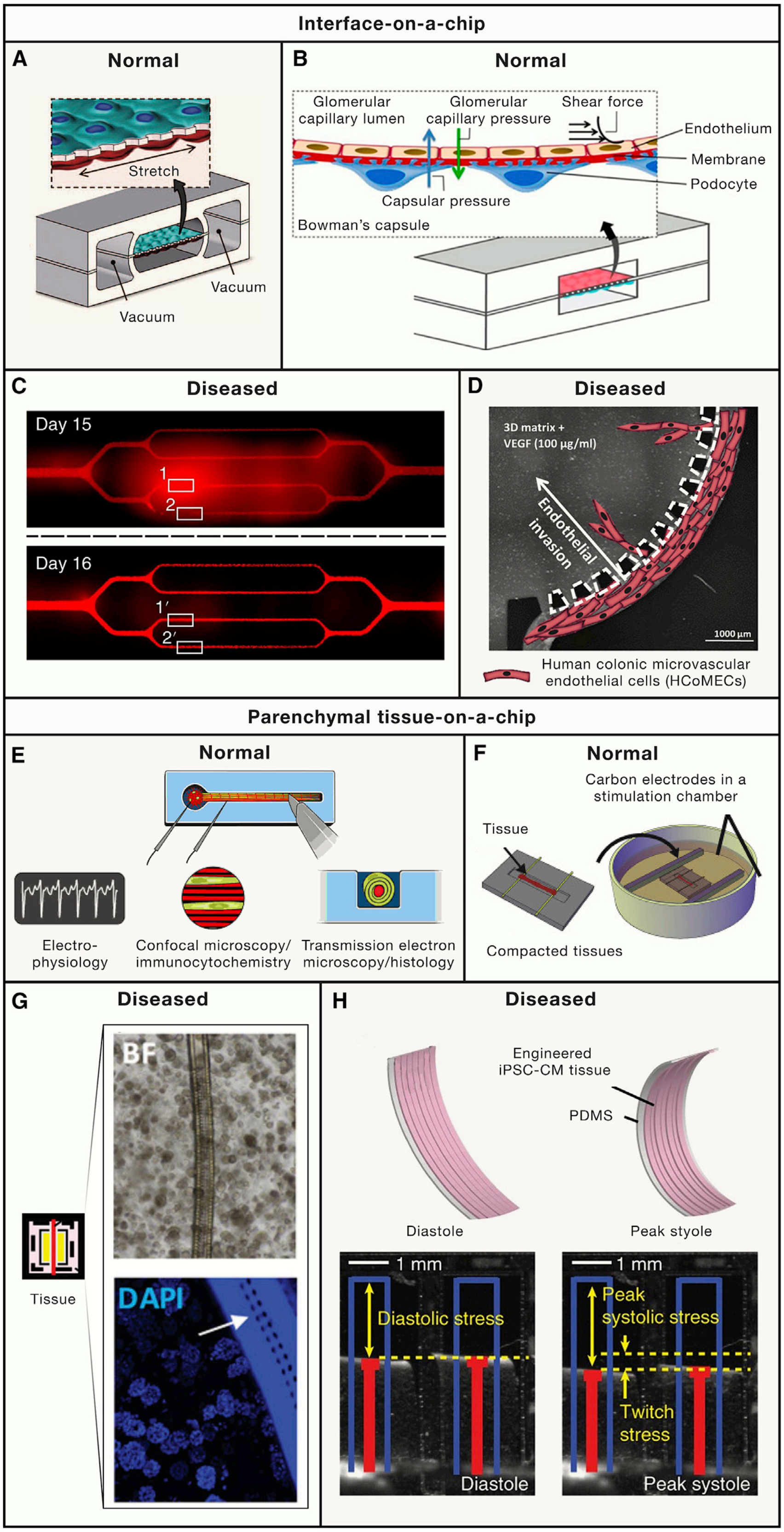



**Figure 4. Representative examples of single OOCs for studies of organ functions**

(A) In the category of interface-on-a-chip devices, the lung-on-a-chip device re-creates epithelial/endothelial barrier function. Reproduced with permission from Huh et al. (2010).

(B) Glomerulus-on-a-chip re-creates podocyte/endothelial barrier function. Reproduced with permission from Zhou et al. (2016).

(C) Interface-on-a-chip devices can be used to study increases in permeability due to disease. Sickle cell occlusion of vasculature-on-a-chip. Reproduced with permission from (Qiu et al., 2018).

