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Review

Organs-on-a-Chip: A Fast Track for Engineered Human Tissues in Drug Development

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https://doi.org/10.1016/j.stem.2018.02.011

Organs-on-a-chip (OOCs) are miniature tissues and organs grown *in vitro* that enable modeling of human physiology and disease. The technology has emerged from converging advances in tissue engineering, semiconductor fabrication, and human cell sourcing. Encompassing innovations in human stem cell technology, OOCs offer a promising approach to emulate human patho/physiology *in vitro*, and address limitations of current cell and animal models. Here, we review the design considerations for single and multi-organ OOCs, discuss remaining challenges, and highlight the potential impact of OOCs as a fast-track opportunity for tissue engineering to advance drug development and precision medicine.

Modeling integrated human physiology *in vitro* is a formidable goal with the potential to transform biological research and eventually healthcare. Current studies largely rely on simple human cell cultures or rodent models. While the reductionist approach to determining organ-level behavior in cell culture is directly scalable and robust, its predictive power is limited by the lack of biological functionality. In contrast, animal models replicate organ- and multi-organ-level function but are inherently flawed due to differences between animal and human physiology. Organs-on-a-chip (OOC) platforms seek to combine the best of both models by culturing human cells in tissue-specific tridimensional settings designed to recapitulate the multifaceted cellular and extracellular cues—molecular, structural, and physical—that are found *in vivo* for a given organ system.

While OOCs evolved from tissue engineering, the goal is not to build a whole living organ but rather to establish a minimally functional unit that can recapitulate certain aspects of human physiology in a controlled and straightforward manner. For example, cells cultured on membranes can recreate interfaces between different tissues, such as alveolar-capillary interface (Huh et al., 2010) or blood-brain barrier (BBB) (Booth and Kim, 2012; Brown et al., 2016; Griep et al., 2013), while multicellular patterns can be designed to enable communication between different cell types (Khetani and Bhatia, 2008; Cho et al., 2010). For most tissues, OOCs need to incorporate physical forces—hydrodynamic (Moya et al., 2013), mechanical (Marturano-Kruik et al., 2015), and electrical (Nunes et al., 2013; Tandon et al., 2009)—to enable the organ-specific functionality and subsequent maturation necessary for physiological relevance of the measured data. Multiple organs can be integrated by linking individual OOCs through microfluidic channels with volume ratios and flow distribution that mimic physiological coupling *in vivo* to create *in vitro* models of subsystems of the human body (Wikswo et al., 2013b).

Organs-on-a-Chip: Addressing Unmet Needs

Overall, the twin incentives of de-risking drug development and personalizing patient treatment can be realized through the use of OOCs that capture the diversity of human genetics, physiology, and pathology. The first attempts to integrate cell culture with microfluidics, resulting in the precursors of today's OOCs, were introduced in 2003 (Park and Shuler, 2003). Today's OOC platforms, utilizing microfluidics and tridimensional cell culture to engineer micro-sized human tissues and organs, can help accelerate drug development by resolving the discrepancies in drug safety and efficacy observed between animal models, cell culture, and clinical studies, as summarized in several recent reviews (Bhatia and Ingber, 2014; Gintant et al., 2016; Gobaa et al., 2011; Passier et al., 2016; Polini et al., 2014; Zhang and Radisic, 2017). The quantitative and mechanistic data collected using these human OOC models could also revolutionize clinical trials, the costliest and riskiest stage at which many drugs fail. Instead of treating future patients as a collective group, the use of patient-specific cells allows capturing the important differences that are due to the genetic diversity, ethnicity, sex, and age of the patients. The same precision medicine approach can enable the development of *in vitro* clinical trials for patient populations unfit for standard clinical trial designs (i.e., rare and pediatric diseases) or to develop drug regimens that are optimized for specific patient biology. Additionally, the adoption of OOCs by industry will facilitate current efforts to reduce, refine, and ultimately replace animal models (3Rs) with more ethical options (Russell and Burch, 1959; Holmes et al., 2010)

Design Principles for Organs-on-a-Chip

What Are Organs-on-a-Chip?

OOCs are designed to guide the collections of cells to assemble into tridimensional tissue constructs representing simplified yet realistic models of their organ-level counterparts with a functionality that matches the intended application (Ahadian et al., 2018; Huh et al., 2010; Lelièvre et al., 2017). OOCs seek to combine the ease of traditional human cell culture with the high levels of biological fidelity inherent to whole organ systems using simplistic approaches that yield functional tissue units capable of predicting organ-level responses. To accomplish this, OOCs take advantage of control strategies and multiparametric approaches designed for microfluidic systems (Park and Shuler, 2003; Rothbauer et al., 2018; Smith et al., 2013; Xu et al., 2016). These

microfabrication methods were initially designed for the electronics industry, hence the "chip" portion of the OOC name.

Design Considerations

When designing OOCs, the first steps are to determine the set of functional characteristics of the organ being modeled, the parameters of the specific question being addressed, and the readouts required to answer the intended question or serve the intended application. An appropriate level of the OOC complexity is then defined, while being mindful of compatibility with drug screening approaches and ease of use. Online monitoring assays can increase throughput and enable evaluation of functional changes and drug interactions, over periods of weeks to months. As OOCs enable concentration-response studies, they can be used to determine appropriate dosing regimens. Here, we summarize some of the key factors involved in the design of human OOCs.

Human Cell Types. The types of human cells used in OOCs are largely determined by cell availability and the ability to form functional tissues. Ideally, all organ units within an OOC would be made from the same source of cells. The advantages and disadvantages of using primary cells, cell lines, and derivatives of induced pluripotent stem cells (iPSCs) may further vary from the design of one organ to another.

For most human organs, primary cells are difficult to obtain, limited in quantity, and cannot be expanded in culture, preventing the development of OOCs using cells from the same individual to provide a genetically uniform background. The advantage of primary cells is that they are phenotypically mature and functional. While their functionality typically declines with time in culture, certain methods can help preserve the phenotype (such as cell co-culture or perfusion) (Abaci et al., 2015; Ataç et al., 2013; Esch et al., 2016). In contrast, cell lines are relatively easy to culture and expand, but typically lack the phenotypic function characteristic of the organ they intend to represent. While improvement can be achieved using perfusion and/or co-culture (Shah et al., 2016; Esch et al., 2012), OOCs generally use cell lines when better options are not available.

iPSCs could be an ideal and unlimited source of cells for OOCs as they are derived from a small cell or tissue sample (such as blood), are patient specific, and can be expanded and selectively differentiated into multiple lineages (Takahashi et al., 2007). The use of a single iPSC line for generating all tissue units in an OOC would enable separation of the effects of genotype and phenotype, as the measured patho/physiological responses will depend on the genetic makeup of the cells. Genetic homogeneity is also advantageous for modeling how drugs will affect the individuals or groups of patients (Burridge et al., 2016; Liang et al., 2016), including those carrying mutations that influence drug efficacy and toxicity. Further, generation of isogenic controls by gene editing of iPSCs to introduce or remove a disease-related mutation enables mechanistic studies and target-specific drug development. However, not all cell lineages can be effectively derived from the same iPSC line, and the development of robust protocols for iPSC differentiation and maturation remains one of the challenges of the field. In general, iPSC-derived lineages lose epigenetic markers during derivation (Kim et al., 2010) and are immature (Rajamohan et al., 2013). The maturity and phenotypic stability of engineered tissues can be increased by physical conditioning and the inclusion of organ-specific supporting cells such as fibroblasts, mesenchymal stem cells (MSCs), and endothelial cells (Michalopoulos et al., 1979; Kostadinova et al., 2013; Nunes et al., 2013).

