Organs-on-Chips in Clinical Pharmacology: Putting the Patient Into the Center of Treatment Selection and Drug Development

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There have been rapid advances since Organs-on-Chips were first developed. Organ-Chips are now available beyond academic laboratories with the initial emphasis to reduce animal experimentation and improve predictability of drug development through better prediction of safety and efficacy. There is now a huge opportunity to use chips to understand efficacy and disease variability. We propose that by 2030, Organs-on-Chips will play a key role in clinical pharmacology as part of the diagnostic and treatment workflow for some diseases by informing the right drug and dose regimen for each patient.

Foundational work published in 2010[1] followed by early funding from the US Food and Drug Administration (FDA), the Defense Advanced Research Projects Administration (DARPA), and the National Institutes of Health (NIH) to many academic teams, led to an explosion in the field of Organs-on-Chips. Since this landmark publication that demonstrated the biological complexity that could be achieved with Organs-on-Chips, there have been many advances with groups in academia and industry developing new design concepts, new organ systems, and engineering new products for applications across many different industries. Here, we discuss the potential impact of Organs-on-Chips on the future of clinical pharmacology and how this technology could help the field move toward a more patient-centric approach to drug treatment.

Organs-on-Chips are human-relevant, micro-engineered, fluidic systems that can emulate critical aspects of the *in vivo* cellular microenvironment required for human cells to demonstrate organ-level function. The engineering approach of the technology allows the control and tuning of key drivers of cell function, differentiation, and gene expression, including *in vivo*–relevant intercellular interactions, spatiotemporal gradients, vascular perfusion, and mechanical forces.

Organs-on-Chips have been developed for multiple organ systems, including liver, kidneys, intestine, lungs, blood–brain barrier, blood vessel, skin, heart, muscles, lymphatics, eyes, and bone marrow, with many other organs in development.[2] These models range from early proof-of-concepts in academic laboratories through prototypes to products on the market. In addition to the normal organ systems, many groups in academia and industry are developing disease models with the chips using patient derived–cells to recreate human disease states, including asthma, chronic obstructive pulmonary disease, diabetes, nonalcoholic steatohepatitis, fibrosis, ulcerative colitis, and neurodegenerative disease.[2,3] Due to the fluidic nature of the systems, multiple chips from different organs can be linked by vascular-like channels to build more complex systems with researchers linking up to 10 separate organs in a single platform.[4,5] However, more developmental work is needed with the goal that these sets of interconnected Organs-on-Chips will recreate the different organ interactions and communication to emulate a "human-on-a-chip."[6]

The cellular complexity of these systems is created using an engineering approach, which builds complexity in a very controlled and stepwise manner. In most cases, researchers do not try to recreate the entire organ but rather the smallest functional unit of that organ. A chip is usually first developed using the most basic cells and microenvironment parameters needed to achieve a simple function. Once that system's baseline phenotype is characterized, additional complexity can be built in. For example, one might begin with a model that includes epithelial and endothelial cells and characterize the response to a stimulus. As a next step, resident immune cells can be added to the chip, and recharacterized to see how the response changes. This can be repeated to add additional cell types, exogenous factors, micro-environmental parameters, such as stromal component, etc., until a relevant level of complexity is created. The differential response elucidates how each specific component contributes to the overall response of the system. A typical Organ-on-Chip design has multiple microscale compartments that may be separated by micropillars,[7] hydrogels,[8] or porous polymer membranes.[1] Each compartment can contain a homogeneous or heterogeneous mixture of cells that can be perfused with cell culture media, blood, intestinal fluid, or air, depending on the organ being modeled. The perfusion rates and media composition can be altered over time to allow delivery of nutrients, cytokines, hormones, and drugs, and the removal of waste products in a physiologically meaningful manner. Some chip designs even incorporate mechanical forces, such as those generated by stretching, for example, to recreate the physiological, mechanical forces from intestinal peristalsis[9] or from a pulsatile vasculature and flow. Chip surfaces can be decorated with extracellular matrix (ECM), with different compositions created for specific cell types. The multichambered nature of many systems means that different ECM compositions can be tuned and used for the different cell types that make up the

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Received August 16, 2019; accepted October 15, 2019. doi:10.1002/cpt.1688

distinct tissue types within an organ. It is also possible to modify the porosity of ECM coatings, their stiffnesses,[10] and the microstructure of the underlying substrate[11] to more closely match the *in vivo* cellular microenvironment in healthy and disease states.