(D) Endothelial invasion in a colorectal tumor. Reproduced with permission from (Carvalho et al., 2019a).

(E) In the category of parenchymal tissue devices, peripheral-nerve-on-a-chip can be used to study electrophysiological properties due to drug toxicity. Reproduced with permission from (Sharma et al., 2019a).

(F) The Biowire II platform established functional hallmarks of human ventricular and atrial myocardium. Reproduced with permission from (Zhao et al., 2019).

(G) Parenchymal-tissue-on-a-chip devices can be used to re-create the pancreatic cancer microenvironment. Reproduced with permission from (Lai et al., 2020).

(H) Cardiomyopathy of a genetic disease, Barth syndrome. Reproduced with permission from Wang et al. (2014).

vasculature in collagen hydrogel (Mandrycky et al., 2020), an important consideration for epithelial/endothelial barriers. A great degree of complexity in shape control was achieved by 3D printing of a proximal tubule-on-a-chip (Homan et al., 2016), microfluidic spinning of glomerulus-on-a-chip (Xie et al., 2020), and stereolithographic printing of microvasculature and lung alveoli in photopolymerizable hydrogels using food colors as photoabsorbers (Grigoryan et al., 2019).

### Parenchymal function

A nerve-on-a-chip system consisting of a spheroid embedded into a PDMS-based well with a single extending channel enabled co-culture of human Schwan cells and neurons with robust myelin formation enabling delineation of electrophysiological responses upon application of various signals and agents (Sharma et al., 2019a) (Figure 4E).

Heart muscle has been extensively studied in OOC devices, since cardiovascular disease still kills more people than all cancers combined. The need for human cardiac tissue models is highlighted by drug recalls due to cardiotoxicity (e.g., Micturin, Fen-phen, Seldane, Vioxx, and Avandia) that were due, at least in part, to the low ability of animal models and cell cultures to predict the patients' responses (Piccini et al., 2009). Immature phenotypes of human heart muscle derived from iPSCs have limited their utility.

Human heart muscle has been grown from iPSCs in hydrogel anchored at the two ends (Figure 4F), which also allowed investigators to measure contraction force through the deflection of PDMS posts (Hinson et al., 2015) or cantilevers (Wang et al., 2014) that provide resistance to contractions (Figure 4H). In later studies, PDMS has been replaced by inert plastics (Zhao et al., 2019) (Figure 4F). Electrical stimulation of heart muscle formed from early-stage iPSC-derived cardiomyocytes at a gradually increasing frequency markedly advanced tissue maturation (Ronaldson-Bouchard et al., 2018, Nunes et al., 2013). Such "intensity training" of heart muscle resulted in adult-like gene expression profiles, oxidative metabolism, remarkably organized ultrastructure with physiologic sarcomere length and density of mitochondria, networks of transverse tubules, positive force-frequency relationship, and functional calcium handling (Ronaldson-Bouchard et al., 2018). The atrial versus ventricular specification was achieved by ramping the stimulation frequency at two different rates (Zhao et al., 2019) and allowed modeling of left ventricular hypertrophy using cells from patients.

Anchoring the forming tissue also enabled engineering of skeletal muscle and neuromuscular junction (NMJ). In one study, patient-derived muscle progenitors were mixed with iPSC-derived motor neurons (Afshar Bakooshli et al., 2019). In another study, the first patient-specific model of NMJ was established using muscle cells and optogenetically edited motoneurons to model myasthenia gravis, an autoimmune disease resulting in NMJ dysfunction (Vila et al., 2019). Similar to cardiac models, skeletal muscle models can also be matured using electrical stimulation (Khodabukus et al., 2019) and derived from human iPSCs (Rao et al., 2018).

Liver-on-a-chip is one of the most commonly used OOCs as a model of metabolic function and drug toxicity. The importance of heterotypic interactions between hepatocytes and fibroblasts was recognized early on (Hui and Bhatia, 2007), resulting in a commercialized Hepatopac system. Sensing of drug injury was demonstrated through incorporation of Kupffer cells into Organovo's 3D-printed liver spheroids (Norona et al., 2019). Emulate designed a rat, dog, and human Liver-Chip containing primary hepatocytes and liver sinusoidal endothelial cells, with or without Kupffer and stellate cells. This study found liver toxicity and fibrosis from compounds that were discontinued upon rodent studies, proving that OOCs can detect similar effects in rodent-based devices and predict liver injury in humans (Jang et al., 2019). Recent PDMS-free OOCs with separate hepatic and vascular channels (incorporating primary human hepatocytes and sinusoidal endothelial cells and stellate and Kupffer cell lines) recapitulated the liver acinus and oxygen zonation (Li et al., 2018).