Biomimetic Cues for Engineering and Maturation of Functional Tissue and Organ Units. For each OOC, the characteristic signals present *in vivo* need to be replicated *in vitro* via engineering methods in order to direct cell differentiation, tissue assembly, and functional maturation. Tridimensional cell culture improves physiological responses (Ma et al., 2012). The OOC microenvironment can be designed to deliver passive or dynamic stretch, electrical or optical signals, fluid shear, and biochemical and hormonal cues. To the extent possible, the important features of each tissue system should be recreated in a simple manner, to retain the ability to adapt the OOC for high-throughput studies (Lelièvre et al., 2017; Loskill et al., 2015; Park and Shuler, 2003; Sart et al., 2017; Schepers et al., 2016; Sung et al., 2013; Villasante et al., 2014, 2017a). The inclusion of environmental control elements sensing and delivering biophysical stimuli would enable feedback control of biomimetic cues that drive physiological responses. The ability for extended culture times enables obtaining important data, as many side effects of drugs are not immediate. Similarly, the ability to perform dose-response studies repeatedly can inform the design of clinical dosing regimens.

Online Readouts of Cell-, Tissue-, and Organ-Specific Functions. For dynamic studies of tissue responses to environmental signals, online readouts of the physical, metabolic, and molecular status of the cells and integrated tissue responses are of great interest. Such non-destructive methods enable longitudinal studies of the simultaneous effects of multiple variables, experimentation in large parameter spaces, and comprehensive assessment of therapeutic interventions (Zhang et al., 2017). The measurement of functional data using optical imaging requires the use of optically transparent materials, suitable working distances, and image-processing software. Ideally, the imaging assays should be designed for direct translation into high-throughput industry settings.

Configurability and Integration of the OOC Platforms. The most frequently used fabrication material is poly(dimethylsiloxane) (PDMS), in spite of significant nonselective absorption of hydrophobic molecules, including oxygen and many drugs (Shirure and George, 2017; Xu et al., 2016). The benefits of PDMS include its biocompatibility, ease of use for microfabrication approaches, optical transparency, and autoclave sterilization. Methods developed to continue the use of PDMS include coatings that reduce its permeability (van Meer et al., 2017) and design considerations that minimize drug absorption and unintended mixing (Shirure and George, 2017). Alternative materials include glass, polycarbonate, and polyurethane, as well as many other biocompatible polymers.

For configurability and high throughput of analytical measurements, the external footprint of integrated OOC platforms should be compatible with formats commonly used in drug development (e.g., multi-well plate and glass-slide) while the interior design should be tissue specific and enable connections between single OOCs. While the fluidic integration of multiple OOCs is critical for enabling organ-organ crosstalk, methods to meet this requirement while preserving individual organ functionality are still being developed. A popular and straightforward approach of using common medium capable of supporting all

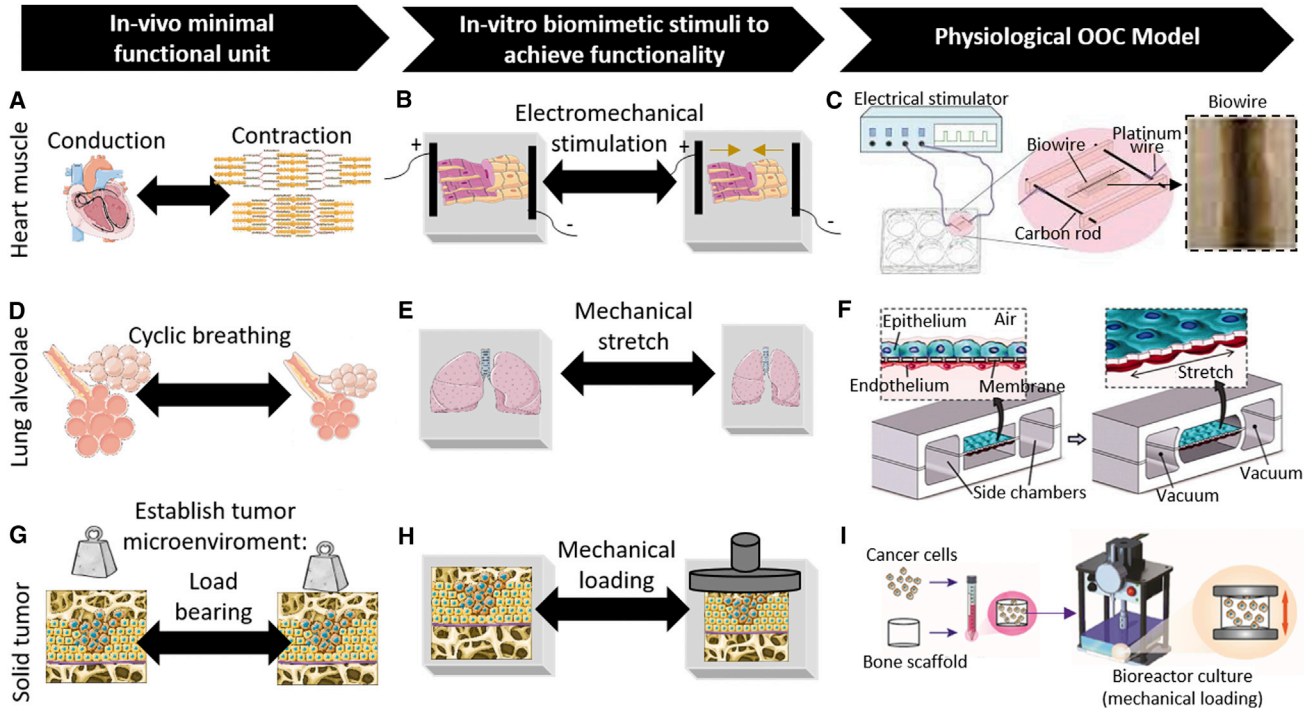

Figure 1. OOC Design

(A–C) Design considerations for an OOC of heart muscle involve mimicking the (A) *in vivo* functions of conduction and contractility by defining the minimal functional unit as a strip of cardiac tissue and using (B) electromechanical stimulation *in vitro* to achieve functionality. (C) An example is the cardiac biowire OOC, consisting of a strip of human cardiomyocytes in a hydrogel that can be electromechanically stimulated. Reproduced with permission (Nunes et al., 2013; Sun and Nunes, 2016).

(D–F) Design considerations for an OOC of lung alveolae involve mimicking the (D) *in vivo* functions of cyclic breathing by defining the minimal functional unit as a single lung alveolus and using (E) cyclic mechanical stretch *in vitro* to achieve functionality. (F) An example is the lung OOC, consisting of layers of epithelium and endothelium on two sides of a membrane that is mechanically stretched by the application of vacuum. Reproduced with permission (Huh et al., 2010).

(G–I) Design considerations for an OOC of a solid tumor involve mimicking the (G) *in vivo* tumor microenviroment and the load-bearing bone niche for bone cancer, and using (H) mechanical loading *in vitro* to achieve functionality. (I) An example is the tumor OOC, consisting of Ewing sarcoma cancer cells embedded in a bone scaffold that can be cyclically compressed in a mechanically loaded bioreactor. Reproduced with permission (Marturano-Kruik et al., 2018).