Organs-on-Chips have the ability to provide rich datasets through high content imaging, the measurement of biochemical factors and clinically relevant biomarkers in the chip effluent, cell-based assays, such as RNA sequencing, and functional end points, such as barrier function. It is important to note that most of these analyses can be done separately for the distinct cell types residing in different fluidic compartments. For example, cytokine release can be independently measured in the effluent of an epithelial fluid channel and an endothelial fluid channel giving the ability to distinguish the response of the specific cell types, while still allowing different cell types to interact in a physiological manner through a membrane or gel. This enables one to answer complex questions by overlaying morphological, biochemical, functional, and gene-expression data in a single experimental context.

One of the low hanging fruit for the application and impact of the technology is to study drug effects on individual organs or combinations of organs without requiring *in vivo* studies. This includes studying preclinical safety and performing risk assessments in target organs.[12] Today, such chips will not replace good laboratory practice animal studies, but they can help select drugs with promising activity or eliminate potentially toxic drugs before the need for testing in animals.[13] As chip technology advances and their adoption grows, it is to be expected that they will play a more significant part in safety testing for drug candidates prior to human studies, especially for highly specific molecules, such as some biologics, with no activity against the molecular target in toxicology species. Organs-on-Chips will also be very important in providing detailed data on the absorption, distribution, metabolism, and excretion properties to be expected in humans, supporting improved physiologically-based pharmacokinetics.[14] Multi Organ-Chip systems can study the interplay between pharmacokinetics, including tissue-level pharmacokinetic interactions, and on-target and off-target, beneficial, and adverse effects of both the parent drug and metabolites.[15]

To date, Organs-on-Chips technology has mostly been developed as a means to improve the predictability of drug discovery and preclinical development, to provide experimental data for the development of improved *in silico* models, and to support reduction, refinement, and replacement of animal experimentation. These are all important and valuable goals that will greatly benefit clinical pharmacologists. Yet, there is more that can be achieved, and in the future, we suggest Organs-on-Chips will be an important technology for understanding response variability and enabling precision medicine in drug development and clinical use.

FUTURE OPPORTUNITIES—PERSONALIZED SAFETY, PERSONALIZED EFFICACY, AND PRECISION MEDICINE

Early work in Organs-on-Chips primarily utilized cell lines, whereas next-generation models are now being populated with either induced pluripotent stem cells (iPSCs)–derived or organoid-derived patient-specific cells.[16,17] Organoids are self-organizing 3D structures composed of specific cell types derived from pluripotent stem cells or directly from patient biopsies. The ability of organoids to retain patient phenotypes[18] have been demonstrated across many studies (e.g., a panel of individual patient organoids derived from ovarian cancer have been used to identify tumor subtypes responsive to platinum chemotherapy[19] and rectal organoids from patients with cystic fibrosis correlated with clinical effects).[20] Although a promising technology, there are several challenges that can limit the utility of using organoids. These include difficulties with controlling drug exposure, variability in their size and shape, and the lack of flow or mechanical forces required for *in vivo*–relevant function. By using dissociated organoids as a cell source for Organs-on-Chips we can overcome many of these limitations of organoids and leverage the advantages of both systems to more closely approximate the patient pathophysiology and drug response.[21] Initially, organoids were thought to be a competing technology to Organs-on-Chips. However, more recently, many researchers in the field are exploring the combination of these two technologies.[21] A view is developing that organoids and iPSCs are not competing systems to the Organs-on-Chips, but rather complementary approaches that provide patient-specific cell sources for clinical chip applications. This is an exciting development offering potential for disease modeling in the chips by using organoids from patients with different disease states and widening the potential for applications in clinical pharmacology with patient-specific cells.

Organs-on-Chips applications can also be advanced by applying genetic manipulation (gene editing) of the component cells, introducing resident or circulating immune cells into the chips, inducing inflammation to create disease models, and creating patient-specific chips from different individuals. Such disease model chips have the potential to be much more representative of the range of disease pathophysiology in different patients with the same disease than can be achieved with current *in vivo* or *in vitro* models. Studying chips developed using tissue from different individual patients that retain or recreate the specific biology of that patient will allow greater understanding of the impact of molecular and cellular heterogeneity on cellular, tissue, and organ functioning, and the variability in clinical manifestation and response to treatment of many diseases. Using "Diseases-on-Chips" for compound selection should further increase the predictability of drug development, improve understanding of the relevance of disease heterogeneity as a cause of drug response variability, and thereby contribute to better targeting of drugs to the patients most likely to respond or away from those at risk of serious adverse effects. In a recent example, a between-patient variation in thrombus formation in a blood vessel chip after administration of a monoclonal antibody to soluble CD40L was suggested to be related to differences in endogenous soluble CD40L, which could be a potential prognostic marker.[22] Exploring dose and exposure response in Diseases-on-Chips would provide insights into the relationship of disease heterogeneity and dose response, potentially guiding rational identification of which patients need lower or higher doses of the same drug to best treat their disease.