Lymph-node-on-a-chip was modeled using a PDMS channel with two inlets and two outlets, supporting a monolayer of dendritic cells to which T cells can be bound during flow in an antigen-specific manner (Moura Rosa et al., 2016).

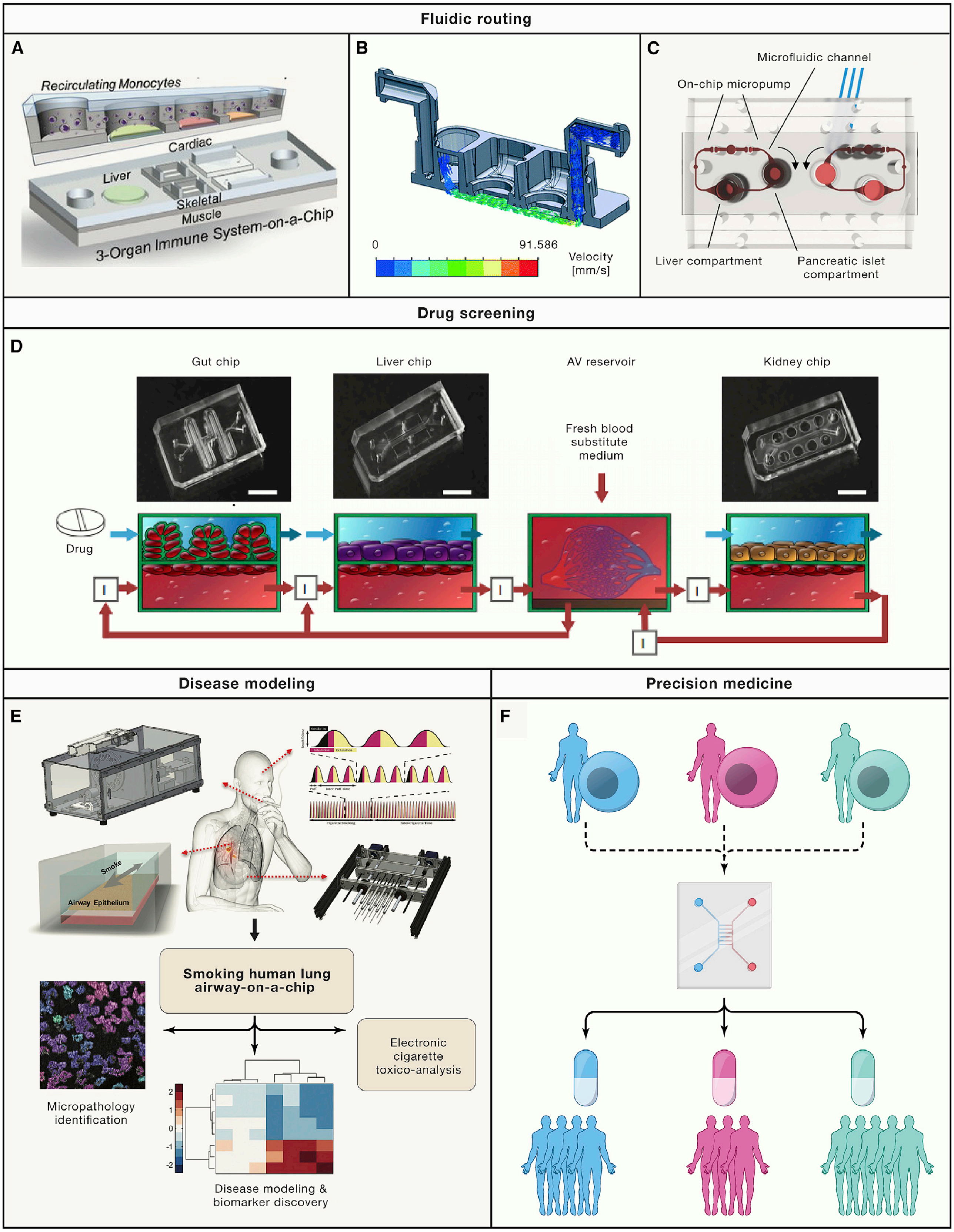
Human-eye-on-a-chip presents the next frontier in OOC, capturing imagination at the same time. The challenge is particularly difficult due to the concavely hemispherical retina structure, a wide field of view, high resolution, and adaptivity to the optical environment. Human corneal and conjunctival cells were combined in a hemispherical device in the shape of the eye. The patterned cells are periodically lubricated by a hydrogel lid to create a blinking-eye-on-a-chip (Seo et al., 2019). A mimic of human retina with all seven cell types was created in an OOC with organoids and microvascular channels (Achberger et al., 2019).