OOCs within the integrated system is limited to tissues that are already matured and phenotypically stable (Zhang et al., 2017; Tsamandouras et al., 2017). Alternatively, each tissue compartment could be separated from the vascular flow by an endothelial barrier to mimic the tissue-blood separation in the body. This way, tissue-specific media could be maintained in each compartment to support and mature each tissue in an optimal manner, while enabling the crosstalk among the tissue units via vascular connections (e.g., by cytokines and cell-secreted vesicles such as exosomes).

Because cell metabolism may change from organ to organ, and with cell maturity and time in culture, the basis for determining the physiological flow rate for each organ and tissue compartment is not entirely clear. Several factors are under consideration, including the distribution of blood flow, relative sizes of the organs, and metabolic rates in the body (Wikswo et al., 2013b). The configurability of the platform to allow changes in relative volumes of the individual OOCs, the order in which they are connected, and OOC perfusion rates is critical for determining the flow configuration in multi-organ OOCs.

How Simple Is Complex Enough?. This question is fundamental for the whole field of tissue engineering and it reflects the conflicting requirements for sufficient biological fidelity of the tissue/organ models and maintenance of simplicity, allowing manipulation and control. As for engineered tissues in general, the complexity of individual OOCs and the need for multi-OOCs depends on the problem being studied. In some cases, the complexity of biological functions can be provided using simple bioengineering tools. For example, a micro-pump can be used to mimic pulsatile blood flow (serving the role of the heart, the body's pump). In studies of drug metabolism, provision of biocatalytic reactions or microsomes can provide some of the metabolic roles of liver. The ability to vary the complexity of OOCs helps determine "how simple is complex enough" and optimize designs for various types of studies, such as drug toxicity, efficacy, and mechanism of action. In the subsequent sections, we provide examples of how single and multi-OOC design and operation change from one application to another.

Single Organs-on-a-Chip

Design principles for OOCs are based on the goal to recapitulate the physiology of the organ system being studied. Ideally, a minimally functional (simplest possible) unit of each organ system should be used to create the OOC environment. The resulting OOC should incorporate biophysical stimuli (hydrodynamic, mechanical, electrical, and chemical) that drive and control the establishment of the organ model and its minimally acceptable functionality (Figure 1). This reductionist approach for recreating

Table 1. Single-Organ OOCs

Organ Type (Functional Unit)	Design Considerations	Cell Types Used Currently	Readouts	References
Heart	contractility and electrical activity	iPSCs	beat rate, force, excitation threshold, maximum capture rate, and contractility	Agarwal et al., 2013; Giacomelli et al., 2017; Hirt et al., 2014; Mannhardt et al., 2016; Marsano et al., 2016; Zhang et al., 2016; Mathur et al., 2015; Nunes et al., 2013
Lung (alveoli)	air-liquid interface and pulmonary drug absorption	cell lines	cell imaging and dissolved gas concentration	Huh et al., 2010; Long et al., 2012; Benam et al., 2017
Liver (hepatic lobule)	drug metabolism, cytochrome P450 interaction, and hepatocyte and fibroblast co-culture	cell lines and iPSCs	albumin and urea production, cytochrome P450 enzymatic activity, metabolite conversion, and drug-induced liver injury (DILI)	Schepers et al., 2016; Lee et al., 2013; Cho et al., 2010; Khetani and Bhatia, 2008; Lee et al., 2007; Ramaiahgari et al., 2014; Rennert et al., 2015; Ware et al., 2015; Bhise et al., 2016; Domansky et al., 2010; Kostadinova et al., 2013
Kidney (nephron and proximal tubule)	drug clearance and proximal tubule epithelium exposed to shear stress	primary and cell lines	filtration, reabsorption, urea concentration, epithelial cell polarization, albumin transport, glucose reabsorption, alkaline phosphatase activity, and permeability glycoprotein efflux transporter	Jang et al., 2013; Kim et al., 2016; Weinberg et al., 2008
Gut	drug absorption, requires a large surface area via villi and microvilli formation, mucosa barrier, and symbiotic bacteria present	cell lines	transepithelial transport, absorption, toxicity, cytochrome P450 3A4 isoform drug metabolism, and responses to bacteria	Shah et al., 2016; Kim and Ingber, 2013; Esch et al., 2012
Brain/BBB	selective drug penetration and interactions between endothelium, pericytes, and astrocytes/neural cells	primary, cell lines, and iPSCs	transendothelial resistance (TEER), permeability, and drug transport	Booth and Kim, 2012; Brown et al., 2015; Griep et al., 2013; Lancaster et al., 2013; Wang et al., 2017
Skin	air-liquid interface and dermal drug absorption	primary, cell lines, and iPSCs	transdermal transport, immunohistochemistry, and gene expression	Abaci et al., 2015; Ataç et al., 2013; Gledhill et al., 2015; Petrova et al., 2014
Vasculature	barrier functionality and thrombosis	cell lines, human MSCs, and iPSCs	permeability, response to shear stress, TEER, and FITC-dextran assay	Fernandez et al., 2016; Kurokawa et al., 2017; Moya et al., 2013
Cancer	tumor microenvironment and metastasis	cell lines	tumor cell phenotype, tumor cell extravasation, and vascular permeability	Marturano-Kruik et al., 2018; Marturano-Kruik et al., 2015; Villasante et al., 2014, 2017a, 2017b; Villasante and Vunjak-Novakovic, 2015; Chen et al., 2013; Jeon et al., 2015

the complex milieu of signals occurring *in vivo* enables the development of OOCs that are physiologically relevant but still represent controllable environments for scientific studies. Here, we provide examples of how biomimetic signals can be used to design physiologically relevant human OOCs for some of the most studied organ systems (Table 1). As the field is rapidly evolving, we had to leave out a number of meritorious reports on these and other organs.

Heart

Cardiovascular toxicity accounts for the majority of phase I drug failures (Onakpoya et al., 2016). Animals have different ranges of heart rates than humans, making them a poor surrogate for QT prolongation since the QT interval varies with the heart-beating rate (Gintant et al., 2016; Olsson and Edwards, 1992). These differences drive the development of human cardiac OOC models. The heart pumps blood by contracting in response to

excitatory electrical signals from the cardiac conduction system (Figure 1A). A bundle of contractile cardiac muscle fibers could thus be considered a minimal functional unit of the heart muscle, as long as it is able to contract and generate force in response to depolarizing electrical signals. Electrical stimulation mimics the function of the pacemaking sinoatrial node, eliciting a key function of the heart with high level of control. Synchronous cell contractions caused by electrical stimulation lead to the physiologically relevant cardiac OOC models (Figure 1B).

For cardiac OOCs, design considerations include anisotropic alignment of human iPSC-derived cardiomyocytes (hiPS-CMs), incorporation of supporting cell types (fibroblasts and endothelial cells) (Giacomelli et al., 2017), electromechanical stimulation (Nunes et al., 2013; Mannhardt et al., 2016), and real-time readouts of cardiac contractility and electrophysiology. Methods to derive hiPS-CMs have become increasingly efficient (Burridge et al., 2015). Because cardiac OOCs remain limited by the immature phenotype of iPS-CMs, emerging maturation protocols continue to advance the field. A recent study (Nunes et al., 2013) demonstrated the use of a novel platform to form highly aligned cardiac tissues ("biowires") and to mature these microtissues by electrical stimulation to acquire functional properties approaching those of native human cardiac muscle (Figure 1C). Mechanical stimulation via cyclic strain of cardiomyocytes in hydrogels stretched around flexible posts has also improved maturation (Mannhardt et al., 2016). Electrical and mechanical stimulation can be combined toward enhancing maturation and enabling physiological drug responses (Hirt et al., 2014; Marsano et al., 2016).