Ultimately, there is the potential for Organs-on-Chips to be integrated into a diagnostic and treatment selection workflow (**Figure 1**). A chip derived from an individual patient's tissue biopsy (organoids) or iPSCs could be used to screen a range of drugs, drug combinations, and doses to identify which has the potential to be most effective in that patient. In a potentially

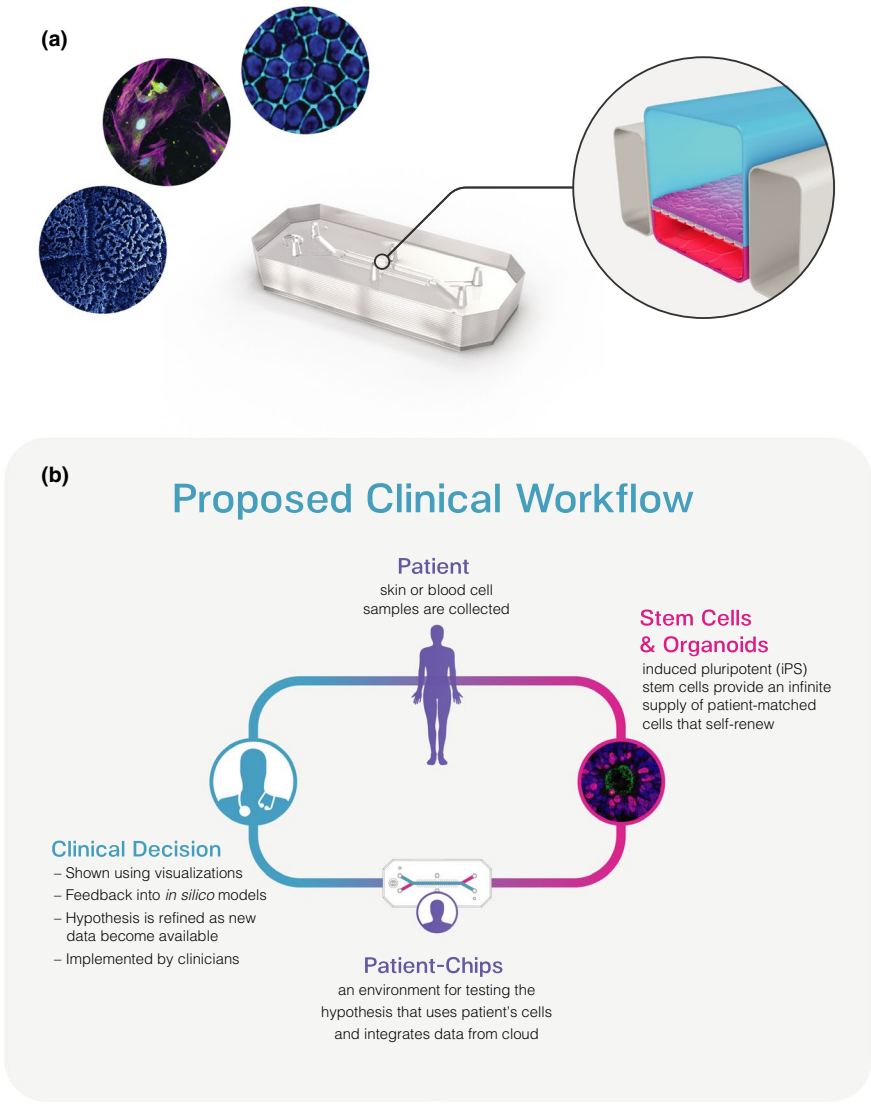

Figure 1 (**a**) Cross section of a typical Organ-on-Chip. This chip is composed of a transparent elastomeric polymer the size of an AA battery containing two hollow channels separated by a porous, flexible membrane with vacuum chambers on either side to allow for stretch when applicable. (**b**) Diagnostic and treatment selection workflow. Here, we provide an overview of the potential impact of Organs-on-Chips on the future of clinical pharmacology and how this technology could move toward a more patient-centric approach to drug treatment.

landmark trial, a bioprinted glioblastoma on a chip model using patient-derived cells was shown to be predictive of patient-specific resistances for chemoradiation with temozolomide and could be used to determine drug combinations associated with superior tumor killing.[23] We envision a 2030 where we can populate chips with an individual patient's cells, mature these to functional chips within ~ 3–7 days (the maturation time will vary depending on the organ system and cell source), and use these during a diagnostic and treatment response workup prior to initiation of treatment, even for diseases such as cancer where there is a need to start treatment quickly. Such an approach to identify the most effective treatment for an individual patient could be combined with an assessment of the risk of developing adverse events using Organs-on-Chips developed from the same patient's iPSCs or organoids to ensure choice of treatments with the best individual benefit:risk assessment.