### Microvasculature

AngioChip was shown to support the assembly of different types of parenchymal cells in a mechanically tunable matrix surrounding a perfusable microchannel network lined with endothelial cells. The design of AngioChip decouples the engineered vessel network and parenchymal tissue, enabling extensive remodeling while maintaining open-vessel lumens. The incorporation of nanopores and micro-holes in the vessel walls enhances intercellular crosstalk in vascularized heart and liver OOCs (Zhang et al., 2016). This platform can be scaled down to a footprint of a 96-well plate (Figure 4G) using the InVADE system, requiring only 100,000 cells per tissue and enabling facile connections of tumor and liver tissues (Lai et al., 2017; Lai et al., 2020). Up to 128 independent vascularized colons-on-a-chip were formed in a 384-well-plate platform with gravity flow that supported modeling of colon inflammation with innate immune function (Rajasekar et al., 2020). An OOC model of vascularized bone was also created by inducing microvascular flow into the mineralized ECM populated by bone-forming cells (Jusoh et al., 2015). Recent studies are also focusing on capillary, venous, arterial, and lymphatic vascular networks (Grigoryan et al., 2019).

### Studies of injury and disease

Whereas endothelial barrier can capture hallmark functions of some organs, studies of cell trafficking and crosstalk require the formation of perfusable vasculature (Figures 4E–4G). Endothelial networks can be maintained for a month, using a branching multichannel device with interpenetrating hydrogel

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recapitulating the blood vessel intima, to study the inflammatory mediators of barrier function and sickle cell occlusion (Qiu et al., 2018) (Figure 4C).

As fibrosis affects essentially all organs as the end stage of various diseases and injuries, recent OOC studies have focused on recapitulating fibrosis (Hayward et al., 2021). This approach is especially important in modeling the cancer microenvironment due to the recognized role of cancer-associated fibroblasts in driving and promoting cancer metastasis. A multi-layered lung-on-a-chip with patterned vasculature and lung channels was used to form cystic-fibrosis-like epithelium and study neutrophil migration (Mejías et al., 2020). To generate fibrotic interstitium, fibroblasts from donors with idiopathic pulmonary fibrosis and healthy fibroblasts treated with transforming growth factor $\beta 1$ (TGF-$\beta 1$) were incorporated into the fibrin/collagen hydrogel at the air/liquid interface.

The effects of ionizing radiation and myeloerythroid toxicity after chemotherapy were studied in a bone marrow OOC, created using an approach similar to the lung OOC, with two parallel channels separated by the porous membrane. The vascular compartment was lined with endothelial cells, whereas the bone marrow compartment was created by filling the parallel channels with fibrin-gel-encapsulated CD34$^+$ cells that supported differentiation and maturation of multiple blood cell lineages over 4 weeks (Chou et al., 2020).

The alcoholic fatty liver disease was modeled by exposing an Emulate liver OOC to the ethanol levels consistent with those in the human blood, resulting in structural changes in bile canaliculi and accumulation of lipid in hepatocytes (Nawroth et al., 2020).

A tumor OOC was used to generate organotypic patient-specific blood vessel models using normal and renal-carcinoma-associated primary CD31$^+$ cells. RNA sequencing of blood vessels allowed selection of candidate drugs, ultimately leading to the testing of sirolimus and nintedanib (Virumbrales-Munoz et al., 2020). Such models are becoming invaluable for assessing the efficacy of drug-loaded nanoparticles in emerging therapies (Figure 4D) (Carvalho et al., 2019a).

A Synovium OOC containing embedded organic-photodetector arrays has been developed to study rheumatoid arthritis. Patient-derived primary synovial organoids were cultivated in the absence and presence of tumor necrosis factor by noninvasive detection of changes in tissue architecture via light scattering (Rothbauer et al., 2020).