Medium perfusion increases the functionality of cardiac OOCs, demonstrated by the positive inotropic effects of isoproterenol on cardiac muscle thin films, developed by seeding cardiomyocytes on cantilevers that subsequently deflect during cardiac contraction (Agarwal et al., 2013). Perfusion also enables prolonged culture of cardiac muscle stretched between PDMS posts connected to outer perfusion channels to support mass transport and enable physiological toxicity screening (Mathur et al., 2015). Finally, perfusion enables the incorporation of supporting vasculature and has been achieved by bioprinting of aligned endothelial cells and cardiomyocytes (Zhang et al., 2016).

Lung

The respiratory regions of the lung expand and relax cyclically as they fill with air to maximize the surface area available for gas exchange (Figure 1D). When considering the alveolus (where the vascular endothelium and the pulmonary epithelium are separated only by a basement membrane) as the minimal functional unit of the lung, one can mimic the cyclic expansion by applying mechanical stretch to the gas exchange surface (Figure 1E). The resulting lung-on-a-chip model (Figure 1F; Huh et al., 2010) pioneered the development of the biologically inspired OOCs we have today (Benam et al., 2017). A porous membrane was seeded on one side with human alveolar epithelial cells exposed to air and endothelial cells on the other side exposed to vascular perfusion. The membrane was incorporated into a chip that enabled the implementation of mechanical stretch to recapitulate the breathing motion experienced by the lung alveolae. The resulting lung-on-a-chip was the first of its kind and has successfully demonstrated organ-level behavior. Mathematical modeling was used to optimize a similar design of a lung OOC chamber with liquid phase flow, resulting in controlled gas concentrations within both the gas and liquid sides of the alveolus-capillary interface while providing a means to measure changes in gas transfer between the compartments (Long et al., 2012).

Liver

The liver is responsible for drug metabolism, detoxification, glycogen storage, and plasma protein synthesis. The minimal functional unit consists of the hepatic lobule or liver sinusoid, containing hepatocytes responsible for drug metabolism. Culture of hepatocytes reveals decreased functionality over time, which can be addressed by including co-cultures of fibroblasts (Michalopoulos et al., 1979), other supporting cells (Kostadinova et al., 2013), and perfusion (Domansky et al., 2010; Bhise et al., 2016). Liver OOCs contain hepatocytes and fibroblasts arranged in meaningful ways by patterning (Khetani and Bhatia, 2008; Cho et al., 2010) or cultured in aggregates (Ramaiahgari et al., 2014; Bhise et al., 2016). Recreation of the microarchitecture of the hepatic lobule via micropillar arrays enabled the formation of bile canalicular structures with polarized cells (Goral et al., 2010). Liver sinusoid OOCs have been created by incorporating microchannels containing densely packed hepatocytes connected to an endothelial barrier (Lee et al., 2007) or using membrane co-cultures (Rennert et al., 2015) supported by fluid flow. Liver OOC models typically use primary human hepatocytes (Godoy et al., 2013), which have a limited lifetime and availability, or cell lines, which demonstrate limited functionality (Lübberstedt et al., 2011). Future work toward developing models using iPSC-derived hepatocytes shows promise (Schepers et al., 2016; Ware et al., 2015) and will enable studies of diverse patient backgrounds.

Kidney

Responsible for filtration and reabsorption, the kidney is a site of frequent toxicity during drug development (Schetz et al., 2005). The minimal functional unit of the kidney is the nephron, consisting of the glomerulus, proximal convoluted tubule, and the loop of Henle. A nephron OOC developed containing various cell types within each of the three compartments demonstrated functional glomerus filtration, proximal tubule reabsorption, and urea concentration within the loop of Henle (Weinberg et al., 2008). The use of fluid flow within OOCs of the proximal tubule containing primary cells improves functionality, with studies showing *in vivo* pathophysiology and drug toxicity (Jang et al., 2013). Microfluidics can also be used to mimic drug delivery mechanisms and their subsequent toxicities, such as when comparing the effects of continual versus bolus dosing within a kidney OOC (Kim et al., 2016).

Gut

The development of gut OOCs enabled studies of the absorption, metabolism, and transport of drugs delivered orally. Gut OOCs were designed to mimic the large surface area of the gut provided by the villi and microvilli, while also including symbiotic microbial flora, by adapting the lung-on-a-chip platform, where the cyclic mechanical strain was used to mimic peristaltic motion (Kim and Ingber, 2013). The membrane-based gut OOCs have been modified to contain 3D structures for villi formation, while also providing access to the apical and basolateral sides of the gut epithelium (Esch et al., 2012). Other gut OOCs used peristaltic motion and fluid flow to induce human Caco-2 cells

to form intestinal villi and achieve functionality superior to static transwell systems, approaching human functionality (Shah et al., 2016). Methods to create gut models using iPSCs include the development of gastric organoids using the principles inherent to biological development (i.e., 3D self-assembly, differentiation, and morphogenesis; Shirure et al., 2017). The organoid-based approach may accelerate the adoption of iPSCs for other organ systems as it enables the multicellular 3D generation of organoids via the principles of developmental biology.

Brain and Blood-Brain Barrier

The BBB selectively controls the passage of drugs into the CNS. OOCs emulating the function of BBB would allow testing whether a drug designed to treat neuro-related diseases can actually pass through the BBB to act on its intended target. Current models are based on the culture of cells on a membrane containing endothelial cells on one side and astrocytes with or without supporting pericytes on the other side (Wang et al., 2017; Booth and Kim, 2012). Using transendothelial resistance (TEER) as a functional readout, the use of microfluidic perfusion has been shown to physiologically increase barrier function and provide more predictive drug responses (Griep et al., 2013). The neurovascular unit (NVU) OOC was designed to couple a vascularized chamber with a brain chamber through a porous membrane in an effort to develop a more faithful BBB model (Brown et al., 2015). In addition to modeling the BBB, models of the brain are also important per se, as the complexity of the human brain makes it difficult to study in non-human models. The development of 3D cerebral organoids from iPSC-derived neuroectodermal tissues shows promise as an *in vitro* model of brain development (Lancaster et al., 2013; Kelava and Lancaster, 2016).

Skin

As the largest organ in the body, skin is crucial both for testing the cutaneous effects of drugs and for modeling percutaneous drug absorption. Skin OOCs can be used to determine the bioavailability of a drug crossing through the stratum corneum of the epidermis and into the endothelium and bloodstream. When designing a skin OOC for studying percutaneous drug absorption, it is thus critical to reproduce the multiple layers of the skin (Abaci et al., 2015). The OOC should be engineered to enable an air-liquid interface so that the topical stratum corneum layer is exposed to air, while the dermal layer is exposed to media or, ideally, vasculature. Current skin OOCs are based on primary cells or cell lines (keratinocytes within the epidermis and fibroblasts within the dermis) (Gangatirkar et al., 2007). Recent efforts to use iPSCs have revealed results similar to those of primary cells (Petrova et al., 2014) and are aimed at including all relevant cell types (e.g., melanocytes; Gledhill et al., 2015). However, methods to derive all skin cells from iPSCs are not yet established, with the ability to derive dermal papilla cells needed for hair generation notably absent (Lim et al., 2016). Subcutaneous explants can be used, although they have limited availability, and both cultures of explants and *in vitro* skin OOCs show benefit from the incorporation of perfusion (Ataç et al., 2013; Abaci et al., 2015).