CHALLENGES

There are several challenges to overcome to achieve this vision. One of the most critical is the development of products that can be manufactured at scale and applied in a reproducible and robust manner across clinical laboratories. Historically, micro-engineered and fluidic-based technologies, such as Organs-on-Chips have been associated with complex instrumentation, convoluted workflows, and chips that could not be manufactured at large scale. Several commercial companies have successfully made the required scale-up of chip manufacture and careful attention should be paid to differentiate products manufacturable at scale from proof-of-concept or prototypes from academic laboratories.

The high biological functionality of Organs-on-Chips usually comes at the price of low throughput and further automation is required for broader use in clinical laboratories and to enable the conduct of clinical tests to support diagnostic applications. Automation

needs to extend beyond the culturing of chips to the entire experimental workflow of chip preparation, study execution, and data capture and analysis. Chips have already been adapted to fit microplate formats used in high-content screening technologies for the collection and analysis of large numbers of microscope images.[24] It is probable that software tools for data interpretation (e.g., for calculating the apparent permeability of an intestine-chip) will need to be adapted or new ones developed and standardized because existing tools for static culture systems may not be appropriate for dynamic flow systems.

It is important that excitement about the technology does not create hype and overpromises. The path toward clinical application needs to be meticulous and will require careful qualification for the specific applications. Collaboration with regulatory authorities will be essential to meet regulatory requirements as the platform moves toward diagnostic applications.

Sourcing of patient tissue samples (organoids) and iPSCs and maturation of chips to a biological state that is ready for use in testing is also a challenge. To be useful for clinical decision making, chips used in a diagnostic or prognostic testing workflow need to be ready for experimentation within days of seeding. Biopsy-derived individual patient chips is the current approach, as described earlier, but currently this often requires weeks to months of preparation, although shorter times have been reported.[23] A more radical approach is that all patients have biobanks of their iPSCs or organoids that are ready for use if needed. Logistics, infrastructure, appropriate patient consent, and reimbursement will have to be overcome in order to make this feasible.

Finally, the technology must demonstrate value through case studies enabling biological insights not feasible with current systems, showing improved predictability, and reduced drug development attrition rates, or supporting patient diagnosis and optimal treatment or regimen selection. Organs-on-Chips must show analytical validity and good enough predictive value and cost-effectiveness. The regulatory authorities will need to play a central role with initiatives such as guidelines to industry and education. We must ensure that the qualification data and case studies are not just being generated in academic settings with prototypes but with real, scaled, and robust products (data reproducible across laboratories) that can translate into regulatory and clinical workflows.

VISION FOR THE FUTURE

As healthcare practices and medical research become more data-rich through advances in inexpensive sequencing, molecular blood diagnostics, high-resolution imaging, electronic medical records, and behavioral tracking, they must also advance the means to organize and interpret these signals. Academic and industrial development of machine learning is powering our ability to find patterns in very sparse and noisy high-dimensional datasets, which were previously intractable. However, the machine learning correlations coming from these data streams will likely need to be qualified before use as diagnostic, or prognostic, or predictive tests guiding clinical decisions. Organs-on-Chips could play an important role here by providing mechanistic understanding to these statistical correlations. The chips can also provide low-noise data (with well-defined boundaries and tightly controlled variables) to *in silico* and machine-learning tools to improve their own algorithms and predictive power. The coupling of machine learning, wearable technologies that monitor patients constantly, and Organs-on-Chips could greatly improve the predictive power of clinical pharmacology for better patient outcomes.

Successful drug discovery and development is dependent on the translation of data from preclinical studies to the clinic. Organs-on-Chips have the potential to enable a paradigm shift in clinical pharmacology. Our vision for 2030 is that the application of this technology platform will help provide individualized diagnosis and treatment selection. Clinical pharmacology would become much more patient-centric with patient-specific biology in the early stages of drug discovery, personalized efficacy, and personalized safety, all the way through development and clinical trials toward the treatment of patients in an individualized manner—start with the patient and end with the patient.

FUNDING

No funding was received for this work.

CONFLICTS OF INTEREST

Richard Peck is an employee of and shareholder in F. Hoffmann la Roche. Geraldine A. Hamilton and Christopher D. Hinojosa are employees of and shareholders in Emulate, Inc. As an Associate Editor for *Clinical Pharmacology & Therapeutics*, Richard Peck was not involved in the review or decision process for this paper.

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