OOCs have been invaluable for the studies of SARS-CoV-2 and other viral infections associated with organ dysfunction (Tang et al., 2020). Emulate's lung OOC was used for screening US Food and Drug Administration (FDA)-approved protease inhibitors to curb the injury resulting from influenza and inhibit pseudotyped SARS-CoV-2 viral entry (Si et al., 2020). The heart OOC demonstrated profound contractile dysfunction (Marchiano et al., 2020), consistent with the recent reports of cardiac side effects in asymptomatic and recovering coronavirus disease 2019 (COVID-19)-infected individuals (Topol, 2020). Importantly, OOCs uniquely allow delineating the effects of SARS-CoV-2 infection on the main functional cell types in the organ from indirect effects of inflammatory cytokines.

## MULTIPLE OOC MODELS OF DISEASE

Studies of human physiology necessitate the use of holistic models that capture how cells, tissues, and organs work in conjunction with one another during normal homeostasis or disease. Multi-OOCs rely on connection via recirculating media that enables communication via secreted factors, extracellular vesicles, and circulating cells. Here, we describe the multi-OOCs used to study the systemic development and function of multiple connected tissues, progression of disease, and their utility for therapeutic screening. An overview of multi-OOC models is shown in Figure 5.

### Combining single-organ models to create multi-organ models

The single-organ models can be fluidically connected to create multi-organ models by co-culture in shared media (Wikswo et al., 2013), external media transfer (Vernetti et al., 2017), recirculation using pneumatic pressure-driven actuation (Satoh et al., 2017), and peristaltic pumps (Chramiec et al., 2020) (Figures 5A–5C). Advanced circuit designs account for the blood flow each organ receives *in vivo* (Edington, 2018)

Most multi-organ settings include liver, as the primary site of drug metabolism, required for prodrug transformation into its active form. More advanced systems are now being developed to provide a tissue-specific niche for each tissue module, mimic the systemic vascular network, and include routing of culture media and biosensors for online readouts (Achberger et al., 2019; Trapecar et al., 2020; Xiao et al., 2017). Such systems allow scaling of organ volumes and blood flow to match the *in vivo* situation while enabling high-throughput screening and extended culture times (Marx et al., 2016). Linking multiple tissues by vascular perfusion has impact on each tissue (by increasing its biological fidelity through cellular crosstalk within *in vivo* like tissue environment) as well as on the entire multi-organ model (by providing systemic components such as metabolism, immunity, and clearance that are needed for physiological studies). The advantages of microfluidic multi-organ models support their continued development despite additional complexities (Sung et al., 2010).

### Modeling development, physiology, and systemic disease

Multi-organ models are designed for a range of applications in drug screening, disease modeling, and precision medicine studies (Figure 5).

**Figure 5. Representative examples of multi-OOC devices and their utility**

(A–C) Single OOCs can be connected by fluidic routing to facilitate inter-organ communication via recirculating shared media perfused above cells (A) (reproduced with permission from Sasserath et al., 2020), pump-driven recirculation below engineered tissues (B) (reproduced with permission from Chramiec et al., 2020), and on-chip micropumps (C) (reproduced with permission from Bauer et al., 2017).

(D–F) These multi-OOC devices can be used for human drug screening (D) (reproduced with permission from Herland et al., 2020), disease modeling (E) (reproduced with permission from Benam et al., 2016), and precision medicine approaches (F).

To disentangle the complexity of the human bone marrow, a multi-OOC was developed that connected the periarterial, perisinusoidal, mesenchymal, and osteoblastic components of the human bone marrow niche (Aleman et al., 2019).

The female reproductive system has been modeled in a multi-OOC to study hormonal signaling during the menstrual cycle and pregnancy-like endocrine loops by connecting ovary, fallopian tube, uterus, cervix, and liver modules via microfluidic routing (Xiao et al., 2017).

Using microfluidics to connect a module containing human pancreatic islet microtissues with a module containing liver spheroids, hormonal feedback between the two organs could be modeled *in vitro* (Bauer et al., 2017). These advances show promise for decoupling the behaviors of individual organs during homeostasis and identifying factors that drive metabolic and hormonal diseases.