Vasculature

Vasculature is critical for delivering nutrients and removing metabolic products, as well as providing a selective barrier for drugs introduced via the circulatory system. Vasculature OOCs can be formed by seeding endothelial cells onto a preformed supporting structure (i.e., via injection molding, 3D printing, and using a sacrificial network onto which endothelial cells can be injected) or by embedding cells into a hydrogel and subsequently inducing sprouting between larger channels. Human tissue-engineered blood vessels were formed by seeding MSCs in collagen, coating the vessel with endothelial cells, and perfusing for a week to enable maturation capable of functional vasoconstriction and vasodilation in response to drugs (Fernandez et al., 2016). Microvascular networks have been formed by seeding fibrin gels containing endothelial cells with supporting fibroblasts and inducing sprouting via mechanical (flow and pressure) and chemical factors (VEGF, hypoxia, and nutrient deprivation) between two outer channels to ultimately create a perfusable microvasculature system (Kurokawa et al., 2017; Moya et al., 2013). Interestingly, flow regimens were necessary to create perfusable vascular networks (Moya et al., 2013).

Cancer

Cancer drug development using animal models, in particular the mouse, has long served as an invaluable tool for studying the roles of genes and the resulting disease phenotypes. As a result, considerable success has been achieved in curing cancer in mice. However, the inability of these discoveries to translate into humans is alarming. The adoption of OOC cancer models could provide a systematic approach to the study of human cancers *in vitro*. Cancer OOCs aim to test drug efficacy, model cancer metastasis, and provide personalized cancer models. The development of cancer OOCs that recreate the complexity of the microenvironment in which the tumor occurs (e.g., bone niche for a bone sarcoma or breast cancer metastasis) enables studies of increased physiological relevance that mimic the tumor phenotype, the emergence and progression of cancer and its complex interactions with the surrounding tissues, and drug resistance. One example is the incorporation of sarcoma cells into a living bone environment and the subsequent exposure of the forming microtumors to mechanical stimuli to create an OOC model of bone cancer (Figures 1G–1I). Moreover, by mimicking bone-like mechanical signals within the 3D model, we rescued the ERK1/2-RUNX2 signaling pathways leading to drug resistance (Marturano-Kruik et al., 2018). Investigations into patient-specific bone sarcoma models were able to mechanistically explain the lack of improvement observed in clinical response to receptor tyrosine kinase inhibitors. Thus, tumor OOCs can provide insights into the links between mechanobiological factors and the development of cancer drug resistance.

Tumors are surrounded by vasculature to support the increased metabolic demands. The inclusion of vasculature in cancer OOCs provides opportunities to predict metastatic potential. Ideally, the vasculature should provide nutrients to the outer regions of the tumor, while maintaining the hypoxic regions similar to those formed *in vivo* that promote the invasion of cancer cells into surrounding tissues and tumor metastasis. For example, the vascularized neuroblastoma model was able to functionally recapitulate vasculogenic mimicry and the resulting formation of drug resistance (Villasante et al., 2017b). The study further revealed a potential role of SOX2 in the development of drug resistance, thereby suggesting a potential mechanistic role for therapeutics targeting SOX2 in neuroblastoma treatment. Depending on the drug study design, a cancer OOC can be designed to include the additional complexity of vasculature

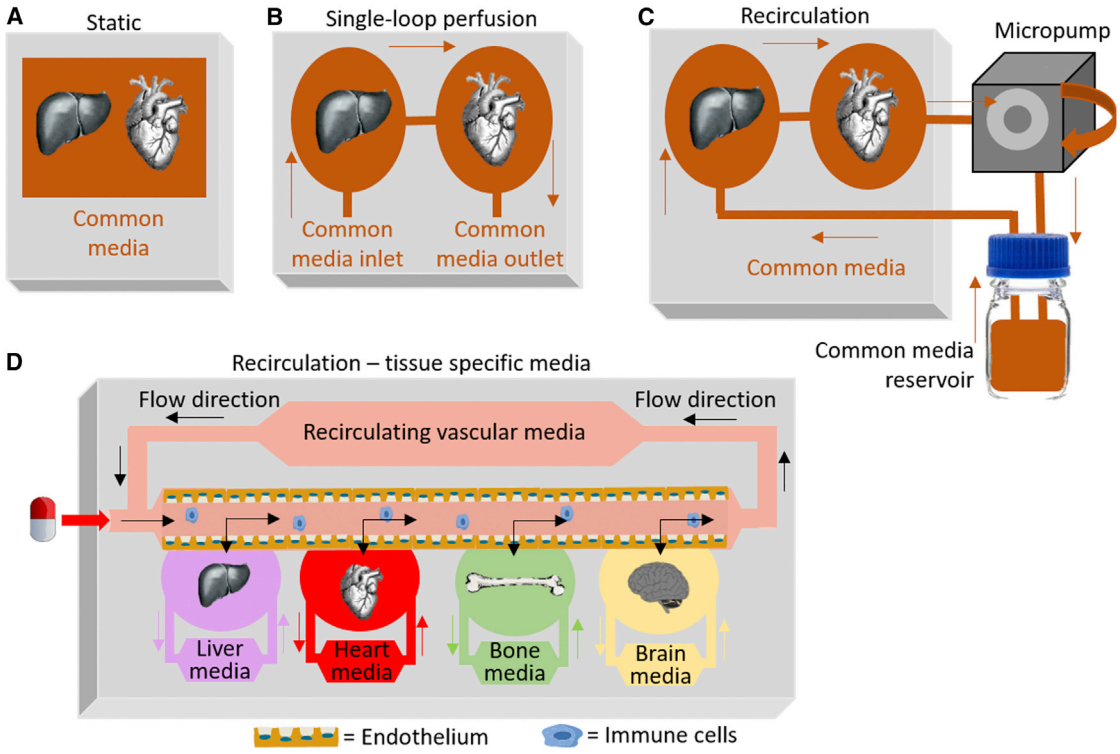

Figure 2. Integrating Multiple OOCs toward a Body-on-a-Chip
(A–C) Methods to integrate multiple OOC systems include (A) static culture, (B) single-loop perfusion, or (C) recirculation of a common media capable of supporting all organ systems.
(D) The development of individual OOCs connected to a selective membrane barrier, such as an endothelial layer, would enable integration of OOCs with perfusion that connects all OOCs while preserving the tissue-specific media composition for each OOC. The recirculating media can include more biomimetic components, such as circulating immune cells.

for studies of metastasis or vasculogenic mimicry, or these complexities can be left out to facilitate the ease of use. Thus, the minimal functional unit described for each OOC will change depending on the question asked.

Integration of Multi-organ OOCs

Cells and organ systems communicate by secreting soluble factors and extracellular vesicles that mediate peripheral crosstalk with the circulatory system (Li et al., 2017; Yin et al., 2017). Thus, biomimetic integration methods are important for establishing physiological organ-organ interactions within OOC platforms. Connecting individual OOCs to one another through microfluidics mimics the *in vivo* role of vascular perfusion and enables control over the culture environment to recapitulate some aspects of homeostasis. These connections enable crosstalk between organs of interest and facilitate a more physiological approach to drug delivery and uptake. The methods for integration can be categorized as static (Figure 2A), unidirectional single-pass (Figure 2B), or recirculating (Figure 2C), with specific examples of each detailed in Table 2.