Systemic diseases provide a real opportunity for the use of multi-organ platforms, as their mechanisms are not adequately recapitulated in animal models. An excellent example is the physiomimetic multi-organ platform containing gut and liver modules connected by fluidic circulation containing circulating Treg and Th17 immune cells to create a human multi-organ model of ulcerative colitis (Trapecar et al., 2020). The system was paired with multi-omics to reveal that short-chain fatty acids either improved or worsened the disease, and that these opposing responses were dictated by CD4+ T cell effector function. This study uniquely demonstrated how human multi-organ systems can be leveraged to better understand the immune and metabolic regulation of human pathophysiology.

The development of degenerative brain diseases like Alzheimer's or Parkinson's disease has been connected with gut health (Alkasir et al., 2017). Recapitulation of the inter-organ brain-gut-immune axis using multi-OOC allowed studies of how these organ systems evolve in response to one another. While the progression from a healthy to diseased state is difficult to model *in vivo*, multi-organ platforms provide human tissues and bioengineering tools to do just this. The inclusion of a patient's microbiome into multi-organ models allows predictive insights into how a patient will respond to a drug therapy (McCracken et al., 2014).

Cancer metastasis is another systemic disease that would greatly benefit from multi-OOCs. Cancer cells have a complex interplay with their surrounding microenvironment, immune system, and metastatic sites. The advanced control offered by multi-OOCs enables decoupling of these networks and identification of key drivers of cancer progression, immune evasion, and drug resistance. Microfluidic models allow studying various components of the metastatic process, including cancer cell escape into the bloodstream (intravasation), changes that occur while in circulation, and cancer cell invasion into target tissues (extravasation). Cancer cell intravasation has been studied using cancer, immune, and vascular multi-organ models, revealing the role of tumor necrosis factor $\alpha$ (TNF-$\alpha$) in increasing endothelial permeability and cancer cell escape (Zervantonakis et al., 2012). Multi-organ models provide tissue-specific insight into cancer cell extravasation (Xu et al., 2016), as lung cancer cells acquire an invasive phenotype when co-cultured with healthy lung cells, to populate distant bone, brain, and liver organs within the multi-OOC. Breast cancers have similarly been modeled to show propensity for metastasis to bone (Jeon et al., 2015), a result commonly seen clinically but difficult to model in animal systems.

## Human OOCs for therapeutic screening

Multi-OOCs provide methodology to evaluate therapeutic safety, efficacy, immunogenicity, and pharmacokinetics/pharmacodynamics (PK/PD) in physiologically relevant human settings, prior to clinical trials, allowing refinement of clinical strategies to better direct the right drug, at the right dose and right time, to the right patient. Multi-organ models usually include the target organ of interest (i.e., tumor) and the organs related to drug metabolism (i.e., liver and kidney) and off-target toxicity (i.e., liver, heart). An example is the model with microfluidically linked liver, tumor, and marrow established by the OOC pioneer Schuler, using flow rates and residence times matching those in the human body (Sung and Shuler, 2009). The liver module was able to metabolize the prodrug tegafur into 5-fluoroucil, and this active drug induced cell death, as expected. Notably, liver cells cultured in a standard 96-well plate were unable to show this expected response.

## Models of human drug PK/PD (phase 1)

The initial driver for developing these OOC models was the need to identify human drug toxicities and optimize drug dosing to rapidly identify human therapeutic indexes—drug concentrations that are high enough to show an effect but low enough to avoid toxicity. OOCs accurately describe drug adsorption, distribution, metabolism, elimination, and toxicity (ADMET) as seen clinically. For example, naphthalene was converted in the liver module into its reactive metabolites and transferred to the lung module, where it depleted cellular glutathione levels and caused the accumulation of hydrophobic compounds in the fat module (Viravaidya et al., 2004).

The inclusion of endothelium can support the functionality of many OOC systems (Schepers et al., 2016) by separating tissue compartments while enabling their communication and is critical in modeling drug transport. A multi-organ human neurovascular unit developed by coupling blood-brain barrier module (containing human astrocytes and pericytes above a channel lined with brain microvascular endothelial cells) with the brain module (containing primary human brain neurons) recapitulated the blood-brain barrier's response to methamphetamine, revealing its selective penetration and the previously unknown metabolic coupling between neurons and the microvascular brain endothelium (Maoz et al., 2018).

Lung OOCs enable studies of inhaled drugs, pollution, and smoking (Benam et al., 2016). Similarly, skin OOCs enable drug entry through the skin, allowing screening of drug delivery and the safety of chemicals and cosmetics in contact with the skin (Pires de Mello et al., 2020). Combining computational models with experimental multi-organ systems, PK parameters were measured for cisplatin and nicotine delivered intravenously or orally to match the PK values demonstrated clinically (Herland et al., 2020).