Static Organ Chambers

Static microfluidic connections between individual organs rely on physical proximity of the organ chambers instead of convective flow. The transport of soluble factors and cell-cell communication are facilitated by culturing all cells and organs in the same well (Li et al., 2012). Multiple single OOCs can be cultured within shallow wells each containing a tissue-specific medium and then connected via the media within a larger well (Li et al., 2004). Co-culture models can also be used to integrate multiple organ systems using the same culture medium. To develop a model of oral bioavailability, human Caco-2 cells were cultured on a transwell to create a gut OOC while human hepatocytes were cultured in the well underneath to create a simple liver OOC. This simple system yielded clinically relevant predictions for 22 out of 24 known compounds (Lau et al., 2004).

Single-Pass Perfusion

Unidirectional perfusion through microfluidic vasculature connecting multiple organ chambers enables modeling of drug transport as it enters the vascular system and travels from one organ chamber to another. These culture systems can be designed to arrange the individual chambers in parallel, in series, or both. However, the unidirectional flow only enables crosstalk to organs located downstream, thereby eliminating upstream feedback typical of the native circulatory environment. Multiple organs can be integrated via fluid paths, as demonstrated by vascular networks within tissue chambers connected to larger vessels that enabled downstream flow in a user-friendly manner, and validated by successfully screening anti-cancer and anti-vascular drugs (Phan et al., 2017).

While the use of a platform with fluidic routes may facilitate high throughput, configurable connections provide the ability

Table 2. Multi-organ OOCs

Organs Included	Perfusion	Comments	References
Static			
Liver-fibroblast	none	increase in toxicity when metabolically active hepatocytes are present	Li et al., 2012
Liver-kidney-lung-neural-vasculature-cancer	none	individual organs cultured in shallow wells connected within a larger well via media	Li et al., 2004
Gut-liver	none	transwell-based model containing Caco-2 cells (gut OOC) within transwell and hepatocytes below	Lau et al., 2004
Single-Pass			
Vascularized tumor	hydrostatic pressure	vasculogenesis within tissues enables connections to larger channels perfused via hydrostatic pressure	Phan et al., 2017
Heart-heart	diffusive transport	modular OOCs integrate linearly via plug-and-play connectors	Loskill et al., 2015
Gut-liver	gravity-driven unidirectional flow	single OOCs cultured separately, then connected by unidirectional flow driven via gravity and passively controlled hydraulic resistances	Esch et al., 2016
Intestine-liver-kidney-BBB	manual transfer of media supernatant	functional coupling of culturing OOCs in separate labs within their specific media and linked using supernatant transfer for drug studies	Vernetti et al., 2017
Recirculating			
Cardiac-muscle-neuronal-liver	pumpless, gravity-driven flow	serum-free media and electrical and mechanical readouts	Oleaga et al., 2016
Liver-pancreas	on-chip micropump	allometric scaling and functional crosstalk regulated glucose levels	Bauer et al., 2017
Liver-intestine and liver-skin	on-chip micropump	successfully incorporated barrier tissues (intestine and skin) with parenchymal organ (liver) and endothelialized microfluidic channels to mimic vasculature	Maschmeyer et al., 2015
Liver-cancer	peristaltic pump	demonstrated importance of 3D over 2D cultures in drug dosing studies	Ma et al., 2012
Gut-liver	on-chip pumps remotely actuated through pneumatic tubing and pneumatic manifold within plate	enabled pharmacokinetic studies (no PDMS), utilized common media, and flexibility of drug dosing route (orally by injection to apical side of gut OOC; intravenously by injection into mixing chamber)	Tsamandouras et al., 2017
Liver-lung-kidney-fat	peristaltic pump	developed common media capable of supporting all organ systems	Zhang et al., 2009
Liver-heart and liver-cancer-heart	microfluidics-controlling breadboard	integrated online sensors for measurements of environmental parameters, immune biosensors, and miniature microscopes	Zhang et al., 2017

to form and mature organs separately via organ-specific biophysical stimuli, and combine them into multi-organ OOC systems. This approach was recently introduced through the μOrgano system, a modular microfluidic system that connects single organ chambers to one another in a plug-and-play fashion (Loskill et al., 2015). A similar approach involved connecting single OOCs on separate chips with porous membranes via gravity flow controlled by passive valves to maintain unidirectionality (Esch et al., 2016). These approaches enable customizable formation and maturation of the individual organs, elimination of failed culture chambers, and flexible study designs.

Other approaches to functional coupling of organs include physical transfer of supernatant from one organ chamber to another via robotic or manual pipetting (Vernetti et al., 2017). While these approaches fail to recapitulate physiological flow between organ systems, they do enable crosstalk from one organ chamber to the next one via secreted factors. Fluid transfer between the chambers also provides flexibility for integration of specialized single-organ chambers developed in individual labs into functional multi-organ platforms that can be configured for a range of study designs. However, because these connections result in mixing of different and highly specialized culture media, they are limited

to the use of organs that can be supported by the same common media.

Recirculating Microfluidic Flow

Microfluidic connections within OOCs with continuous perfusion more closely mimic blood circulation. These setups are based on recirculation of the media from a single-pass flow, so that organ-organ communication occurs both downstream and upstream. The fluidic connections can be established through predetermined microfluidic paths or via flexible approaches described for single-pass systems. Gravity-driven flow can be utilized to facilitate crosstalk between individual OOCs connected via microfluidic channels (Oleaga et al., 2016). As the first study to use serum-free media when integrating multiple OOCs, the resulting platform provided a simple, modular approach that successfully predicted drug toxicity within four interconnected OOCs. To demonstrate the feasibility of combining multiple OOCs with barriers controlling drug transport, multi-OOCs consisting of barrier tissues (separated via porous membranes) and parenchymal tissues (within chambers connected via recirculation through microchannels) were connected via a fixed platform (Smith et al., 2013).

The importance of using tridimensional cultures for drug screening was demonstrated in a multi-OOC liver cancer model. The study revealed that cells within the tridimensional cultures were more resistant to drugs than the same cells cultured in monolayers (Ma et al., 2012). The recirculation of drugs and their metabolites also enables long-term studies of pharmacokinetics (PK). However, such OOCs should be designed to exclude drug-permeable materials (such as PDMS), minimize culture media volumes (to avoid dilution of secreted factors), and enable media sampling throughout the experiment, coupled with mathematical models to facilitate experimental design and data interpretation. The gut-liver multi-OOC is an excellent example of this approach, in which computational modeling was used to investigate the PK properties of a drug in two organs responsible for drug bio-distribution and bioavailability (Tsamandouras et al., 2017). By using polysulfone (PSF) instead of PDMS, drug absorption was eliminated, while small media volumes and an open platform configuration enabled media sampling and high-content measurements. Mathematical models can inform the experimental designs for drug testing and rationalize the selection of drug dosing regimens. The continued development of multi-OOC models capable of PK and pharmacodynamics (PD) investigations will be crucial for translating preclinical *in vitro* studies toward clinical relevance.

By developing platforms with integrated sensors, real-time information can feed the control feedback algorithms for evaluating drug responses over extended time periods. Such a platform was recently developed, with physical sensors for monitoring the extracellular microenvironment, electrochemical sensors to measure soluble protein biomarkers, miniature microscopes to facilitate analysis of morphological changes, and a microfluidic breadboard to route fluids in a timed manner (Zhang et al., 2017). As many as 13 OOCs have been combined using common media, supporting a combination of barrier and non-barrier tissues (Miller and Shuler, 2016). Alternatively, the organs could be separated from one another, while being connected via vascular circulation, by selective barriers (e.g., a membrane with endothelium that allows the transport of molecules while maintaining separation of the organ-specific media and circulating vascular-specific media) (Figure 2D). Organ-specific media mimic the *in vivo* paradigm and support the individual organ systems for maximal functionality and maturation.