### Models of human safety and efficacy (phase 2/3)

Multi-OOCs have been able to accurately model both the on-target effects of cancer therapies and off-target heart and liver toxicity for drugs involving liver metabolism (McAleer et al., 2019). The system was designed so that the drug first passed through the liver module, where it was converted into its active form and subsequently delivered to downstream tissue modules via fluidic recirculation.

Multi-organ model screening of the anti-cancer drug linsitinib recapitulated clinical findings, including limited efficacy of the drug in treating bone tumors and limited cardiac toxicity in the human setting (Chramiec et al., 2020). These results are in direct contrast to animal models that showed both high efficacy and high potential for cardiac toxicity. Notably, clinical trials more closely matched with the OOC, supporting the utility of this model for predictive insights into the potential of drugs before clinical trials. Insights into which patients are more likely to respond to a drug could help refine the scope of clinical trials.

### Personalized medicine

The top 10 clinical drugs in the United States only work in 4%–25% of patients (Schork, 2015). For systemic diseases (i.e., cancer, autoimmune diseases, fibrosis, infection, and inflammation), patient variability in disease presentation and therapeutic outcomes complicates the development of successful interventions. The use of patient-derived cells in multi-OOCs will equip biologists with tools to mechanistically interrogate and understand disease onset, progression, and treatment in a personalized manner. The utility of multi-organ models for such work has been demonstrated already, including the role of gut health in immune regulation of patients with ulcerative colitis (Trapecar et al., 2020) and responses to smoking in patients with chronic obstructive pulmonary disease (COPD) (Benam et al., 2016).

## PERSPECTIVE AND CHALLENGES

Looking forward, the key question is what is necessary for OOCs to become broadly used. We summarize here the challenges and prospects for realizing the full potential of OOC in biological research.

### OOC availability

Current OOCs are developed in academic labs as artisanal devices, handmade at a production throughput of just a set of devices per day. Standardization and quality control that are essential for reproducible functionality of these devices vary from one design to another and are limited. To support the broad use in biological research, standardized OOCs need to become as available as are cell culture plates and reliably manufactured on an industrial scale. The first steps in this direction have been made, as several companies (Emulate, inSphero, Mimetas, Tissuse, Nortis, and CN Bio) now offer OOCs for culturing one or more tissue types, enabling entry into the field for new groups. Biological experimentation is expected to continue driving the development of OOCs and advancing their performance, which require mass production of standardized, inexpensive, and configurable devices.

### Inert OOC materials

PDMS is currently commonly used for making the entire OOCs or their components (such as elastic anchors), including the commercial OOCs listed above, because of its ease of processing. As PDMS absorbs hydrophobic compounds, most critically oxygen and many drugs, it hinders control of their concentrations in the cellular environment (Toepke and Beebe, 2006). While the need to replace PDMS with inert plastics has been long recognized, some progress has been made only recently by accounting for drug absorption (Herland et al., 2020) or applying inert coatings (Herland et al., 2020).

### Online functional readouts

Noninvasive data acquisition in longitudinal studies where the same biological sample is repeatedly evaluated over time are invaluable for increasing consistency of experimentation and capturing the biological dynamics. This way, each sample becomes an experiment by itself ("n=1 study"; Lillie et al., 2011), allowing monitoring of the evolution of biological events, or responses to perturbation. Because these studies generate large volumes of data, OOCs are now starting to attract the artificial intelligence and machine-learning approaches to data interpretation and experimental design. Also, the concept of a "digital twin" (a physical or computational replica of the actual system) is being extended to OOCs to advance studies of emergent cellular behaviors. Cells themselves can be tracked in space and time using inducible reporters of cell state or function (Mathur et al., 2015) or on-demand activation (e.g., by optogenetic methods; Vila et al., 2019). Many studies have taken advantage of measuring in real time the contraction frequency and force generation of heart and skeletal muscle from deflection of the anchors at the two ends of the tissue (Mannhardt et al., 2016). Likewise, calcium flux measurements are already well established for a number of tissue systems (Goldfracht et al., 2019).

### Establish and maintain mature tissue phenotypes

An essential requirement for utility of OOCs is the authenticity of molecular, structural, and functional tissue phenotypes, which in turn involves cell types, maturity, and long-term culture with tissue-tissue interactions.