Current Landscape and Future Outlook

By faithfully mimicking the patient's genetics and pathophysiology *in vitro*, OOCs can be used for predictive, patient-specific modeling of human health and disease, providing new types of models that can inform drug development and enable precision medicine. The development of new technologies, such as machine learning and control algorithms, to interface with the OOC systems may further advance their use in systems biology and precision medicine.

Ongoing Initiatives

Since 2012, the NIH has led the development of OOC technologies (https://ncats.nih.gov/tissuechip/projects#modeling), through the initial development of tissue chips (2012–2014), integration of OOC platforms (2014–2017), independent validation of the current OOC platforms (2016–2018), two initiatives for chips in space (in partnership with the Center for the Advancement of Science in Space, CASIS), and the ongoing initiative for the use of tissue chips for disease modeling and drug efficacy testing. The 3R's drive increased the support OOC technologies, with efforts also in Japan and Europe (Holmes et al., 2010). The ongoing collaborations between the government, industry, academic research labs, commercial entities, and regulatory agencies are expected to accelerate the adoption of OOCs into standard industry workflows.

De-risking Drug Development

Drug development is an inefficient, resource-intensive process that in most cases does not lead to development of a new medicine. Due to many drugs failing in phase III trials or demonstrating severe side effects after release to the market (Gobaa et al., 2011), the average cost of developing a new drug often exceeds $1 billion (DiMasi et al., 2003). The limited predictability of animal models results in ~90% of candidate drugs failing in human trials (Gintant et al., 2016). Equally damaging is the cautious elimination of potentially curative new drugs because their adverse effects in animals do not necessarily translate into humans. These false-positive and false-negative readouts create an enormous financial burden, resulting in decision-making in which the potential profitability of a drug is leveraged against the potential risks, rather than on the drug's potential to improve disease outcomes (Jekunen, 2014).The potential of OOCs to advance drug development lies in their ability to inform decisions about which drugs should be advanced into further development and testing, and which should be halted, by providing humanized drug toxicity information. The earlier this decision can be made for a drug that would ultimately harm people if entering the market, the more health benefit, money, and time can be saved (Jekunen, 2014).

Preclinical Drug Studies

In preclinical screening of drugs, OOCs can confirm both the absence of toxic events (e.g., by assessing cardiovascular, liver, or skin toxicity) and the effectiveness in treating the intended target pathways (Figure 3). Multi-organ OOCs with microfluidic vasculature are suitable for preclinical determination of off-target toxicities. Early identification of off-target effects would enable

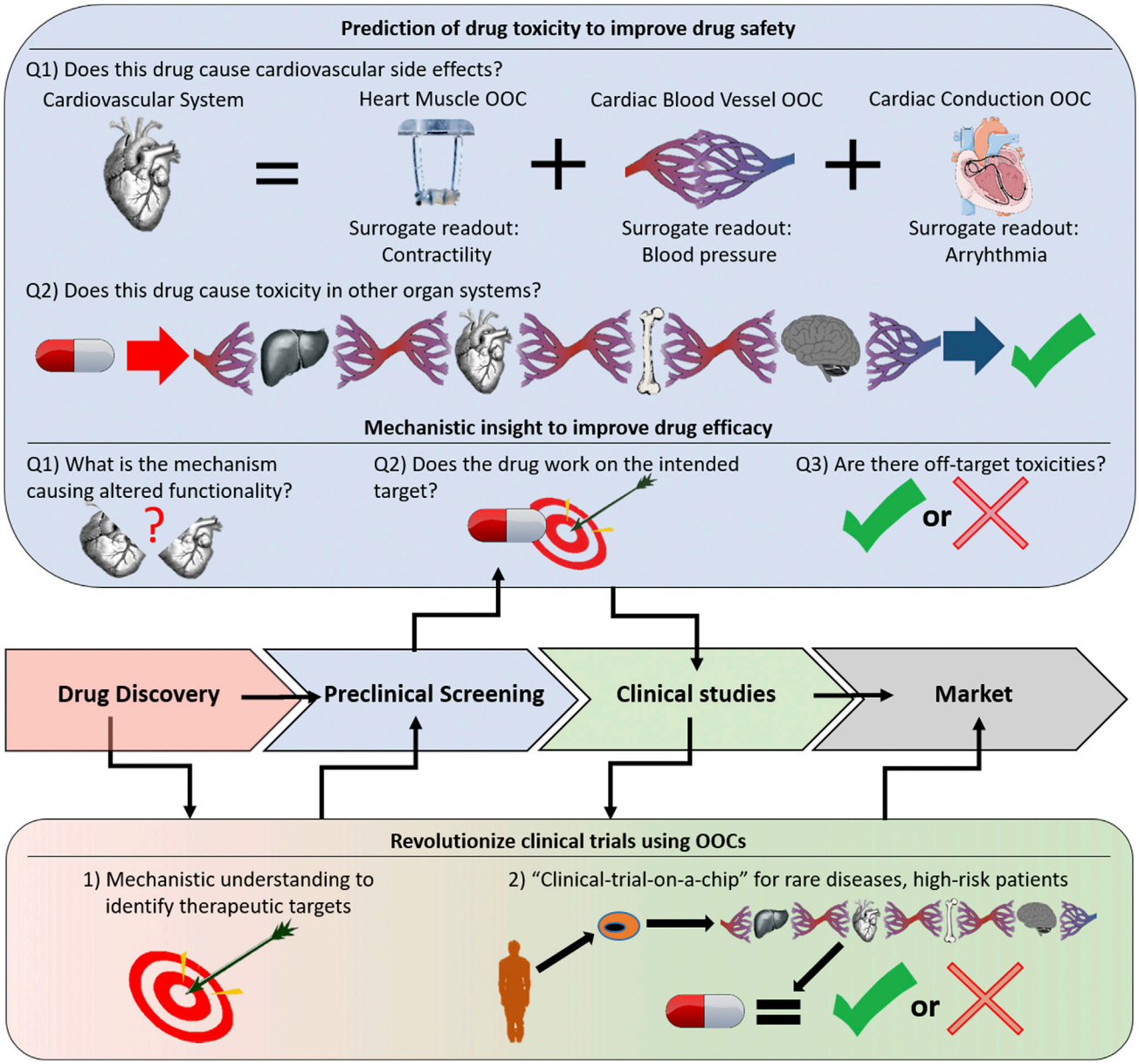

Figure 3. Potential of OOCs to Disrupt Drug Development
The use of OOCs can disrupt drug development at multiple points: mechanistic studies of drug action, preclinical trials of drug toxicity and efficacy, clinical studies using patient-specific OOCs for models of patient diversity, and the development of a "clinical-trial-on-a-chip" to discover therapeutic options for rare diseases.

the redesign of the drug to minimize the risk to patients and decrease the cost of development. However, the barrier to entry for preclinical toxicity screening may be higher than for other applications. While regulatory agencies recognize that stagnant drug development results in part from the insufficient predictability of animal models, their use in preventing harmful drugs from getting to market remains. The International Council for Harmonization (ICH) and FDA guidelines concerning cardiovascular screening have not let an arrhythmic drug get to market since being instated. Therefore, OOCs must be truly predictive and value-adding if they are to be adopted by industry and regulatory agencies.