Primary cells have a well-established identity and heterogeneity, but access to these cells can be limited (e.g., neural and heart cells), thus not allowing patient-specific studies. In contrast, iPSCs are routinely derived from small samples of blood and differentiated into a number of lineages that can be used to derive tissues of interest. iPSCs provide consistency, comparisons across the labs, and patient-specific OOCs that allow parsing out genetic and environmental factors, studies of genetic diseases, and biologic diversity. A long-standing limitation of iPSCs, the immaturity of the resulting tissues, is being addressed by inclusion of supporting cells and metabolic and physical regulatory factors (Aleman et al., 2019; Alimperti et al., 2017; Bauer et al., 2017; Benam et al., 2016; Blundell et al., 2016; Keung et al., 2014; Kostrzewski et al., 2019; Lin et al., 2019; Musah et al., 2017; Ronaldson-Bouchard et al., 2018; Trapecar et al., 2020; Zhao et al., 2019).

The required level of maturity and proper benchmarks are not clearly defined and depend on the biological question. The molecular, structural, and functional features can develop at different rates and to a different extent. An example is the engineered heart muscle matured by electromechanical conditioning with the force generation and conduction developing more slowly and to a lesser extent than structural features (Ronaldson-Bouchard et al., 2018; Ronaldson-Bouchard et al., 2019).

Maintaining the individual tissue phenotypes for weeks to months while allowing their communication remains a challenge. The new OOC designs that utilize physiological principles of tissue and organ communication are needed to overcome the limitations of sharing medium by all tissue types.

### Biological complexity

A great value would come from establishing vascular connections, innervation, immune system, and tissue interfaces in OOCs and the ability to build complexity on demand. The biological fidelity will also depend on adding innervation, immune system, and microbiome. The immune and endocrine systems are particularly important, as they interact with all organ systems in the body and are poorly modeled in animals (Habert et al., 2014). A major challenge is the derivation of innate, adaptive, and tissue-resident immune cells from iPSCs. Bone marrow OOCs with functional multipotent hematopoietic pool of stem cells (HSCs) (Chou et al., 2020) could provide a renewable source of immune cells in OOCs. Current OOCs rely on adding immune cells into the tissues or perfusate. For myeloid cells, one does not need to match the tissues with the donor immune cells, but with lymphoid cells, matching may be needed. Another approach is to use a common HLA-null iPSC line to generate "agnostic" tissues that can be combined with the patient's immune cells. Recent studies also started establishing patient-specific microbiota in OOCs.

### Address patient diversity

Humans are living longer than ever before, and healthcare is entering the era of healthy aging and precision medicine. Forward-thinking healthcare would greatly benefit from OOCs, which can provide patient-specific models of human pathophysiology. Notably, OOCs enable systematic studies of the diversity of population with respect to racial/ethnic background, sex, and age to help address current health disparities. By looking for commonalities between the clinical and *in vitro* data, we could identify shared mechanisms related to disease risk, discover early-stage biomarkers, monitor disease progression, and determine optimal therapeutic treatment regimens in a personalized manner.

## SUMMARY

Our goal was to explore the use of OOCs in biological research and explain why they have so rapidly evolved in recent years. We distinguish OOCs from organoids and describe the design and applications of OOCs representing a single-tissue unit (e.g., bone marrow, neuromuscular junction, lung alveolum) and multiple units linked to recapitulate more complex physiological functions (e.g., cancer metastasis, infection). Importantly, OOCs do not recapitulate the entire organ but instead approximate just one or few organ-level functions, such as barrier function of the lung, contractile function of the heart, or filtration in the kidney.

We propose that OOCs are en route to becoming broadly accepted in biological research, as they can offer biologic fidelity along with experimental control not provided otherwise, while still being sufficiently easy to use. We believe that OOCs will realize this promise through the development of (1) standardized user-friendly designs that are readily available at low cost; (2) real-time measurements; (3) maintenance of tissue phenotypes over weeks to months, while allowing their communication by vascular perfusion; and (4) incorporation of innervation, immune system, metabolism, and microbiome, if needed. Biological experimentation requires that these features are complemented by versatility of design configurations and operating conditions allowing the basic designs to be customized for addressing a specific question.

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## DECLARATION OF INTERESTS

G.V.-N., K.R.-B., and M.R. are co-founders and equity holders of TARA Biosystems that uses Biowire II platform for commercial drug testing. M.R. and G.V.-N. receive consulting fees and royalty from TARA Biosystems.

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