Clinical Drug Studies

Advancing a drug to the clinical trial stage could be expedited by incorporating OOCs. The current paradigm treats patients in a manner that normalizes their diversity to determine how a drug will work in a patient overall (Lu, 1998). Instead, OOCs provide the means to embrace the diversity inherent in patient subgroups (i.e., ethnicity, gender, and age) and in a more individualized manner (i.e., genetic makeup) so that the most efficacious and safe drugs get to the patient that they will benefit the most (Aneesh et al., 2009). OOCs have potential to provide a "clinical-trial-on-a-chip" for special groups of patients currently excluded from clinical trials and enable development

of individualized therapeutic regimens, and can help uncover therapeutic targets. This approach may also allow effective repurposing of drugs to specific patient populations, since OOCs can be used to determine the efficacy at an earlier stage, and without risks to the patient. The use of patient-specific cells enables systematic modeling of the individual patients or subpopulations, so that the risks of unwanted drug effects or drug-drug interactions can be readily identified. Patient-specific OOCs can also be used to understand negative results observed in clinical trials. Such studies could determine if there are underlying patient-specific drug sensitivities unaccounted for or to redirect the design of the clinical trial to include the specific cohorts of patients (Figure 3).

The unexpected side effects and fatalities following the release of the drug cisapride are an example of where such an OOC approach may have been effective. Follow-up studies revealed that 56% of the patients experiencing side effects were also taking medications that inhibited the cytochrome P-450 3A4 enzyme within the liver, thereby impacting cisapride metabolism, resulting in increased drug levels in patient serum (Ahmad and Wolfe, 1995). Other subsets of the affected patient population had underlying cardiac disease or a history of arrhythmia that made them more susceptible to cisapride-induced proarrythmias (Olsson and Edwards, 1992). Such negative and potentially fatal outcomes could be avoided by using patient-specific cells within physiologically relevant OOC systems to provide predictive evaluations of how a potential drug would affect each patient, or a group of patients. The drug could then be released to the market with appropriate information for prescribing physicians, so that the potential drug-drug interactions and underlying comorbidities can be avoided from the very beginning. Additionally, the adoption of OOCs by industry will facilitate current 3R efforts (Russell and Burch, 1959; Holmes et al., 2010).

Disease Modeling

Because OOCs can be used to investigate both on-target and off-target mechanisms in multiple human organs, they can provide more realistic models for human diseases (Ingber, 2016). Surprisingly, the potential for industry adoption of OOCs may be realized most quickly for mechanistic studies of disease modeling. hiPSCs allow the use of patient-specific cells and gene editing techniques such as CRISPR-Cas, enabling studies into how specific gene mutations affect the organ functionality, and how subsequent therapeutic treatments interact mechanistically. The allure of these OOC models lies in their capacity to provide new insights into the disease progression and mechanisms of drug action (Figure 3). The OOCs can be tailored to follow the needs of the drug pipeline and can thus provide advantage over both animal models and general OOC models of healthy organs for toxicity screening. Additionally, because these OOCs can be validated in a straightforward manner, the implementation of OOCs in mechanistic studies may create a fast-track opportunity for initial industry adoption. Early adoption of OOCs for mechanistic studies may also be more feasible with respect to OOC throughput capacity, as they screen a smaller number of compounds.

Challenges Ahead

Studies conducted over the last decade convincingly demonstrate the potential of OOCs to faithfully model human organs *in vitro* (Ingber, 2016). However, many challenges remain, and they are driving further improvements in OOC design and operation and increasing the complexity of biological questions being addressed. We summarize here some of the challenges the field is currently facing.

Modeling Drug Absorption, Distribution, Metabolism, and Excretion (ADME)

The path of a drug involves its absorption into the bloodstream, subsequent distribution throughout the body, metabolism, and excretion. For each drug, the known or assumed path informs the order of organs in the OOC. For drugs under investigation, animal models and clinical data are very helpful for determining the layout of organ systems and the directions of flow in OOC systems. In principle, the path(s) identified *in vivo* would be recapitulated *in vitro*. Intravenously injected drugs would travel through the vasculature, cross the endothelial barrier, and become distributed to the various organs. In the liver, the drug is metabolized, and in many cases the metabolic products have different safety and efficacy profiles than the original drug. Following the liver, the drug is supplied via vasculature to other organs for determining the direct and off-target effects, and ultimately to the kidneys, where it is excreted. Clearly, the challenges include the presence of confluent, stable, and functional endothelium to serve as a selective barrier for transport of drugs and bioactive factors, and the need for a set of organs that can match the critical functions needed for the drug study. Drug distribution is a main cause of unwanted drug side effects and remains a challenge to model in currently available systems (Upadhyay, 2014). The ability of OOCs to model drug ADME will inform clinical studies and may also provide insights into the physiological arrangement of OOCs in the order that mimics distribution within them in a similar way as within the body (Kimura et al., 2015; Vernetti et al., 2017).

Scaling of Organ Sizes and Vascular Flow

It has been proposed that the scale of OOCs should be based on organ sizes within the body, and the OOCs have been arranged based on the respective masses of human organs (Wikswo et al., 2013a, 2013b). While this may be the case, it may prove that the use of functional scaling (e.g., based on the blood flow or metabolic rates) is more appropriate, with the necessary volume of each organ determined from the required level needed to support functionality. For example, multiple liver organ chambers may be combined to achieve an appropriate level of drug metabolism per fluidic pass. The corresponding volume of the heart module required to elicit the expected functional responses would then be determined using known drug actions. The discrete numbers of the individual modules that can be used to compose a larger organ compartment present limitations to fine-tuning of the relative volumes, and are thus an additional design consideration. The ability to combine modular functional units of each organ system into OOCs allows design optimization for investigating the particular question of interest.

Missing Components

Future efforts to increase the physiological relevance of OOCs should recapitulate the immune and endocrine systems, as they directly interact with the health and functionality of all organ systems in the body and are poorly modeled in animals (Habert et al., 2014). Recent research on the importance of the microbiome (Cho and Blaser, 2012) suggests another major

physiological component that should be included in future generations of OOC systems. The establishment of patient-specific microbiota in their corresponding patient-specific OOCs may be necessary to faithfully recapitulate the complex interactions between these systems. Incorporation of neurological control of tissue function may also be required to model systemic diseases resulting from neurological disorders in which the vast interactions and disease mechanisms are largely unknown.

Conclusion

The use of human OOCs capable of predicting the human physiological responses provides an attractive approach to drug development, disease modeling, and precision medicine. In addition to the utility of available primary cells and cell lines in these models, hiPSCs are of particular interest as they provide unlimited sources of patient-specific cells for multi-organ OOCs. Further, gene editing technologies that enable modifications to insert mutations into healthy cells or correct mutations in diseased cells can be used, with isogenic cell controls, to methodically investigate the role of genetic mutations in the resulting phenotypic behaviors. OOC platforms designed to emulate the functions of either a single organ or a multi-organ subsystem formed from iPSCs derived from the same individual could be transformative both to biological research and to the current drug development paradigm. In both cases, the translation of current advances in our understanding and application of hiPSCs require tissue/organ models of high biological fidelity. The clinical significance of such translation is in the patient-specific models of human disease that would accelerate and de-risk drug development. The commercial translation of tissue engineering methodologies originally designed to serve the needs of regenerative medicine into the OOC models of human pathophysiology to improve the drug development process is already underway (Zhang and Radisic, 2017).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the funding of OOC work by the NIH (grants EB025765, HL076485, and EB002520) and NSF (grant 16478).

DECLARATION OF INTERESTS

The authors are co-founders of Tara Biosystems, a Columbia University startup company commercializing organs-on-a-chip with human heart muscle.

WEB RESOURCES

NIH NCATS Tissue Chip Initiatives & Projects, https://ncats.nih.gov/tissuechip/projects#modeling

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